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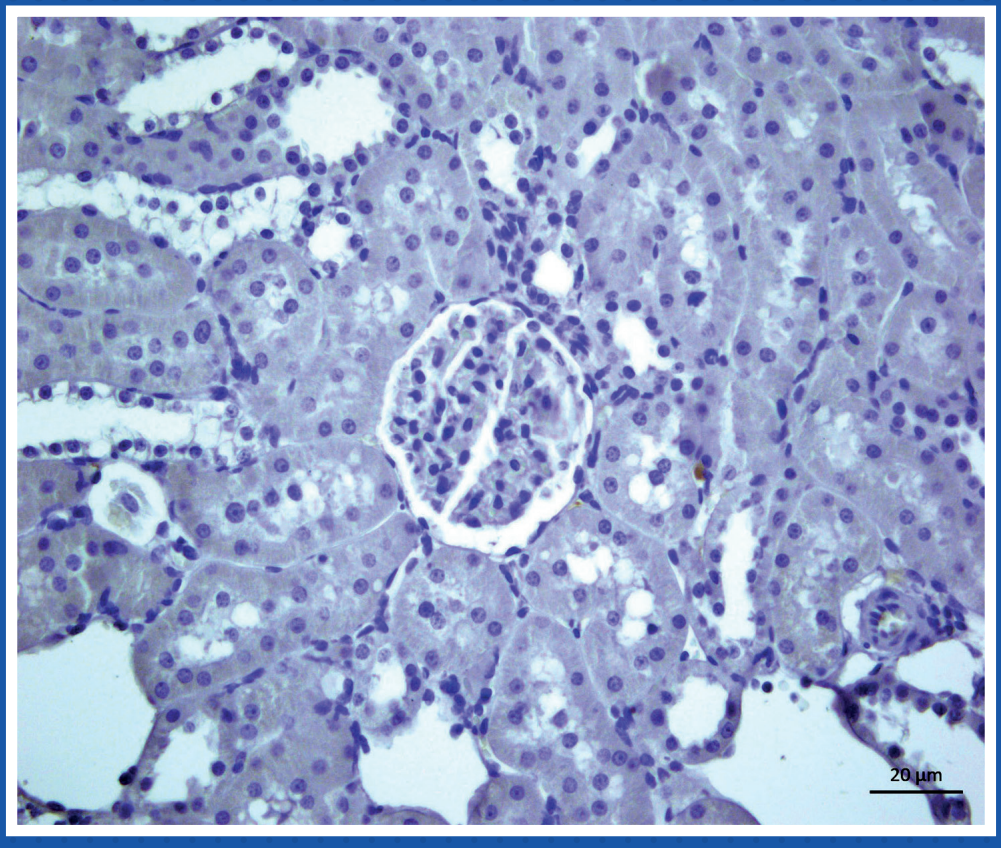


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# Northwestern Medical Journal

## Editorial Board

# Northwestern Medical Journal

## Advisory Board

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# Editorial

Dear colleagues,

It gives me great pleasure to report that the Northwestern Medical Journal will publish its second issue in 2024. Healthcare professionals are becoming more and more familiar with our journal.

Nine original articles are included in this issue. Çelik et al. examined the neuroimaging findings in children who admitted to pediatric emergen-cy clinic with acute neurologic complaints. Aytaç evaluated the antibiotic resistance rates of Klebsiella pneumoniae strains isolated from urine cultures. Yakıt Yeşilyurt investigated the pelvic floor knowledge, awareness and healthcare seeking in women with urinary incontinence. Optical coherence tomography angiography changes in patients with hemoglobinopathy was assessed by Özer et al. Soylu et al. evaluated hydroxytyrosol affects antioxidant Nrf2 expression in diabetic rat kidney. The relationship between infection parameters and urine volume in acute kidney injury was studied by Oruç et al. The prevalence of dry eye in patients using topical antiglaucoma medications was reported by Küçük et al. Seçgin et al. analyzed an anatomy laboratory for microbiological contamination, while Gökbulut et al. evaluated the satisfaction in patients performed oral glucose tolerance test.

We sincerely appreciate the thoughtful criticism that our readers, writers, publishers, and reviewers have given us. We anticipate your substantial contributions to our upcoming issues.

Best regards,
**Prof. Ahmet Ural**, M.D.
Editor-in-chief

NORTHWESTERN MEDICAL JOURNAL

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RESEARCH ARTICLE

# Evaluation of neuroimaging findings in children who admitted to pediatric emergency clinic with acute neurologic complaints

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## ABSTRACT

**Aim:** Acute neurological complaints are one of the reasons why children present to pediatric emergency departments. Neuro-imaging techniques gain more importance in children since physical examination and anamnesis do not provide sufficient information due to insufficient cooperation. We aimed to determine the distribution of patients who applied to the pediatric emergency department with non-traumatic acute neurological complaints and underwent neu-roimaging, and the clinical benefit of neuroimaging in these patients.

**Methods:** The information and records of the patients who applied to the Konya City Hospital Pediatric Emergency Clinic between January 1 and October 1, 2022, due to acute neurological complaints and underwent neuroimaging were retrieved and analyzed retrospectively. Acute neurological complaints of the patients were classified according to the International Classifi-cation of Diseases-10 diagnostic coding.

**Results:** This study included 180 (50.5% male) patients. The median age of the patients was 120 (interquartile range: 45-180) months. Afebrile convulsion was the most common reason for admission in 69 patients (38.3%). Cranial computed tomography (CCT) was performed in all 180 patients. Of the patients, 68 (37.8%) only underwent CCT scan, while 90 (50%) had diffusion magnetic resonance imaging (MRI), 20 (11.1%) had brain+diffusion MRI, and 2 (1.1%) had brain+diffusion+spinal MRI. Neuroimaging abnormalities were statistically higher in patients with abnormal physical examination findings than in patients with normal physical examination findings ($p<0.001$).

**Conclusion:** Neuroimaging results are mostly normal even in the presence of symptoms such as seizures, headaches, and impaired consciousness. Therefore, neuroimaging should be planned by considering not only the acute neurological complaint on admission but also the physical examination findings.

**Keywords:** acute neurological complaints, neuroimaging, pediatric emergency

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## INTRODUCTION

Emergency outpatient clinics have high patient admission rates and require urgent diagnosis and planning of treatment approaches based on the patient's physical, social, and psychological conditions (1). Acute neurological complaints such as seizures, acute changes in consciousness, headache, and acute neurological deficits are among the reasons why pediatric patients apply to pediatric emergency outpatient clinics (2). Initiating the necessary treatment to prevent or minimize acute brain damage in patients who apply to the pediatric emergency outpatient clinic with acute neurological complaints as soon as possible requires an accurate and rapid approach. The first thing to do is obtain a detailed medical history and perform a physical examination. However, laboratory tests and imaging techniques gain more importance in pediatric patients since physical examination and anamnesis do not provide sufficient information due to insufficient cooperation. Today, many imaging techniques, such as direct radiography, ultrasonography (USG), cranial computed tomography (CCT), and magnetic resonance imaging (MRI) are used in the evaluation of central nervous system (CNS) diseases. The patient's clinical picture, age, pre-diagnosis, and the physical conditions of the hospital are determinants in the decision of the imaging techniques (3,4). CCT is very successful in evaluating brain anatomy and pathology and is the first-line imaging technique in life-threatening conditions that require immediate intervention, such as brain tumors, head trauma, cerebral hemorrhage, and hydrocephalus. However, despite its clinical importance and widespread use, CCT contains high radiation, and the risk of radiation-induced malignancy may be higher in young children than in adults (5,6). Therefore, CCT should be used only when necessary. Besides brain CT, structural abnormalities, neuro-metabolic diseases, and tumors are also visualized using MRI. However, the use of MRI in urgent clinical evaluation is limited due to its high cost, long scan time, and the possible need for anesthetic sedation.

This study aimed to determine the distribution of patients who applied to the pediatric emergency outpatient clinic with non-traumatic acute neurological complaints and underwent neuroimaging (cranial MRI, spinal MRI, CCT, USG, etc.), according to indications, the frequency of emergency intervention (medical/surgical) due to abnormal clinical and radiological findings, and the clinical benefit of neuroimaging in these patients, as well as the frequency of excessive and unnecessary use.

## MATERIAL AND METHOD

In this study, the information and records of the patients who applied to the Konya City Hospital Pediatric Emergency Outpatient Clinic between January 1 and October 1, 2022, due to non-traumatic acute neurological complaints and underwent neuroimaging were retrieved from the hospital automation system and analyzed retrospectively.

Acute neurological complaints of the patients were classified as syncope, acute altered consciousness, headache, seizure, and acute neurological deficit according to the International Classification of Diseases-10 (ICD 10) diagnostic coding. The age, gender, time of admission, complaint on admission, accompanying symptoms, previous diseases, physical examination findings, neuroimaging results reported by radiology, consultation reports of related branches, diagnoses, and surgical or medical treatments applied, were retrieved from the patient record system and files of the patients included in the study.

### Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics Version 22.0 for Windows statistical software package (IBM Corp., Armonk, NY). Numbers and percentages were reported for discrete variables; continuous variables were expressed as mean and standard deviation for data with normal distribution and as the median and interquartile range (IQR) for non-normally distributed data. The chi-square (X2) test was used to compare nonparametric data; the Mann–Whitney U test was used to compare non-normally distributed continuous data and the independent samples t-test for normally distributed continuous data. The P value < 0.05 was considered statistically significant.

## RESULTS

A total of 269 patients who applied to the Pediatric Emergency Department of our hospital between January 1 and October 1, 2022, with acute neurological complaints and underwent neuroimaging were identified. Of these patients, 89 were excluded due to the lack of information in the patient record system and suspected trauma history. The present study included 180 patients, 91 of whom were male (50.5%). The median age of the patients was 120 (interquartile range: 45-180) months.

Afebrile convulsion was the most common reason for admission to the emergency department in 69 patients (38.3%). This was followed by headache in 36 (20%) patients and febrile convulsions in 34 (18.9%) patients (Table 1). Of the patients, 154 (85.6%) applied to the emergency outpatient clinic outside working hours.

Abnormal physical examination findings were recorded in 36 patients (20%). These findings were confusion in 25 patients (13.9%), speech disorder + limb weakness in four patients (2.2%), peripheral facial paralysis in three patients (1.7%), double vision in two patients (1.1%), neck stiffness in one patient (0.6%), limb weakness in one patient (0.6%), and ataxic gait + speech disorder in one patient (0.6%). Neuroimaging results were abnormal in 13 (36%) of 36 patients with pathological physical examination findings, whereas neuroimaging results were abnormal in only three (2%) of 144 patients with normal physical examination findings. Neuroimaging abnormality was statistically higher in patients with abnormal physical examination findings than in patients with normal physical examination findings ($p<0.001$).

**Table 1.** Characteristics of patients.

| | |
|---|---|
| **Age (month)** | |
| Median (interquartil range) | 120 (45-180) |
| **Sex, n(%)** | |
| Male | 91 (%50.5) |
| Female | 89 (%49.5) |
| **Acute neurological complaints, n(%)** | |
| Afebrile seizure | 69 (%38.3) |
| Headache | 36 (%20) |
| Febrile seizure | 34 (%18.9) |
| Acute change in consciousness | 32 (%17.8) |
| Acute neurological sequelae | 9 (%5) |
| **Physical examination findings, n(%)** | |
| Normal | 144 (%80) |
| Abnormal | 36 (%20) |

CCT was performed in all 180 patients. Of these patients,68 (37.8%) underwent CCT scan only, while 90 (50%) had diffusion MRI, 20 (11.1%) had brain+diffusion MRI, and two (1.1%) had brain + diffusion + spinal MRI.

### Analysis of patients' imaging results according to their complaints on admission

### Neuroimaging results of patients presenting with afebrile convulsions

A CCT scan was requested for all 69 patients, and 7 (10.1%) of them displayed abnormal findings. Of the seven patients, three had ventriculoperitoneal shunt dysfunction, two had hydrocephalus, one had Dandy-Walker malformation, and one had cerebral atrophy. Diffusion MRI was performed in 44 (63.7%) of these patients, diffusion + brain MRI was performed in 10 (14.5%) patients, and abnormalities were detected in nine of them. Seven of the nine patients were patients who previously had abnormalities on the CCT scan. The other two patients had normal CCT, one had posterior reversible encephalopathy syndrome (PRES) on brain and/or diffusion MRI, and the other had an arteriovenous malformation. Three patients with ventriculoperitoneal shunt dysfunction underwent emergency surgery.

### Neuroimaging results of patients presenting with headache

CCT was performed in all 36 patients and abnormal findings were observed in one (2.8%) patient. This patient had an intracranial mass. Eleven of the patients had diffusion MRI, and two of them had brain+diffusion MRI. Abnormalities were detected in two of these patients. One of the patients was the patient with a CCT showing abnormality. The other patient had a

normal CCT, exhibited an increase in the amount of CSF around the optic nerve on brain+diffusion MRI, and was later diagnosed with idiopathic intracranial hypertension. The patient with an intracranial mass underwent emergency surgery.

### Neuroimaging results of patients presenting with complicated febrile convulsions:

All 34 patients underwent CCT and 16 patients underwent CCT+diffusion MRI. None of the patients exhibited abnormal findings.

### Neuroimaging results of patients presenting with acute altered consciousness

Of the 32 patients who were diagnosed with syncope by history and physical examination, all underwent CCT, 16 had CCT+diffusion MRI, and 2 had CCT+diffusion MRI+brain MRI. None of the patients had abnormal findings. All eight patients who presented with acute altered consciousness without syncope underwent CCT. Bleeding was detected in one patient. CCT+diffusion MRI was performed in six patients, and acute ischemia was detected on the diffusion MRI in one of these patients. CCT+diffusion MRI+brain MRI were performed in two patients, and demyelinating plaques were detected on the brain MRI in one of the patients.

### Neuroimaging results of patients presenting with acute neurological deficit

All nine patients underwent CCT+diffusion MRI, and five patients underwent CCT+diffusion MRI+brain MRI. Acute infarction was detected in one of the patients on diffusion MRI and brain MRI.

The rates of abnormalities in neuroimaging results according to the patients' complaints on admission are summarized in Figure 1.

## DISCUSSION

In acute neurological conditions, the aim of the emergency services should be to make an accurate and rapid diagnosis, initiate treatment immediately, and ensure that neuronal loss or damage is as much as possible (3). The lack of cooperation in most children necessitates laboratory tests and neuroradiological imaging methods in addition to anamnesis and physical examination findings.

In our study, the most common complaint on admission was afebrile convulsion, which was seen in 38.3% of the patients. Epilepsy is a predisposition to recurrent seizures. Epilepsy is the occurrence of at least two seizures without a demonstrable cause and recurs for more than 24 hours, and reflex seizures are included in this definition (7). Emergency CCT imaging is recommended by the American Academy of Neurology in cases of prolonged post-ictal period or focal neurological deficit in children who have had a febrile seizure for the first time (8). A previous cohort study of 155 pediatric patients reported that CCT was performed in 46.5% of patients presenting with a first afebrile seizure. CCT results were normal in 87.5% of these patients. While clinically significant findings (left-sided intracranial calcification and encephalomalacia, subependymal nodules, and focal cerebral edema)

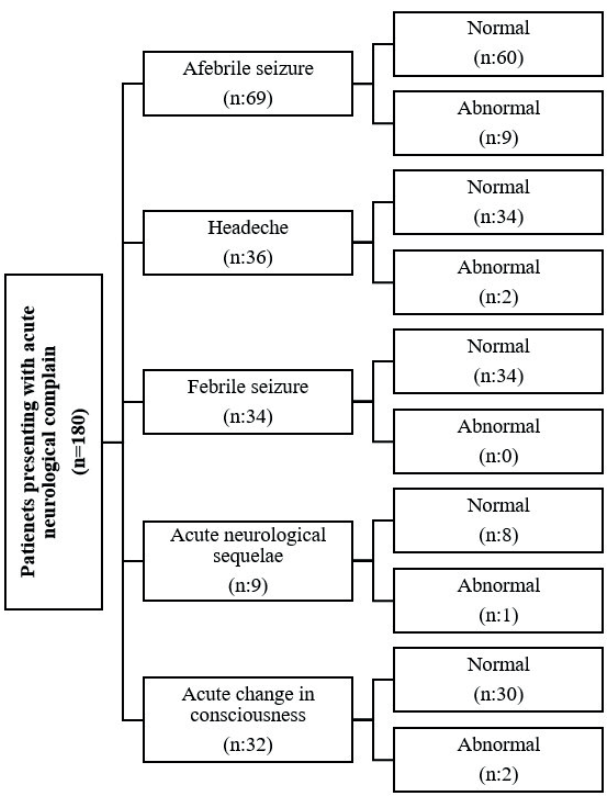


**Figure 1.** Neuroimaging results of patients presenting with acute neurological complaints.

were observed in three patients who had brain CT findings, nonspecific findings were reported in six patients (9). In our study, 38.3% of the patients who underwent neuroimaging presented with a first afebrile seizure. All patients underwent CCT, three had ventriculoperitoneal shunt dysfunction, two had hydrocephalus, one had Dandy-Walker malformation, and one had cerebral atrophy. Diffusion MRI was performed in 44 (63.7%) patients, and diffusion+brain MRI was performed in 10 (14.5%) patients. Anomaly was detected in nine of these patients. Seven of the nine patients were patients with CCT-identified abnormalities. The other two had normal CCT, one had posterior reversible encephalopathy syndrome (PRES) on brain and/or diffusion MRI, and the other had an arteriovenous malformation. None of the patients who presented with the complaint of seizures without any known central nervous system pathology required emergency surgical intervention. In these cases, a brain MRI may be more useful in elective conditions instead of a CCT.

In our study, another complaint that required neuroimaging was headache. The incidence of headache has increased significantly over the past 30 years (10). It is more common in girls and between the ages of 13-19 (11). The International Headache Society (IHS) classification system classifies headaches into two main groups, primary and secondary headache disorders. Primary headache has no underlying cause and is the most common headache in childhood. Headache that develops due to an underlying disease is defined as secondary headache. Although the use of neuroradiological imaging is not recommended for recurrent headaches in children with normal neurological examinations, it is often used to rule out intracranial pathologies and to alleviate the concerns of families (12). In our study, 20% of the patients who underwent neuroimaging had headache complaints. CCT was performed in all patients, and an abnormal finding (intracranial mass) was detected in only one patient (2.8%). Eleven of the patients had diffusion MRI, and two of them had Brain+Diffusion MRI. Abnormalities were detected in two of these patients. One of the patients had a CCT showing an abnormality. The other patient had a normal CCT, exhibited an increase in the amount of CSF around the optic nerve on brain+diffusion MRI, and was later diagnosed with idiopathic intracranial hypertension. In the literature, brainstem glioma was found in only one of 58 pediatric patients who applied to the pediatric emergency department and underwent CCT due to the possibility of secondary headache based on history and physical examination (13). Another study reported that 75.1% of pediatric patients admitted to the emergency department with the complaint of headache had normal brain MRI results. Among patients with abnormal findings, the most common symptoms were sinusitis 7.2%, pineal cyst 2.4%, arachnoid cyst 1.9%, and Chiari malformation (14). Our study and previous studies have shown that the majority of patients who applied to the pediatric emergency outpatient clinic with a complaint of headache and underwent neuroimaging had normal neuroimaging results. This highlights the need for guidelines on which patients presenting to the pediatric emergency outpatient clinic with a complaint of headache should undergo neuroimaging.

According to the International League Against Epilepsy (ILAE), the most common type of seizure in childhood and the most frequent cause of emergency admission is febrile seizure (7). It is a type of seizure accompanied by fever that occurs between the ages of 6 months and 6 years, without a known cause such as severe electrolyte-metabolic disorder, infection, trauma, or poisoning, and without a previous history of afebrile seizures. Of the febrile seizures, 20-30% are complicated febrile seizures, and neuroradiological imaging is decided in these patients after a detailed clinical evaluation (15). Generally, febrile seizures are not associated with intracranial pathologies (16). Although neuroimaging is not recommended in patients presenting with the first simple febrile seizure, there is no guideline for complicated febrile seizures (17). In our study, all patients who underwent neuroimaging due to complicated febrile seizures had normal findings, suggesting that neuroimaging is not essential in these patients.

Syncope is a common cause of altered consciousness. It is particularly common in children over 10 years of age and its incidence is almost twice as high in girls compared to boys (18). In our study, 24 (75%) of 32 patients presenting with acute altered consciousness were suspected to have syncope, but neuroimaging results of these patients were found to be normal.

Consistent with our study, the literature also shows that the CCT findings of the patients who presented to the emergency clinic and were diagnosed with syncope due to acute altered consciousness were indeed normal (19). While EEG is routinely recommended in cases of unexplained syncope in the literature, neuroimaging techniques are recommended only in cases with focal neurologic findings, a history of trauma, or suspected epilepsy (20). On the other hand, of the eight patients who were still unconscious when they were admitted to the emergency department, one had intracranial hemorrhage, one had acute ischemia and one had multiple demyelination plaques. The literature has shown that cranial MRI contributes to the treatment of more than three-quarters of children presenting to the pediatric emergency department with prolonged non-traumatic consciousness changes and may be preferred in acute non-traumatic coma (21).

Another group of patients in our study were those with acute neurological deficits. According to the neuroimaging results, the findings were consistent with acute infarction in one out of nine patients, and the other patients were evaluated as normal. The physical examination findings were found to be normal at the end of the 8-hour emergency follow-up period in all patients who had normal neuroimaging results.

Neuroimaging abnormality was statistically higher in patients with abnormal physical examination findings than in patients with normal physical examination findings. In light of these data, it is clear that neuroimaging contributes significantly to the diagnosis and treatment of patients with abnormal physical examination findings.

The weakness of our study is that there may be subjective findings and interpretations in the physical examinations due to the evaluation of the patients by different doctors. The strength of our study is that it was conducted in a tertiary health center with a rich diversity of patients.

In conclusion, CCT is the most frequently used examination method in the evaluation of acute neurological problems in our pediatric emergency department, followed by brain MRI. Neuroimaging results are mostly normal even in the presence of symptoms such as seizures, headaches, and impaired consciousness. Regardless of the cause, the fact that neuroimaging is normal in most patients suggests unnecessary and excessive use. Therefore, neuroimaging should be planned by considering not only the acute neurological complaint on admission but also the physical examination findings.

### Ethical approval

This study has been approved by the University of Karatay (approval date 23/12/2022, number E-2022/008). We did not collect any data that could be used to identify patients. As this was a non-interventional retrospective study, informed consent forms were considered not necessary.

### Author contribution

Concept: HÇ, İÖ, NB, HKÖ; Design: HÇ, İÖ, NB, HKÖ; Data Collection or Processing: HÇ, İÖ, NB, HKÖ; Analysis or Interpretation: HÇ, İÖ, NB, HKÖ; Literature Search: HÇ, İÖ, NB, HKÖ; Writing: HÇ, İÖ, NB, HKÖ. All authors reviewed the results and approved the final version of the article.

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The authors declare the study received no funding.

### Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

# Antibiotic resistance rates of *Klebsiella pneumoniae* strains isolated from urine cultures

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**Cite as:** Aytaç Ö. Antibiotic resistance rates of *Klebsiella pneumoniae* strains isolated from urine cultures. Northwestern Med J. 2024;4(2):64-69.

## ABSTRACT

**Aim:** Our study aimed to determine the antibiotic resistance rates of *K. pneumoniae* by retrospectively examining the results of urine culture samples studied in our laboratory.

**Methods:** Urine samples with *K. pneumoniae* growth, sent to our laboratory from various wards, outpatient clinics, and intensive care units between July 1, 2018 and December 31, 2022 were included in the study and retrospectively examined.

**Results:** The antibiotic to which *K. pneumoniae* was most resistant was cefixime (53.3%), and the antibiotic to which it was least resistant was imipenem (12.1%). While the lowest resistance rates were observed in the samples of outpatients, the highest resistance rates were observed in the samples of ward patients and to cefixime (81%), amoxicillin clavulanic acid (AMC) (80%), trimethoprim-sulfamethoxazole (TMT/SXT) (74.8%), and ciprofloxacin (72.1%). Ertapenem (48.9%), meropenem (50.2%) and piperacillin-tazobactam (PRP) (57.3%) resistance was found to be higher in intensive care patients.

**Conclusion:** Although fluctuations in resistance rates have been observed over the years, resistance rates have generally been found to be high for antibiotics frequently used in the empirical treatment of urinary tract infections. Re-adjusting treatment according to culture results and keeping resistance rates in mind for empirical treatment will be important for treatment success.

**Keywords:** antibiotic resistance, *Klebsiella Pneumoniae*, urine cultures

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## INTRODUCTION

*Klebsiella pneumoniae* (*K. pneumoniae*) is an important Gram-negative bacterium causing hospital-acquired infections associated with septicemia, pneumonia, and urinary tract infections (1). The Centers for Disease Control and Prevention (CDC) reported in 2019 that the multi-resistance seen in *Acinetobacter baumannii* (43.6%), *K. pneumoniae* (15.6%), *Escherichia coli* (*E. coli*) (7.3%), and *Pseudomonas aeruginosa* (3.9%) was concerning (2). Today, the increase in multi-resistance in bacteria has become an important public health problem, especially in hospital environments, as resistant strains are increasingly spreading in our globalized world (3). According to data from a study published in the Lancet in 2022, based on predictive statistical modeling, *K. pneumoniae* ranks third in deaths associated with antimicrobial resistance. *K. pneumoniae* ranks second in deaths attributed to antimicrobial resistance, and according to this study, the rate of carbapenem-resistant *K. pneumoniae* in Türkiye was reported to be 20–30% (4).

Urinary tract infections (UTI) are currently the most common bacterial infections across all age groups, both within and outside hospital settings (5). Although many bacterial species and fungi cause urinary tract infections, *E. coli* and *Klebsiella* spp. are reported to cause approximately 90% of these infections (6). According to the European Association of Urology Guidelines on Urological Infections, antibiotics with resistance above certain rates are not suitable for use in empirical treatment (7). Therefore, to determine the appropriate antibiotic to be used in empirical treatment, the change over the years and the status of the antibiotic resistance rates of *K. pneumoniae*, which is found to be the most common causative agent of urinary tract infections after *E.coli*, in our region, must be well known. This study aimed to determine the antibiotic resistance rates of *K. pneumoniae* by retrospectively analyzing the results of urine culture samples from patients admitted to our hospital.

## METHODS

Urine samples with *K. pneumoniae* growth sent to our laboratory from various wards, outpatient clinics, and intensive care units between July 1, 2018 and December 31, 2022 were included in the study and examined retrospectively. Urine samples were plated on blood agar and eosin methylene blue (EMB) agar (Oxoid, Basingstoke, United Kingdom) media with a quantitative method using loops capable of holding 0.01 ml of urine and incubated at 37 C in an aerobic environment for 18-24 hours. Identification and antibiotic susceptibility tests were performed on samples with bacterial growth of 100,000 cfu/ml and above and on samples with lower numbers of microorganisms thought to be the causative agent by taking into account characteristics such as the number of colonies grown, the number of species, the presence of leukocytes in the urine sample, and the clinical condition of the patient. Identification of microorganisms and antibiotic susceptibility tests were performed using the VITEK 2 Compact system (bioMérieux-France). Antibiotic susceptibility tests were evaluated according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (8). Extended-spectrum beta-lactamase (ESBL) confirmation tests could not be conducted, which is a limitation of our study; consequently, potential rates were reported based on the results obtained from the automated identification system.

Approval for this study was obtained from Fırat University Faculty of Medicine Non-Interventional Clinical Ethics Committee (Decision No: 04-18 Date: 17.03.2022). The study was conducted in accordance with the principles of the Declaration of Helsinki.

## RESULTS

In our study, significant growth was detected in 20.1% (5,877) of a total of 29218 patients who were admitted to the outpatient clinic and were requested a urine culture. *K. pneumoniae* was detected in 14.1% (830) of the patients whose samples showed growth (Table 1). Among the patients with *K. pneumoniae*, 60% (498) were female and 40 % (332) were male. The mean age of outpatients with *K. pneumoniae* was 27.00±27.57 years.

In our study, a total of 10,093 urine culture samples were sent to our laboratory from the wards. Growth was detected in 11.9% (1200) of these samples.

**Table 1.** *K. pneumoniae*-detected sample distribution by ward and time period N (%).

| | 2018 (last six months) | 2019 | 2020 | 2021 | 2022 | Total |
|---|---|---|---|---|---|---|
| Ward | 18 (16.5) | 99 (21.1) | 39 (14.0) | 64 (17.2) | 85 (16.3) | 305(17.4) |
| Intensive Care | 24 (22.0) | 152 (32.5) | 136 (48.7) | 113 (30.4) | 188 (36.2) | 613(35.1) |
| Outpatient Clinic | 67 (61.5) | 217 (46.4) | 104 (37.3) | 195 (52.4) | 247 (47.5) | 830(47.5) |
| Total | 109 | 468 | 279 | 372 | 520 | 1748 |

*K. pneumoniae* was detected in 25.4% (305) of the patients whose samples showed growth (Table 1). Of the patients with *K. pneumoniae*, 54.1% (165) were female and 45.9% (140) were male. The mean age of the ward patients with *K. pneumoniae* was 61.74±27.16 years.

In our study, a total of 9564 urine culture samples were sent to our laboratory from intensive care units. Growth was detected in 22.3% (2128) of these samples, and *K. pneumoniae* was detected in 28.8% (613) of these samples (Table 1). Of the patients with *K. pneumoniae*, 62.8% (385) were female and 37.2 % (228) were male. The mean age of intensive care unit patients with *K. pneumoniae* was 69.34±31.18 years.

The antibiotic to which *K. pneumoniae* was most resistant was cefixime (53.3%), and the antibiotic to which it was least resistant was imipenem (12.1%). When the resistance rates by year were analyzed, it was found that even though the highest resistance rates for the majority of antibiotics were detected in 2020, resistance rates fluctuated over time (Figure 1).

When the resistance rates were evaluated by clinics, it was found that while the resistance rates were the lowest in the samples of outpatients, the highest resistance to antibiotics was in ward patients and to cefixime (81%), amoxicillin clavulanic acid (AMC) (80%), trimethoprim-sulfamethoxazole (TMT/SXT) (74.8%), and ciprofloxacin (72.1%). Ertapenem (48.9%), meropenem (50.2%), and piperacillin-tazobactam (PRP) (57.3%) resistance were higher in intensive care unit patients (Figure 2).

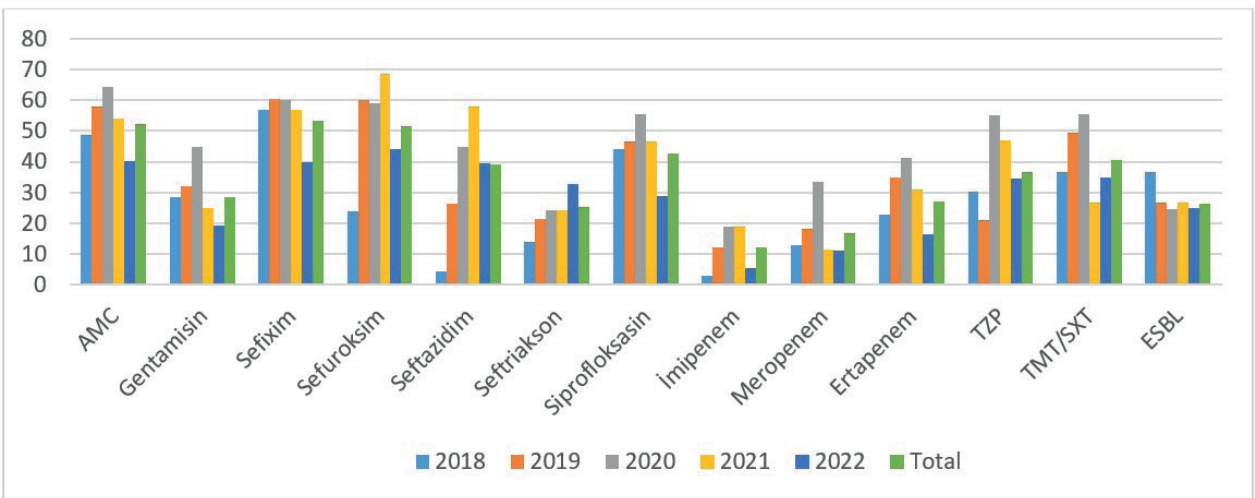


**Figure 1.** Resistance profile of Klebsiella spp. isolates from urine cultures to different antibiotics by year (%).

AMC: Amoxicillin-clavulanate; TZP: Piperacillin-tazobactam; TMP-SXT: Trimethoprim-sulfamethoxazole; ESBL: extended-spectrum beta lactamase.

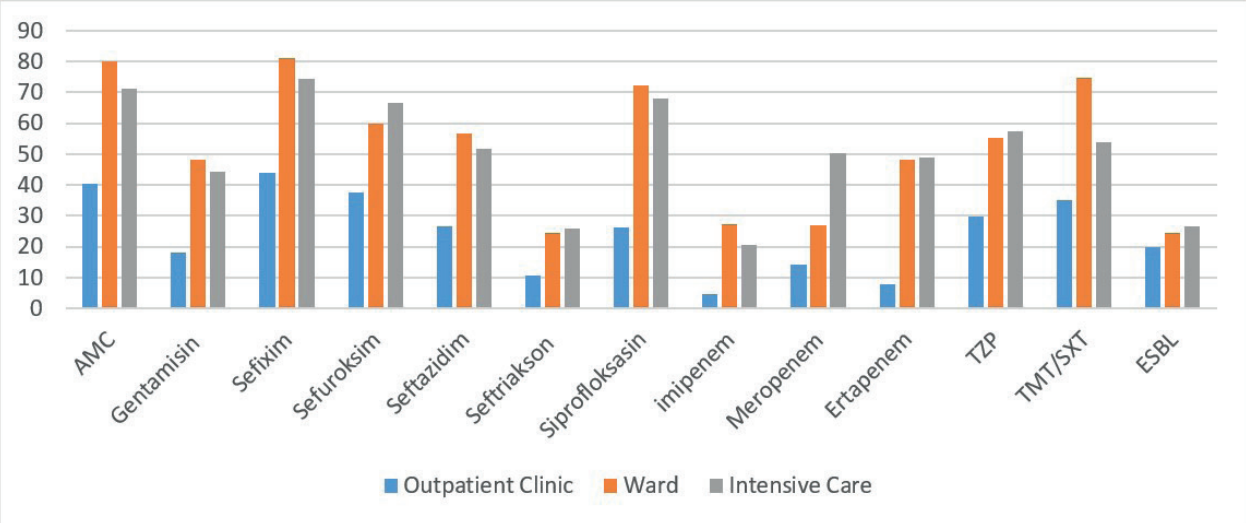


**Figure 2.** Resistance profile of *Klebsiella* spp. strains isolated from urine culture to various antibiotics by clinics (%).

AMC: Amoxicillin-clavulanate; TZP: Piperacillin-tazobactam; TMP-SXT: Trimethoprim-sulfamethoxazole; ESBL: extended-spectrum beta lactamase.

## DISCUSSION

UTIs, which affect 150 million people worldwide every year, are among the most common infectious diseases (9). The distribution of infectious agents and profiles of antibiotic resistance may vary regionally. Additionally, resistance rates in the same region may also change over time. Having a good command of local epidemiologic data and knowing antibiotic resistance rates are important for rational antibiotic use (10). It would therefore be beneficial for centers to conduct research on infectious agents and antibiotic resistance rates in their regions.

*K. pneumoniae* isolates are emerging as community- and hospital-acquired infectious agents with various antibiotic resistance mechanisms. Antibiotic treatment poses a significant problem as *K. pneumoniae* isolates become fully resistant by producing ESBL, high-level AmpC beta-lactamase, carbapenemase, and oxacillinase due to multi-resistant strains, which are especially prevalent in intensive care patients (11).

According to the results of our study, *K. pneumoniae* strains exhibited the lowest level of resistance to carbapenems. Ertapenem was the carbapenem with the highest resistance throughout all years. Meropenem (50.2%) was the most resistant carbapenem among intensive care unit patients when analyzed by ward. According to World Health Organization data for 2021, the percentage of invasive *K. pneumoniae* isolates resistant to carbapenems (imipenem/meropenem) was between 10% and 25% in Türkiye (12). According to National Antimicrobial Resistance Surveillance System (NAMDSS) 2014 data, the level of carbapenem resistance in invasive *K. pneumoniae* strains is 15% (13). In our study, although carbapenem resistance varied over time, ertapenem (41.2%) and meropenem (33.3%) resistance rates were the highest in 2020. Although carbapenem group antibiotics generally exhibited the lowest resistance rates, the fact that carbapenem resistance was significantly higher in 2020 than in other years may be attributable to the increased use of carbapenems, particularly in wards and intensive care units, as a result of the COVID-19 pandemic.

The Infectious Diseases Society of America recommends quinolones, fosfomycin, trometamol, or nitrofurantoin as first-line treatment for UTI if the regional resistance rate is above 20% and trimethoprim-sulfamethoxazole if it is below 20% (14,15). The resistance rates to ciprofloxacin, trimethoprim sulfamethoxazole, and AMC, which are frequently preferred in the treatment of community-acquired UTIs, were 26.1%, 35.1%,

and 40.4%, respectively, in outpatient, and 72.1%, 74.8%, and 80%, respectively, in ward patients. Similar rates were found in other studies conducted for ciprofloxacin, trimethoprim sulfamethoxazole, and AMC in outpatients (16-18). It would be beneficial to avoid these antibiotics in empirical treatment due to high resistance rates.

While antibiotic resistance has been an important problem for hospital-acquired infections, it has also become an important problem for community-acquired agents (19). With their resistance mechanisms, gram-negative ESBL-positive bacteria develop resistance to several antibiotic groups. Studies have shown an increase in ESBL rates over the years. According to the results of a review of 101 articles published in Türkiye, the ESBL rate was reported to be 8.09% in 1996–2001, 10.61% in 2002–2007, and 28.17% in 2007–2012 (20,21). In a study conducted in Türkiye between 2018 and 2019, the ESBL type resistance rate was reported to be 40-47% in *Klebsiella* spp and E. coli strains isolated from community-acquired UTIs (6). In another study conducted between 2020 and 2021, 84.63% of the 423 *K. pneumoniae* isolates examined were ESBL-positive (22). In our hospital, there were no significant changes in ESBL rates, and the average rate was determined to be 26.4%. However, as a limitation of our study, extended-spectrum beta-lactamase (ESBL) confirmation tests could not be performed, and potential rates were reported according to the results obtained from the automated identification system.

## CONCLUSION

In conclusion, this study emphasizes that the rational use of antibiotics is very important, that local epidemiological data should be closely monitored, and that necessary precautions should be taken. Moreover, it should be kept in mind that reassessing each treatment according to the antimicrobial susceptibility profile plays a crucial role in both increasing treatment success and decreasing resistance rates due to the high resistance rates of antibiotics frequently used in the empirical treatment of UTIs.

### Ethical approval

This study has been approved by the Fırat University Non-invasive Research Ethics Committee (approval date 17/03/2022, number 2022/04-18). Written informed consent was obtained from the participants.

### Author contribution

Surgical and Medical Practices: ÖA; Concept: ÖA; Design: ÖA; Data Collection or Processing: ÖA; Analysis or Interpretation: ÖA; Literature Search: ÖA; Writing: ÖA. The author reviewed the results and approved the final version of the article.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

# Investigation of pelvic floor knowledge, awareness and healthcare seeking in women with urinary incontinence: A cross-sectional study

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## ABSTRACT

**Aim:** Healthcare seeking by women with urinary incontinence is affected by many factors. However, the effect of pelvic floor awareness and knowledge on seeking health care is not clear. We aimed to investigate the relationship between pelvic floor awareness, urinary incontinence (UI) and pelvic floor knowledge levels and healthcare seeking in women with incontinence.

**Methods:** A total of 178 women, 96 incontinent and 82 continent, were included in the study. The presence of UI was evaluated with Incontinence Questionnaires (3IQ), incontinence knowledge level with the Prolapse and Incontinence Knowledge Questionnaire (PIKQ-UI), and pelvic floor knowledge with the Pelvic Floor Health Knowledge Quiz (PFHKQ). Pelvic floor awareness and treatment seeking were measured with open-ended questions compiled from the literature. The Mann Whitney U, Chi-square and Kruskal Wallis tests were used. A value of $p<0.05$ was considered statistically significant.

**Results:** There were significant differences between the PIKQ-UI scores of incontinent women who answered yes or no to questions about pelvic floor awareness ($p<.05$) and seeking health care ($p=0.039$). The PIKQ-UI scores of incontinent women were higher than those of continent women ($p=0.033$). Incontinent and continent women had similar PFHKQ

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scores (p>0.05). A difference was observed in the purpose of seeking information about the pelvic floor between women with and without incontinence (p=0.002).

**Conclusions:** The knowledge level of incontinent women with pelvic floor awareness and who seek health care was higher than that of incontinent women without pelvic floor awareness and who do not seek health care. Pelvic floor awareness in incontinent women may contribute to healthcare seeking and increase the level of knowledge about incontinence and pelvic floor.

**Keywords:** awareness, healthcare seeking, knowledge, urinary incontinence

## INTRODUCTION

International organizations such as the International Urogynecological Association and the International Continence Society define the pelvic floor as the structures located within the bony pelvis, i.e., urogenital, and anorectal viscera, pelvic floor muscles and their connective tissues, nerves, and blood vessels. Pelvic floor dysfunction (PFD) is defined as a damage/injury to these structures (1). Urinary incontinence (UI) is one of the most common PFD (2).

UI is defined as the involuntary leakage of urine (1). UI, which is frequently seen in women, reduces the quality of life and has a negative psychosocial impact (3). Despite its high prevalence and being a treatable disease, many affected women do not seek treatment (4). Previous studies have reported that only between 22-50% of women with UI seek treatment (5,6). Lack of knowledge about the causes of UI, treatment options or what it entails, as well as the widespread myth that UI is simply a consequence of childbearing and ageing, all have a negative impact on treatment seeking. Regardless of the progress made in access to information in recent years, misconceptions about UI are still very common, and therefore, this condition should be the focus of health professionals. In addition, the lack of information about UI negatively affects the decision to seek health care (7).

Knowledge of UI and health care seeking are frequently assessed in the literature. However, fewer studies aim to assess women's knowledge and awareness of pelvic floor diseases (2,8). It has been reported that PFD is more common in women with poor pelvic floor awareness and knowledge, and therefore acquiring more knowledge of pelvic floor health might encourage women to seek healthcare (9,10). Previous studies have reported that factors such as severity of incontinence, ethnicity, age, and loss of ablution influence women with UI to seek healthcare (11-13). However, there is a lack of clarity about the effect of pelvic floor awareness and knowledge on health care seeking. Therefore, this study aimed to investigate the relationship between pelvic floor awareness, UI, pelvic floor knowledge levels, and healthcare seeking in women with incontinence.

## MATERIALS AND METHODS

### Study design and participants

This cross-sectional study was carried out as a web-based assessment using an online form (Google forms) due to the Covid-19 pandemic. A total of 178 women, 96 incontinent and 82 continent, were included in the study. These were women aged between 20 and 75 years, who were able to speak and read Turkish, and volunteered to participate in the study. Women who could not use smartphones and computers were excluded from the study. Informed consent was obtained from all individual participants.

This study was performed in line with the principles of the Declaration of Helsinki. The approval was granted by the Bolu Abant İzzet Baysal University Clinical Research Ethics Committee (Date: 13.04.2021/No: 2021/81).

### Sample size

G-Power 3.0.10 program was used to calculate the sample size. Since no equivalent study in the literature with a hypothesis similar to the one in this study, a moderate (w=0.5) effect size would be significant when comparing the incontinent and the continent women. We planned to recruit a total of at least 128 women,

with a minimum of 64 in each group, giving a statistical power of 80% (α = 5%). The study was carried out as a web-based assessment. Due to the estimated dropout rate (the possibility of women misunderstanding the question, leaving it blank, or overlooking it) of 20%, we aimed to recruit at least 154 (77/77) women.

### Data collection instruments

Women's socio-demographic and physical characteristics (age, weight, height, education, occupation, marital status, menstrual status, and obstetric history) were recorded. The presence of UI, incontinence and pelvic floor knowledge levels were assessed using an online form. The presence of UI, incontinence and pelvic floor knowledge levels were evaluated using the 3 Incontinence Questionnaires (3IQ), the Prolapse and Incontinence Knowledge Questionnaire (PIKQ), and the Pelvic Floor Health Knowledge Quiz (PFHKQ), respectively. Pelvic floor awareness and health care seeking were measured with open-ended questions compiled from the literature.

### Presence of urinary incontinence

The presence of UI in women was assessed using the 3 Incontinence Questionnaires (3IQ). This questionnaire consists of three questions and takes approximately 30 seconds to complete (14).

### Incontinence knowledge

Women's knowledge about incontinence was assessed using the Turkish version of the PIKQ. The questionnaire consists of two scales with 12 items. There is a UI scale to assess women's knowledge of UI and a POP scale to assess their knowledge of pelvic organ prolapse (POP). The Cronbach's alpha value was reported as 0.825 for the UI scale and 0.895 for the POP scale. The PIKQ evaluates women's knowledge about the epidemiology, pathogenesis, diagnosis, and treatment of UI and POP. The patients were asked to respond to each questionnaire item with "agree", "disagree", or "I Don't Know". Scoring was based on the percentage of questions answered correctly. Questions answered with "I Don't Know" considered incorrect (15). the UI subscale of the PIKQ (PIKQ-UI) was used in this study.

### Pelvic floor awareness

Pelvic floor awareness was assessed by 4 questions compiled from the literature (16-18). These questions were "Have you ever heard the expression "pelvic floor muscles"? (PFA-1)", "Have you ever received any information about pelvic floor muscles? (PFA-2)", "If you have received information, what was it for (PFA-2a) and from whom did you receive it? (PFA-2b)", "Have you ever done any research on pelvic floor muscles? (PFA-3)", "What sources did you use in your research? (PFA-3a)" and "Have you ever heard of pelvic floor muscle exercise? (PFA-4)". The answers to the questions PFA-1, PFA-2, PFA-3 and PFA-4 were recorded as Yes/No. If the answer to the question PFA-2 was "Yes", women were asked to answer the PFA-2a and PFA-2b questions. The answers to question PFA-2a were "to obtain knowledge", "for educational purposes", "to seek health care for UI", "for pregnancy education". The answers to PFA-2b were recorded as "Physiotherapist", "Gynecology and Obstetrician", "Midwife/Nurse", "Friend/Family", "School", "General Practitioner", "Other", and "Multiple Sources". If the answers to the question PFA- 3 were "Yes", women were asked to answer the question PFA-3a and the answers were recorded as "Physiotherapist", "Books", "Internet" and "Multiple resources". Women were classified according to their answers to questions PFA-1, PFA-2, PFA-3, and PFA-4. The answer "Yes" to the questions about pelvic floor awareness was interpreted as "I am aware.", the answer "No" as "I am not aware.".

### Pelvic floor knowledge

Pelvic floor knowledge was measured using the PFHKQ. The PFHKQ consists of 29 questions, with the available answers "Yes", "No" and "I Don't Know". In the test, there is a dichotomous scoring system. Correctly answered questions receive a score of "1", and incorrect answers and unanswered questions receive a score of "0". The PFHKQ was developed by Al-Deges et al. (19).

### Healthcare seeking of incontinent women

The healthcare seeking behavior of incontinent women was surveyed as stated in the literature (13,20,21). The questions were "Have you ever been treated for

incontinence? (HCS-1)", "Which conditions related to your incontinence bothered you and led you to seek treatment? (HCS-2)", "Who referred you to seek treatment? (HCS-3)". The answers to the question HCS-1 were recorded as "Yes/No". The answers given to the HCS-2 question were grouped as "Wetness/ hygiene etc.", "My social/work/outdoor life was negatively affected", "I have symptoms" and "I did not seek treatment". Responses to HCS-3 were categorized as "Physiotherapist", "Doctor", "Family", "Friend" and "Myself", "I did not seek treatment".

### Analysis of data

The data were analyzed using the Statistical Package for the Social Sciences (SPSS version 21.0) software. For descriptive statistics, numbers and percentages were given in categorical data, mean, standard deviation or median, and minimum-maximum values were given in numerical data. The Kolmogorov-Smirnov test and graphs (box-line graph, histogram, etc.) were used for the assumption of normality. For the comparison of the two group, t-test or Mann Whitney U test was used for independent groups, and one-way analysis of variance or Kruskal Wallis test was used to compare three or more groups. In case of differences, post-hoc tests were used. Relationship analysis of categorical variables was done with chi-square tests. The level of significance was set at $p<0.05$.

## RESULTS

Physical and socio-demographic characteristics of incontinent and continent women are shown in Table 1. The analysis revealed a statistically significant difference between continent and incontinent women in terms of age, BMI, level of education, presence of chronic disease, menstrual status, number of pregnancies, and live births ($p<0.05$).

The answers to the questions PFA-1, PFA-2, PFA-3, and PFA-4 of the continent and incontinent women were similar (p=0.551, p=0.328, p=0.504, p=0.392, respectively) (Table 2). However, there were differences between the purpose of obtaining information about the pelvic floor muscles. It was determined that 40% of the incontinent women sought information for educational purposes, and 50% of them sought information to find a solution to their urinary incontinence problem (p=0.002) (Table 2). In addition, statistically significant differences were found in the PIKQ-UI scores of continent and incontinent women (p=0.033), with incontinent women having higher PIKQ-UI scores. The PIKQ subscales scores were calculated proportionally over 100 points. The PIKQ-UI score of incontinent women, 8 points, was 66.6%, the PIKQ-UI score of continent women, 7 points, was 58.3%. The PFHKQ scores of continent and incontinent women were similar (p=0.294) (Table 2).

A comparison was made between healthcare seeking incontinent women with and without pelvic floor awareness. 36 women with UI who had never heard of pelvic floor muscle did not seek treatment (p=0.002). 61 women with UI who did not seek information about pelvic floor muscles had never been treated before (p=0.035). 40 women with UI, who had not received information about pelvic floor muscles, had never sought treatment. 10 women with UI received information about pelvic floor from physiotherapists (p=0.002). 42 women with UI who had not previously sought information about pelvic floor muscles had never sought treatment before (p=0.042). 33 women with UI who had never heard the term 'pelvic floor exercise' had never sought treatment (p=0.004) (Table 3).

Incontinent women were classified according to their answers to the questions PFA-1, PFA-2, PFA-3, and PFA-4. The PIKQ-UI scores of women who answered 'Yes' to these questions were higher than those who answered 'No'. In addition, those who answered 'Yes' to the questions PFA-1, PFA-2 and PFA-4 had higher PFHKQ scores than those who answered 'No' (Table 4). For the PFA-3 question, similar PFHKQ scores were found for all, regardless of whether they answered 'Yes' or 'No' answers (p=0.123) (Table 4).

It was determined that there was a difference between the PIKQ-UI scores of women with UI who answered 'Yes' and 'No' to the HCS-1 question (p=0.039), however, their PFHKQ scores were similar (p=0.080) (Table 5).

**Table 1.** Comparison of physical and demographic characteristics by incontinence status

| Characteristics | | Incontinence Status | | p |
|---|---|---|---|---|
| | | Continent (n=82) | Incontinent (n=96) | |
| Age (y) | | 42.22 ± 10.89 | 48.75 ± 11.88 | <0.001 |
| BMI (kg/m²) | | 23.96 ± 4.33 | 28.29 ± 4.85 | <0.001 |
| Occupation | Housewife | 32 (39.0) | 52 (54.2) | 0.130 |
| | Officer | 27 (32.9) | 19 (19.8) | |
| | Private sector | 16 (19.5) | 15 (15.6) | |
| | Retired | 7 (8.5) | 10 (10.4) | |
| Education level | Primary school | 11 (13.4) | 34 (35.4) | 0.002 |
| | Middle School | 8 (9.8) | 8 (8.3) | |
| | High school | 17 (20.7) | 16 (16.7) | |
| | Licence | 44 (58.7) | 31 (32.3) | |
| | Graduate | 2 (2.4) | 7 (7.3) | |
| Chronic Disease | No | 75 (91.5) | 64 (66.7) | <0.001 |
| | Yes | 7 (8.5) | 32 (33.3) | |
| | Hypertension | 3 (42.9) | 10 (31.3) | |
| | Heart disease | 3 (42.9) | 1 (3.1) | |
| | Diabetes | 1 (14.2) | 5 (15.6) | |
| | COPD/asthma | 0 (0.0) | 5 (15.6) | |
| | Multiple chronic disease | 0 (0.0) | 11 (34.4) | |
| Menstrual Status | Regular menstruation | 56 (68.3) | 43 (44.8) | 0.003 |
| | Irregular menstruation | 8 (9.8) | 9 (9.4) | |
| | Spontaneous menopause | 17 (20.7) | 34 (35.4) | |
| | Surgical menopause | 1 (1.2) | 10 (10.4) | |
| | | n=66 | n=87 | |
| Gravida (n= 153) | | 2.0 [1.0– 8.0] | 3.0 [1.0 – 11.0] | <0.001 |
| | | n=65 | n=83 | |
| Alive children (n= 148) | | 2.0 [1.0 – 10.0] | 2.0 [1.0 – 8.0] | 0.007 |

BMI: Body Mass Index

## DISCUSSION AND CONCLUSION

Our study revealed some important findings about the relationship between UI and pelvic floor knowledge, pelvic floor awareness, and treatment seeking in incontinent women. It was found that more than half of the women with UI who did not seek treatment lacked pelvic floor awareness. Women with UI who answered "I am aware" to questions about pelvic floor awareness and women with UI who sought treatment had higher levels of incontinence knowledge. Another important result of our study is the identification of differences between incontinent and continent women in terms of their pelvic floor awareness, and pelvic floor and UI knowledge levels.

Based on our results, it was concluded that more than half of the women with UI in our study did not seek

**Table 2.** Comparison of pelvic floor awareness, pelvic floor health, and incontinence knowledge levels by incontinence status

| | | Incontinence Status | | P |
|---|---|---|---|---|
| | | Continent (n=82) | Incontinent (n=96) | |
| PFA-1 | Yes | 40 (48.8) | 39 (40.6) | 0.551 |
| | No | 39 (47.6) | 53 (55.2) | |
| | I Don't Know | 3 (3.7) | 4 (4.2) | |
| PFA-2 | Yes | 21 (25.6) | 31 (32.3) | 0.328 |
| | No | 61 (74.4) | 65 (67.7) | |
| PFA-2a (n=52) | toobtain knowledge | 7 (35.0) | 8 (28.9) | 0.002 |
| | for educational purposes | 8 (40.0) | 6 (20.0) | |
| | to seek healthcare for UI | 1 (5.0) | 15 (50.0) | |
| | for pregnancy education | 4 (20.0) | 1 (3.3) | |
| PFA-2b (n=52) | Physiotherapist | 5 (23.8) | 14 (45.2) | NA |
| | Gynecologist/Obstetrician | 5 (23.8) | 5 (16.1) | |
| | Midwife/Nurse | 1 (4.8) | 0 (0.0) | |
| | Friend/Family | 0 (0.0) | 1 (3.2) | |
| | School | 2 (9.5) | 2 (6.5) | |
| | General Practitioner | 1 (4.8) | 1 (3.2) | |
| | Other | 5 (23.8) | 3 (9.7) | |
| | Multiple Sources | 2 (9.5) | 5 (16.1) | |
| PFA-3 | Yes | 15 (18.3) | 14 (14.6) | 0.504 |
| | No | 67 (81.7) | 82 (85.4) | |
| PFA-3a (n=29) | Physiotherapist | 0 (0.0) | 1 (7.1) | 0.133 |
| | Books | 3 (20.0) | 0 (0.0) | |
| | Internet | 6 (40.0) | 6 (42.9) | |
| | Multiple sources | 6 (40.0) | 7 (50.0) | |
| PFA-4 | Yes | 42 (51.2) | 43 (44.8) | 0.392 |
| | No | 40 (48.8) | 53 (55.2) | |
| PIKQ-UI | | 7.0 [0.0 – 12.0] | 8.0 [0.0 – 12.0] | 0.033 |
| PFHKQ | | 15.0 [0.0 – 27.0] | 16.5 [0.0 – 27.0] | 0.294 |

PFA-1: Have you ever heard the expression "pelvic floor muscles"? PFA-2 Have you ever received any information about pelvic floor muscles? If you have received any information, what was it for: PFA-2a and from whom did you receive it: PFA-2b, PFA-3: Have you ever done any research on pelvic floor muscles? PFA-3a:What sources did you use in your research?? PFA-4: Have you ever heard of pelvic floor muscle exercise ? PFHKQ:Pelvic Floor Health Knowledge Quiz, PIKQ-UI: Prolapse and Incontinence Knowledge Questionnaire-Incontinence subscale, UI: Urinary Incontinence

treatment, probably due to inadequate pelvic floor awareness. Cygańska et al. emphasized that women with UI had a low level of awareness about preventive treatment methods and that they needed awareness education (22). In another study, it was observed that women were aware of the need to do PFM exercises, but many did not know that these exercises should be continued throughout life; rather, these exercises were associated with pregnancy and labor (23). In a study evaluating Qatari women's awareness of UI and

**Table 3.** Comparison of healthcare seeking of incontinent women with and without pelvic floor awareness

| | | PFA-1 | | P |
|---|---|---|---|---|
| | | Yes | No | |
| HCS-1 | Yes | 7 (63.6) | 32 (37.6) | 0.115 |
| | No | 4 (36.4) | 53 (62.4) | |
| HCS-2 | Wetness / hygiene etc. | 10 (55.6) | 8 (44.4) | 0.456 |
| | My social/work/outdoor life has been negatively affected | 7 (43.8) | 9 (56.3) | |
| | I have symptoms | 9 (39.1) | 14 (60.9) | |
| | I did not seek healthcare | 13 (33.3) | 26 (66.7) | |
| HCS-3 | Physiotherapist | 10 (71.4) | 4 (28.6) | 0.002 |
| | Doctor | 3 (7.7) | 1 (4.2) | |
| | Family | 3 (7.7) | 9 (75.0) | |
| | Friend | 4 (57.1) | 3 (42.9) | |
| | Myself | 8 (66.7) | 4 (33.3) | |
| | I did not seek healthcare/nobody | 11 (66.7) | 36 (76.6) | |
| | | PFA- 2 | | |
| | | Yes | No | |
| HCS-1 | Yes | 7 (63.6) | 24 (28.2) | 0.035 |
| | No | 4 (36.4) | 61 (71.8) | |
| HCS-2 | Wetness / hygiene etc. | 8 (50.0) | 9 (50.0) | 0.135 |
| | My social/work/outdoor life has been negatively affected | 5 (31.3) | 11 (68.8) | |
| | I have symptoms | 9 (39.1) | 14 (60.9) | |
| | I did not seek healthcare | 8 (20.5) | 31 (79.5) | |
| HCS-3 | Physiotherapist | 10 (71.4) | 4 (28.6) | 0.002 |
| | Doctor | 2 (50.0) | 2 (50.0) | |
| | Family | 3 (25.0) | 9 (75.0) | |
| | Friend | 3 (42.9) | 4 (57.1) | |
| | Myself | 6 (50.0) | 6 (50.0) | |
| | I did not seek healthcare/nobody | 7 (14.9) | 40 (85.1) | |

healthcare seeking, 70.4% of women reported that UI was an abnormal condition, and that patients should consult a doctor (12). Despite having pelvic floor awareness, many women do not seek healthcare for reasons such as embarrassment, and acceptance of the condition as normal, as reported in many studies (13,24). In our study, 71.4% of the women referred by a physiotherapist for the UI treatment had heard the term "pelvic floor muscle", and 71.4% had recieved information about "pelvic floor muscles". In addition, 78.6% of these women had heard the term "pelvic floor exercises". Therefore, it appears that health professionals have made a significant contribution to the development of women's pelvic floor awareness.

In this study, it was determined that incontinent women who answered 'Yes' (I am aware) to questions about pelvic floor awareness had higher levels of knowledge than those who answered 'No' (I am not aware). Agrawal et al. evaluated the pelvic floor

**Table 3.** Continued

| | | PFA- 3 | | p |
|---|---|---|---|---|
| | | Yes | No | |
| HCS-1 | Yes | 3 (27.3) | 8 (72.7) | 0.199 |
| | No | 11 (12.9) | 74 (87.1) | |
| HCS-2 | Wetness / hygiene etc. | 4 (22.2) | 14 (77.8) | 0.650 |
| | My social/work/outdoor life has been negatively affected | 3 (18.8) | 13 (81.3) | |
| | I have symptoms | 3 (13.0) | 20 (87.0) | |
| | I did not seek healthcare | 4 (10.3) | 35 (89.7) | |
| HCS-3 | Physiotherapist | 1 (7.1) | 13 (92.9) | 0.042 |
| | Doctor | 1 (25.0) | 3 (75.0) | |
| | Family | 0 (0.0) | 12 (100.0) | |
| | Friend | 2 (28.6) | 5 (71.4) | |
| | Myself | 5 (41.7) | 7 (58.3) | |
| | I did not seek healthcare/nobody | 5 (10.6) | 42 (89.4) | |
| | | PFA- 4 | | |
| | | Yes | No | |
| HCS-1 | Yes | 8 (72.7) | 3 (27.3) | 0.059 |
| | No | 35 (41.2) | 50 (58.8) | |
| HCS-2 | Wetness / hygiene etc. | 11 (61.1) | 7 (38.9) | 0.351 |
| | My social/work/outdoor life has been negatively affected | 7 (43.8) | 9 (56.3) | |
| | I have symptoms | 11 (47.8) | 12 (52.2) | |
| | I did not seek healthcare | 14 (35.9) | 25 (64.1) | |
| HCS-3 | Physiotherapist | 11 (78.6) | 3 (21.4) | 0.004 |
| | Doctor | 3 (75.0) | 1 (25.0) | |
| | Family | 3 (25.0) | 9 (75.0) | |
| | Friend | 4 (57.1) | 3 (42.9) | |
| | Myself | 8 (66.7) | 4 (33.3) | |
| | I did not seek healthcare /nobody | 14 (29.8) | 33 (70.2) | |

HCS: Healthcare Seeking, PFA: Pelvic Floor Awareness

HCS-1: Have you ever been treated for urinary incontinence? HCS-2: Which conditions related to your incontinence bothered you and led you to seek treatment? HCS-3: Who directed you to seek treatment? PFA-1: Have you ever hear the expression "Pelvic floor muscle" before? PFA-2: Have you ever received any information about pelvic floor muscles? PFA-3: Have you ever done any research on pelvic floor muscles? PFA-4: Have you ever heard of pelvic floor muscle exercise ?

awareness of women with UI and pelvic organ prolapse by classifying the answers to the questions as "aware", "mis-aware", and "unaware", finding that less than half of women were aware of their pelvic floor muscle (25). Thus, it appears that pelvic floor awareness has a positive effect on the level of knowledge about both UI and pelvic floor health in incontinent and aware women.

Knowledge of UI has been shown to have a positive effect on seeking treatment. Siddiqui et al. reported that women's responses were "We would be more

**Table 4.** Comparison of Knowledge Levels of Incontinent Women with and Without Pelvic Floor Awareness

| | | PFHKQ | PIKQ-UI |
|---|---|---|---|
| PFA-1 | Yes | 20 (2 – 27) | 10 (0 – 12) |
| | No | 13 (0 – 26) | 7 (0 – 12) |
| | p | <0.001 | 0.001 |
| PFA-2 | Yes | 22 (4 – 27) | 10 (3 – 12) |
| | No | 14 (0 - 26) | 7 (0 – 12) |
| | p | <0.001 | <0.001 |
| PFA-3 | Yes | 18.5 (6 – 25) | 9.5 (5 – 12) |
| | No | 16 (0 – 27) | 7 (0 – 12) |
| | p | 0.123 | 0.024 |
| PFA-4 | Yes | 19.0 (2 – 27) | 9.0 (0 – 12) |
| | No | 13.0 (0 – 26) | 7.0 (0 – 12) |
| | p | <0.001 | 0.006 |

PFHKQ: Pelvic Floor Health Knowledge Quiz, PIKQ-UI: Prolapse and Incontinence Knowledge Questionnaire-Incontinence subscale. PFA-1: Have you ever heard the expression "pelvic floor muscles"? PFA-2: Have you ever received any information about pelvic floor muscles? PFA-3: Have you ever done any research on pelvic floor muscles? PFA-4: Have you ever heard of pelvic floor muscle exercise?

**Table 5.** Comparison of Knowledge Levels of Incontinent Women by Seeking Healthcare

| | | Knowledge Levels Tests | |
|---|---|---|---|
| | | PFHKQ | PIKQ-UI |
| HCS-1 | Yes | 19 (2 – 27) | 10 (4 – 12) |
| | No | 16 (0 – 27) | 8 (0 – 12) |
| | p | 0.080 | 0.039 |

HCS:Healthcare Seeking, PFHKQ: Pelvic Floor Health Knowledge Quiz, HCS-1:Have you ever been treated for urinary incontinence? PIKQ-UI: Prolapse and Incontinence Knowledge Questionnaire-Incontinence subscale.

likely to be treated if we were informed about treatment options" (13). Although the rate of UI is high in athletes with more than eight years of experience, women lacked knowledge about this issue and the treatment options. In addition, it was found that the prevalence of UI decreased to 57% in female athletes who had sufficient knowledge about UI (26). Consistent with these results (13,26), we observed that the level of knowledge about incontinence of incontinent women who sought healthcare was higher than the level of those who did not, but the level of knowledge about pelvic floor were similar in both groups. It is very important to underline that having information about incontinence contributes positively to the health care seeking and that information such as pelvic floor function, dysfunction, risk factors for PFD and treatment options should be incorporated into an education program.

Previous studies showed that women's knowledge of pelvic floor and incontinence may change due to different situations (such as being incontinent, pregnant, postmenopausal, and/or having a PFD) (23,24). However, Neels et al. evaluated the pelvic floor knowledge in groups of nulliparous, peripartum, and postmenopausal women, and reported low levels of knowledge in all groups and found that most of the nulliparous women (81%) were not informed about the pelvic floor (18,27). In our study, there were similar levels of pelvic floor knowledge and awareness among continent and incontinent women, but incontinent women's knowledge of incontinence was higher. A systematic review reported that the most commonly used questionnaire to assess the knowledge level of incontinence and prolapse is the PIKQ. (8). In the UI subscale of the PIKQ developed by Shah et al., threshold value of knowledge proficiency was reported to be 80% and above (5,28). In our study, the 80% cut-off value was taken as a reference value, in line with many previous studies (5,24), which allowed making comparison between studies and groups. Our findings showed that although the incontinent women had a higher level of knowledge about incontinence than the continent women, the PIKQ-UI was found to be below the 80% threshold, and insufficient with a rate of 66.6%. In addition, continent women in our study sought information about incontinence for educational purposes, while incontinent women were seeking a solution to their urinary incontinence problem. Kang et al. also stated that the knowledge level and attitudes of American-Korean women with UI were lower than those of the general population, and that even if they are familiar with pelvic floor muscle exercises, they believed that surgery was the best treatment (29). Incorrect or insufficient information about UI and PFD is considered a barrier to accessing treatment, and this issue should be further studied by health professionals (7,30). One of our assumptions is that it may be possible to remove barriers to health care

seeking with extensive training related to pelvic floor and UI, tailored to the age, education, occupation, and socioeconomic status of the groups. In addition, considering the differences between women regarding access to information, we think that informing women at every stage of their education will contribute to the prevention of PFD.

Another finding of our study was that incontinent women are more likely to be older, have a higher BMI, a lower level of education, more comorbidities, a higher number of pregnancies, and also to be postmenopausal. Age, BMI, level of education, presence of comorbidities, menstrual cycle, and number of pregnancies are each independent factors significantly associated with the likelihood of having UI. The physical and socio-demographic data of the incontinent women in this study reflect the UI profile.

### Limitations and strengths

There are some limitations to this study. The first was that women's awareness was assessed using open-ended questions, since there is no universally-accepted standardized method to assess pelvic floor awareness in the literature. The online design of this study limited the number of participants as it could not include those who were unable to use smartphones and computers. However, organizing the study online allowed women from different cities and regions of the country to participate. The strength of the present study was that it allowed making comparison between continent and incontinent women in terms of pelvic floor awareness, pelvic floor knowledge and UI knowledge levels. To our knowledge, it was the first study to investigate the effects of pelvic floor awareness, pelvic floor, and UI knowledge levels, and healthcare seeking behavior among women with UI.

This study showed that women's knowledge of incontinence and awareness of the pelvic floor was unsatisfactory, regardless of their UI status. Therefore, it is very important for health professionals to provide information and raise awareness about the pelvic floor in order to prevent urinary incontinence. Future studies should also investigate the awareness of the related areas - pelvic organ prolapse, fecal incontinence, and sexual function- and the availability of health care for such dysfunction.

### Ethical approval

This study has been approved by the Bolu Abant İzzet Baysal University Clinical Research Ethics Committee (approval date 13.04.2021, number 2021/81). Written informed consent was obtained from the participants.

### Author contribution

Concept: SYY, NÖ; Design: SYY, NÖ, EDY; Data Collection or Processing: SYY, Bİ, EDY, SAT, HÇ, MBG; Analysis or Interpretation: MBG, NÖ; Literature Search: SYY, Bİ, EDY, SAT, HÇ; Writing: SYY, NÖ, MBG. All authors reviewed the results and approved the final version of the article.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

# Optical coherence tomography angiography changes in patients with hemoglobinopathy

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## ABSTRACT

**Aim:** The aim of this study is to evaluate retinal vascular changes in patients with sickle cell disease (SCD) and beta-thalassemia with optical coherence tomography angiography (OCT-A).

**Methods:** For this purpose, 98 patients with SCD, 75 patients with beta-thalassemia, and 100 healthy controls in Mersin University Hospital between January 1, 2020, and November 1, 2021, were included in this study. OCT-A imaging was performed with ZEISS AngioPlex OCT angiography (Carl Zeiss Meditec, Dublin, CA, USA).

**Results:** All OCT-A parameters (FAZ area, perimeter, circularity, vessel, and perfusion density) were found to be statistically significantly different in both patients with thalassemia and patients with sickle cell disease when compared to the controls.

**Conclusions:** In conclusion, retinopathy related to both hemoglobinopathy subgroups can be diagnosed and followed up with OCT-A. It was also found that OCT-A parameters are affected before the development of clinically detectable retinopathy.

**Keywords:** opptical coherence tomography angiography, retinopathy, sickle cell disease, thalassemia

## INTRODUCTION

Hemoglobinopathies, the most common hereditary blood disease in the world, are caused by structural changes in the chains of the hemoglobin. They are divided into two main classes, abnormal hemoglobins and thalassemias. Sickle cell disease (SCD) is a group of inherited hematological diseases in which erythrocytes characteristically distort the biconcave disc shape and take a sickle shape. This situation causes vaso-occlusion and can affect all organs (1).

Retinal hypoxia, ischemia, and neovascularization may develop after microvascular occlusion in patients with SCD. Neovascularization that occurs before the development of vitreous hemorrhage or retinal detachment is the most important precursor (2).

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The most common reasons for vision loss in patients with SCD are proliferative sickle cell retinopathy (PSCR), which is characterized by chronic peripheral retinal microvascular occlusion, and ischemia (3). Retinopathy due to sickle cell disease is divided into proliferative and non-proliferative retinopathy. The grade of proliferative retinopathy is determined by the Goldberg classification (4) (Table 1).

Thalassemia is a genetic disease characterized by a decrease or complete absence in the synthesis of one or more of the globin chains in the structure of the hemoglobin. It is classified according to the reduced or non-synthesized globin chain. The best described types are α-and β-thalassemia (5). The frequency of eye involvement in β-thalassemia was found to range from 41.3% to 85% in various studies (6,7).

In different studies, retinal disorders such as retinal pigment epithelial degeneration and mottling, venous tortuosity, retinal hemorrhage, retinal edema, peripheral and central retinal thinning, cup-to-disk ratio enlargement, and macular scarring have been reported in beta-thalassemia patients. The prevalence of reported retinal disorders varies between studies (8).

The main purpose of this study is to evaluate the retinal changes that may occur in patients with hemoglobinopathy with OCT-A.

## MATERIALS AND METHODS

Written informed consent was obtained from all participants in this study. The study protocol was approved by the Mersin University Clinical Research Ethics Committee. At all stages, this study adhered to the principles of the Declaration of Helsinki.

**Table 1.** Classification of proliferative sickle cell retinopathy (PSCR).

| | |
|---|---|
| Stage I | Peripheral arterial occlusion |
| Stage II | Peripheral arteriovenous anastomoses (hairpin loop) |
| Stage III | Neovascular and fibrous proliferations (sea fan) |
| Stage IV | Vitreous hemorrhage |
| Stage V | Retinal detachment |

Patients who underwent OCT-A (ZEISS AngioPlex OCT angiography, Carl Zeiss Meditec, Dublin, CA, USA) imaging for beta-thalassemia or sickle cell disease at the Department of Ophthalmology, Faculty of Medicine, Mersin University between 01/Jan/2020 and 01/Nov/2021 were included in this study. Fifty healthy controls without any cardiovascular and/or ophthalmologic diseases were included in the study.

Demographic data, hemoglobin values and OCT-A parameters of the patients included in the study were evaluated. The foveal avascular zone (FAZ) (in square millimeters) is an area of the macula that does not contain capillary structures. The axial length of this area is defined as the FAZ perimeter (in millimeters). FAZ circularity (unitless) indicates the proportion of the shape of the FAZ that resembles an ideal circle. Vessel density (in millimeters per square millimeter) is the total vessel length per unit area in the region. Perfusion density (%) is the total area supplied by the total vessels measured per unit area in the region.

All scans were repeated until an ideal quality was achieved. The data for the FAZ were obtained from a 3×3 mm foveally centered scan area.

Continuous data are expressed as mean ± standard deviation. The Shapiro-Wilk test was used to assess adherence to a normal distribution. The means of two independent groups were compared by Student's t-test and the means of more than two groups were compared using ANOVA. Categorical data were expressed as numbers and percentages and chi-square test was used to compare them. Statistical analysis of the study was performed with SPSS 29,0. The level of statistical significance was accepted as $p < 0,05$.

## RESULTS

One hundred healthy controls, 98 patients with SCD and 75 patients with beta-thalassemia were included. The age and gender distribution was similar and is summarized in Table 2.

**Table 2.** Demographic characteristics of all participants.

| | Normal | β-Thalassemia | | | SCD | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | Major | Minor | No Retinopathy | NPSCR | Stage 1 | Stage 2 | Stage 3 | Homozygous (HbSS) | Heterozygous (HbS variant) |
| **N (%)** | **100** | **75 (100%)** | **23 (30,7%)** | **52 (69,3%)** | **61 (62,2%)** | **20 (20,4%)** | **9 (9,2%)** | **5 (5,1%)** | **3 (3,1%)** | **31 (31,6%)** | **67 (68,4%)** |
| Age (years) | 39,8 ± 7,7 | 40,2 ± 15,2 | 41,4 ± 14,6 | 39,7 ± 15,5 | 38,2 ± 10,4 | 34,9 ± 12,6 | 37,1 ± 12,2 | 37,4 ± 9,8 | 44,3 ± 7,8 | 39,4 ± 10,6 | 36,7 ± 10,8 |
| Male | 38 (38%) | 26 (34,7%) | 5 (21,7%) | 21 (40,4%) | 25 (41%) | 7 (35%) | 4 (44,4%) | 3 (60%) | 2 (66,7%) | 7 (22,6%) | 34 (50,7%) |
| Female | 62 (62%) | 49 (65,3%) | 18 (78,3%) | 31 (59,6%) | 36 (59%) | 13 (65%) | 5 (55,6%) | 2 (40%) | 1 (33,3%) | 24 (77,4%) | 33 (49,3%) |

Of the patients with sickle cell disease, 31 (31,6%) were homozygous and 67 (68,4%) were heterozygous. In addition, retinopathy was not detected in 61 (62,2%) patients, non-proliferative sickle cell retinopathy (NPSCR) was found in 20 (20,4%) patients, and proliferative sickle cell retinopathy (PSCR) was found in 17 (17,4%) patients. The distribution of retinopathy in sickle cell patients is shown in Table 3.

The OCT-A parameters and hemoglobin levels of all participants are shown in Table 4. All parameters (FAZ area, circularity, perimeter, vessel and perfusion density) were statistically different across the three groups (for all parameters, $p < 0,05$). When the hemoglobin levels of the all patients and their OCT-A parameters were compared, a statistically significant correlation was found for all parameters in patients with beta-thalassemia major, whereas no statistically significant correlation was found for any parameter in patients with sickle cell disease (Table 5).

When the subgroups of the diseases were compared with the control patients, the difference between the measurements of the patients with thalassemia major and homozygous-sickle cell disease was found to be statistically significant (for all parameters, $p < 0,05$). In addition, no statistically significant difference was found between the measurements of patients with sickle cell disease (for all remaining parameters except vessel density, $p > 0,05$). However, vessel density was statistically different between the patients who had no retinopathy and the control group ($p = 0,038$, $p < 0,05$).

## DISCUSSION

This is the largest study using OCT-A imaging in patients with beta-thalassemia and sickle cell disease reported in the literature. There is no standardized imaging protocol yet, but the use of different imaging devices can help in the diagnosis.

Lynch et al. included fifty-two patients with SCR (19 proliferative and 33 non-proliferative) and 20 healthy controls in their study. FAZ perimeter and a circularity index were significantly higher in SCR eyes compared to controls. In addition, vessel density was significantly lower in SCR eyes than in the control group (9).

**Table 3.** Distribution of retinopathy in sickle cell patients.

| | Homozygous (HbSS) | | Heterozygous (HbS variant) | | Total | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| No Retinopathy | 13 | 41,9 | 48 | 71,6 | 61 | 62,2 |
| NPSCR | 8 | 25,8 | 12 | 17,9 | 20 | 20,4 |
| Stage 1 | 3 | 9,7 | 6 | 9 | 9 | 9,2 |
| Stage 2 | 4 | 12,9 | 1 | 1,5 | 5 | 5,1 |
| Stage 3 | 3 | 9,7 | - | - | 3 | 3,1 |
| Total | 31 (31,6%) | | 67 (68,4%) | | 98 (100%) | |

Zhou et al. included 31 patients with SCR (21 NP-SCR and 10 P-SCR) and 14 healthy controls in their study. All FAZ (area, perimeter, a circularity) measurements were significantly higher in NP-SCR and P-SCR subjects than in healthy controls (10).

Han et al. found in their study of 82 eyes of 46 patients that there was a loss of flow and a decrease in vessel density in patients with sickle cell retinopathy (11).

In another study, the transfusion-dependent thalassemia group (TDT) (thalassemia major) showed a statistically significant decrease in retinal and choriocapillaris vessel density (VD) compared to controls ($p < 0,05$). In our study, vascular density decreased and FAZ area increased in patients in the thalassemia group compared to controls.

This difference is especially more pronounced in the thalassemia major subgroup. This result is consistent with the literature (12).

OCT-A parameters have provided a deeper understanding of retinal involvement, particularly of systemic diseases that produce ischemia and microangiopathy. In a study by Mokrane et al., FAZ area was not significantly different between both genotypes in sickle cell anemia. However, it differed from healthy controls (HbSC $p= 0,034$ and HbSS $p = 0,001$). OCT-A provides useful information about the structural changes associated with microvasculature and retinopathy. It reveals the structural changes in patients who cannot be diagnosed clinically. It is a non-invasive, simple and reproducible imaging technique (13).

Limitations of the study include the relatively small number of patients, reporting from a single center, short follow-up period, lack of evaluation of treatment responses and retrospective design.

In conclusion, it was determined that all FAZ parameters (area, circularity, perimeter) were significantly different in patients with both beta-thalassemia and sickle cell disease, even if retinopathy was not developed clinically. In addition, vessel and perfusion density were found to be lower in both patient groups compared to the healthy controls. However, no significant correlation was found between the severity of retinopathy of the patients and angiography parameters. In addition, a correlation between hemoglobin levels and angiography parameters was observed in patients with beta-thalassemia major.

## Ethical approval

This study has been approved by the Mersin University Rectorate Clinical Research Ethics Committee (approval no 146, date 05.02.2020). Written informed consent was obtained from the participants.

## Author contribution

Concept: ÖÖ; Design: ÖÖ; Data Collection or Processing: ÖÖ; Analysis or Interpretation: ÖÖ; Literature Search: ESG; Writing: ÖÖ. All authors reviewed the results and approved the final version of the article.

## Source of funding

The authors declare the study received no funding.

**Table 4.** The OCT-A parameters of all participants.

| | Normal | β-Thalassemia | | | SCD | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total | Major | Minor | Total | Homozygous (HbSS) | Heterozygous (HbSvariant) | No Retinopathy | NPSCR | Stage 1 | Stage 2 | Stage 3 |
| **N (%)** | **100** | **75 (100%)** | **23 (30,7%)** | **52 (69,3%)** | **98 (100%)** | **31 (31,6%)** | **67 (68,4%)** | **61 (62,2%)** | **20 (20,4%)** | **9 (9,2%)** | **5 (5,1%)** | **3 (3,1%)** |
| Hb (g/dL) | 14,5 ± 3,24 | 7,6 ± 2,9 | 4,8 ± 1,1 | 9,7 ± 1,8 | 8,26 ± 1,61 | 7,9 ± 1,45 | 8,43 ± 1,66 | 8,36 ± 1,77 | 8,69 ± 1,09 | 6,9 ± 1,22 | 8,12 ± 1,31 | 7,6 ± 0,72 |
| FAZ area ($mm^2$) | 0,296 ± 0,088 | 0,327 ± 0,102 | 0,344 ± 0,09 | 0,313 ± 0,081 | 0,315 ± 0,109 | 0,358 ± 0,124 | 0,304 ± 0,089 | 0,299 ± 0,083 | 0,322 ± 0,105 | 0,375 ± 0,08 | 0,384 ± 0,094 | 0,402 ± 0,153 |
| FAZ circularity | 0,84 ± 0,06 | 0,71 ± 0,13 | 0,51 ± 0,14 | 0,8 ± 0,11 | 0,69 ± 0,12 | 0,54 ± 0,17 | 0,77 ± 0,15 | 0,78 ± 0,14 | 0,61 ± 0,18 | 0,46 ± 0,19 | 0,42 ± 0,11 | 0,41 ± 0,17 |
| FAZ perimeter | 2,05 ± 0,43 | 2,29 ± 0,54 | 2,91 ± 0,62 | 2,02 ± 0,37 | 2,47 ± 0,55 | 2,84 ± 0,73 | 2,01 ± 0,69 | 2,06 ± 0,50 | 2,15 ± 0,81 | 3,09 ± 0,62 | 3,05 ± 0,53 | 3,16 ± 0,84 |
| Vessel density | 16,07 ± 1,55 | 14,75 ± 2,86 | 14,38 ± 3,64 | 14,94 ± 4,06 | 14,59 ± 3,53 | 13,18 ± 2,88 | 15,92 ± 4,42 | 14,94 ± 3,11 | 14,31 ± 2,73 | 14,11 ± 3,87 | 12,81 ± 2,61 | 10,26 ± 0,73 |
| Perfusion density | 0,43 ± 0,014 | 0,38 ± 0,077 | 0,33 ± 0,062 | 0,41 ± 0,09 | 0,36 ± 0,06 | 0,29 ± 0,068 | 0,41 ± 0,08 | 0,38 ± 0,046 | 0,35 ± 0,082 | 0,3 ± 0,067 | 0,24 ± 0,07 | 0,21 ± 0,02 |

**Table 5.** Comparison of hemoglobin levels and OCT-A parameters.

| | β-Thalassemia-Major | | β-Thalassemia-Minor | | SCD | |
|---|---|---|---|---|---|---|
| | Hemoglobin levels | | | | | |
| | r | p | r | p | r | p |
| FAZ area ($mm^2$) | -0,866 | **0,0023** | -0,013 | 0,438 | -0,122 | 0,579 |
| FAZ circularity | 0,677 | **0,021** | 0,282 | 0,599 | 0,159 | 0,275 |
| FAZ perimeter | -0,702 | **0,037** | -0,035 | 0,452 | -0,265 | 0,735 |
| Vessel density | 0,753 | **0,044** | 0,288 | 0,362 | 0,248 | 0,469 |
| Perfusion density | 0,725 | **0,0095** | 0,352 | 0,896 | 0,062 | 0,639 |

### Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

# Hydroxytyrosol affects antioxidant Nrf2 expression in the kidneys of diabetic rats

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## ABSTRACT

**Aim:** Diabetic nephropathy is the result of oxidative stress caused by chronic hyperglycemia. Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important transcription factor that responds to oxidative stress. Nrf2 relieves oxidative stress, inflammation, and fibrosis associated with diabetes in the kidneys. In this study, we investigated the effects of hydroxytyrosol, which is a polyphenolic compound with proven antioxidant activity, on Nrf2 expression in diabetic kidneys.

**Methods:** Forty male Wistar rats were used in our study and the rats were divided into four groups as control (sterile water only), hydroxytyrosol (HT) (10mg/kg hydroxytyrosol administered intraperitoneally (ip) for 30 days), streptozotocin (STZ) (diabetes was induced by administering a single dose of 55 mg/kg streptozotocin ip), and streptozotocin + hydroxytyrosol (STZ+HT) (single dose of 55 mg/kg streptozotocin and 10 mg/kg hydroxytyrosol administered ip for 30 days). At the end of the study, Nrf2 expression in kidney tissue was evaluated by immunohistochemistry and Western blot.

**Results:** Immunohistochemistry and Western blot findings of Nrf2 were similar. It was found that while Nrf2 expression increased significantly in the HT group compared to the control group, whereas it decreased significantly in the STZ group ($p<0.001$). In the STZ+HT group, Nrf2 expression was found to be significantly increased compared to the STZ group ($p<0.001$).

**Conclusions:** It was found that hydroxytyrosol with known antioxidant activity increased Nrf2 expression in diabetic rats. These results suggest that hydroxytyrosol may mitigate diabetic nephropathy by Nrf2-induced reduction in oxidative stress, inflammation, and fibrosis.

**Keywords:** diabetes mellitus, hydroxytyrosol, kidney, Nrf2, streptozotocin

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## INTRODUCTION

Diabetes mellitus is a very common disease in society. Diabetes-induced permanent hyperglycemia causes complications such as diabetic nephropathy (DN) (1). DN is a progressive disease that may cause death in developing countries (2). DN is a syndrome that causes death and hypertrophy of glomerular cells, and fibrosis is also observed (3, 4). The most striking phenomenon in DN pathogenesis is the production of reactive oxygen species due to chronic hyperglycemia. Disruption of the balance of reactive oxygen species in the cell causes oxidative stress (5). The balance between the formation and clearance of free radicals in the cells of diabetic patients is disturbed. As a result, a significant increase in lipid peroxidation rate is observed in the cells of diabetic patients (6). This increase may cause damage to many organs of diabetic patients (7). Previous studies show that the development of DN is controlled by oxidative stress, inflammation, and fibrosis. Among these, oxidative stress occurs earliest and causes the activation of pathological pathways in kidney cells (8).

Diabetes-induced oxidative stress causes adverse effects by affecting DNA, proteins, lipids, and cellular pathways (7,9). These adverse effects impair kidney functions by affecting kidney cells. Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important transcription factor that responds to oxidative stress (10). In the oxidative equilibrium state of cells, Nrf2 is found in the cytoplasm bound to Kelch-like ECH-related protein 1 (Keap1). Oxidative stress that occurs due to disruption of oxidative balance causes Nrf2 to detach from Keap1 and translocate to the nucleus. Nrf2 binds to the antioxidant response element (ARE) of antioxidant genes in the nucleus and initiates transcription of antioxidant genes such as superoxide dismutase (SOD) and heme oxygenase-1 (HO-1) (11). A large number of studies have shown Nrf2 activation against DN to be effective in protecting the kidneys (12-19). The triple mechanism including oxidative stress, inflammation, and fibrosis may aggravate DN. However, many studies have shown that Nrf2 activated by different agents can regulate the above-mentioned mechanisms in DN treatment (12). Nrf2 activation has an important role in decreasing oxidative stress, inflammation, and fibrosis (13-15). Nrf2 stimulation reduces renal damage and extracellular vesicular accumulation by decreasing malondialdehyde levels and increasing SOD activity (16). Increased Nrf2 expression reduces renal tissue damage and apoptosis by reducing inflammation and oxidative stress through HO-1 signaling (17, 18). Increased Nrf2 signaling inhibits apoptosis, angiogenesis, inflammation, and oxidative stress by inducing HO-1 and relaxes DN (19). Nrf2 activation is protective against albuminuria and glomerular remodeling observed in DN (15). Herbal remedies used in traditional complementary medicine are widely used to treat diabetes and its complications. The roles of herbal agents should be explored to obtain a DN-effective drug. Mediterranean diet is a source of important antioxidant compounds in the prevention of DN (20). Olive oil is the main component of the Mediterranean diet. Olive oil contains monounsaturated fatty acids and lots of polyphenolic bioactive compounds. The most remarkable of these polyphenolic compounds is hydroxytyrosol with its antioxidant effects (21). Hydroxytyrosol shows its antioxidant effects by reducing inflammation, thrombocyte aggregation, and oxidized low-density lipoprotein (ox-LDL) production. It also shows insulin-like and insulin resistance-reducing effects (21-23). Hydroxytyrosol has also been reported to reduce proteinuria by 67-73% and glomerular volume and glomerulosclerosis by 20-30% (24).

Developing new alternative treatment options is crucial as diabetes is a chronic disease with inadequate treatment options and high treatment costs. Compounds obtained from plants are among the alternatives. Hydroxytyrosol is an anti-oxidative polyphenolic compound with known protective effects on the kidney, and has been shown to have protective and relaxing effects on diabetic nephropathy. We investigated the effects of hydroxytyrosol on Nrf2 expression.

## MATERIALS AND METHODS

### Animals and chemicals

We used 40 Wistar male rats, aged 3-month-old, with a weight of 250-300 grams. Power analysis was used to determine the number of animals in the study. It was determined that there should be 10 animals in each

group. The animals were housed at 24 ± 2 °C, 50 ± 5% humidity, and a cycle of 12 hours light: 12 hours dark. Food and water were provided *ad libitum*. The animals were divided into four groups, each consisting of 10 rats. These groups were as follows: the control group was only injected with sterile water intraperitoneally (ip); the hydroxytyrosol (HT) group was administered ip injection of 10 mg/kg hydroxytyrosol (Cayman, 70604) dissolved in sterile water for 30 days; streptozotocin (STZ) group was administered single dose ip 55 mg/kg streptozotocin injection dissolved in 0.05 M citrate buffer (pH 4.5) to induce diabetes at the beginning of the experiment; streptozotocin+hydroxytyrosol (STZ+HT) group was administered single dose ip 55 mg/kg streptozotocin injection (25) to induce diabetes at the beginning of the experiment and animals with diabetes were administered 10 mg/kg hydroxytyrosol ip injection for 30 days. Hydroxytyrosol dose was determined by considering the doses used in many previous studies (26,27). Blood glucose levels of the animals were measured from tail blood by using an Accu-Chek Nano Performa glucose meter 48 hours after streptozotocin injection. Animals with a blood glucose level ≥250 mg/dl were considered as diabetic and included in the study (25). At the end of the study, the animals were anesthetized with 90 mg/kg ketamine + 10 mg/kg xylazine, their kidney tissues were removed, and they were euthanized by cervical dislocation. Half of each removed kidney was placed in a 4% formaldehyde solution for immunohistochemistry and the other half was placed in cryotubes for Western blotting and kept at -80 ° C. This study was approved by the Düzce University Animal Experiments Local Ethics Committee with decision number 2022/12/05.

### Immunohistochemistry

After the tissues were fixed in 4% formaldehyde for 24 hours, they were dehydrated by passing through increasing degrees of alcohol. Subsequently, the tissues were embedded in paraffin and cleared with xylene. The specificity of the Nrf2 antibody was determined using human breast carcinoma tissue. Negative control immunochemistry was performed by using IgG isotype instead of primary antibody. Sections of 4 µm thickness were taken to positively charged slides from paraffin blocks. The sections were deparaffinized and rehydrated. They were boiled in citrate buffer (pH 6.0) in a 750-watt microwave oven for 7 minutes. They were cooled at room temperature for 20 minutes. The sections were washed with phosphate-buffered saline (PBS). They were incubated in a 3% hydrogen peroxide solution for 15 minutes. The sections were then washed with PBS 3 times for 5 minutes. Their borders were drawn with a hydrophobic pen. They were incubated for 7 minutes in a humidified chamber with UltraV block (Thermo Scientific™ TL-125-UB) and incubated in a humidified chamber overnight at +4 °C with Nrf2 rabbit polyclonal primary antibody (1:100 dilution, Abcam; Cat# ab137550). The next day, the primary antibody was removed and the sections were washed 3 times for 5 minutes with PBS. Biotinylated secondary antibody (Thermo Scientific™ TL-125-PB) was dropped on the sections and they were washed 3 times for 5 minutes with PBS after being incubated at room temperature for 30 minutes. They were incubated for 15 minutes in a humidified chamber at room temperature with peroxidase-labeled streptavidin (Thermo Scientific™ TL-125-PH) and washed. Immunohistochemical reaction was performed with diaminobenzidine. The sections were counterstained with hematoxylin, backtracked, and closed with entellan. Reaction photos were taken with an AxioCam Zeiss digital camera attached to an Olympus Cx41 microscope. Staining intensities in the photos were analyzed using ImageJ Version 1.8.0 software (http://imagej.nih.gov/ij/). The same procedures were applied in our previous study (28).

### Semi-quantitative evaluations

Nrf2 expressions and immunostaining densities in kidney cells were evaluated semi-quantitatively. The scores are shown in Table 1.

### Western blotting

The tissues kept at -80 °C were taken on ice and melted. After the tissues were mechanically lysed, they were incubated for 1 hour by mixing with lysis buffer (Tris–HCl (pH 7.4), Naorthovanadate, sodium dodecyl sulfate (SDS)) and protease inhibitor cocktail (Roche cOmplete™, Mini Protease Inhibitor Cocktail, Cat# 4693124001) and vortexed every 15 minutes. The lysates formed were centrifuged at +4 °C and 15000 g for 10 minutes, the resulting supernatants were taken and protein measurement

**Table 1.** Semi-quantitative distribution of Nrf2 labeling in control, HT (hydroxytrosol), STZ (streptozotocin) and STZ+HT. 0: negative; (+): weak positive; +: positive; ++: dense positive; +++: very dense positive.

| Cells | Nrf2 | | | |
|---|---|---|---|---|
| | Control | HT | STZ | STZ+HT |
| Distal Convoluted Tubule Cells | ++ | +++ | (+) | ++ |
| Proximal Convoluted Tubule Cells | + | +++ | (+) | + |
| Macula Densa Cells | + | + | (+) | + |
| Podocyte Cells | (+) | + | 0 | + |
| Mesangial Cells | 0 | 0 | 0 | 0 |
| Distal Straight Tubule Cells | (+) | +++ | 0 | ++ |
| Proximal Straight Tubule Cells | + | +++ | (+) | +++ |

was performed using Pierce™ BCA Protein Assay Kit (Cat# 23225). The determine the protein content of the samples, they were mixed with 4x lamella buffer (Biorad, Cat# 1610747) and sterile distilled water to equalize the protein content and boiled at 95 °C for 5 minutes. The boiled samples were loaded into SDS polyacrylamide gel wells (50 µg/ml protein in each well). Electrophoresis was performed at 100 V for 2-3 hours. The samples which were then transferred to gel were transferred to polyvinylidene fluoride membranes (PVDF) (EMD Millipore) by performing overnight blotting at 30 V at +4 °C. The membranes were blocked with 5% skimmed milk powder prepared with tris-buffered saline (TBST) containing 0.05% tween-20. The membranes were incubated overnight with Nrf2 rabbit polyclonal primary antibody (1:1000 dilution, Abcam; Cat# ab137550) and internal loading control β-actin rabbit primer antibody (1: 2000 dilutions, Cell Signalling Technology; Cat# 4970). The next day, they were incubated for 2 hours at room temperature (1:2000 dilutions, Cell Signalling Technology, Cat # 7074s) with horseradish peroxidase (HRP) conjugated secondary antibody compatible with primary antibody. Bands were obtained by using a chemiluminescence-based substrate kit (Pierce, Rockford, IL, USA) which reacted with HRP. The bands formed were exposed to X-ray film, scanned, and transferred to the computer. The bands were analyzed by using ImageJ software (http://imagej.nih.gov/ij/). The same procedures were applied in our previous study (29).

### Statistical analysis

The immunochemistry and Western blot results were analyzed with GraphPad Prism 9 (GraphPad Software). After the Shapiro-Wilk test was used to assess the normality of the data obtained with ImageJ, they were expressed as mean ± standard deviation (SD). All data were analyzed with one-way analysis of variance (ANOVA) and Tukey's test was used for the post-hoc test. $P<0.05$ was considered as the level of significance.

## RESULTS

Since we examined the effect of hydroxytyrosol on blood glucose values in detail in another study, we did not repeat it in this study (30).

### Nrf2 immunohistochemistry results;

In the kidney cortex and medulla cells, the Nrf2 immunostaining pattern was both cytoplasmic and nuclear (Figure 1A). In the control group, Nrf2 expression was densely positive in distal convoluted tubule cells, and positive in proximal convoluted tubule, macula densa, and proximal straight tubule cells (Table 1, Figure 1A (1 and 2)). It was weakly positive in podocyte cells and distal straight tubule cells (Table 1, Figure 1A (1 and 2)). Nrf2 expression in the HT group was higher than control and STZ+HT groups ($p<0.001$) (Figure 1A and 1B). Nrf2 expression was very dense positive in distal and proximal

convoluted tubule and distal and proximal straight tubule cells of the HT group (Table 1, Figure 1A (4 and 5)). Nrf2 expression was positive in macula densa and podocyte cells of the HT group (Table 1, Figure 1A (4 and 5)). Nrf2 expression in the STZ group was found to be significantly decreased compared to control and HT groups ($p$<0.001) (Figure 1A and 1B). Nrf2 expression was weakly positive in the distal and proximal convoluted tubule, macula densa, and proximal straight tubule cells of the STZ group (Table 1, Figure 1A (7 and 8)). Nrf2 expressions were negative in podocytes and distal straight tubule cells (Table 1, Figure 1A (7 and 8)). In addition, vacuolization was observed in some distal convoluted tubule cells of the STZ group (Figure 1A (7 and 8)). In the STZ+HT group, Nrf2 expression was significantly increased compared to the STZ group ($p$<0.001) (Figure 1A and 1B). However, no significant difference was found between the STZ+HT group and control group ($p$=0.648) (Figures 1A and 1B). In the STZ+HT group cells, Nrf2 expression was dense positive in distal convoluted and straight tubule cells, positive in proximal convoluted tubule, macula densa, and podocyte cells, and dense positive in proximal straight tubule cells (Table 1, Figure 1A (10 and 11)). In mesangial cells of all groups, Nrf2 expression was negative (Table 1, Figure 1A (1, 2, 4, 5, 7, 8, 10, and 11)).

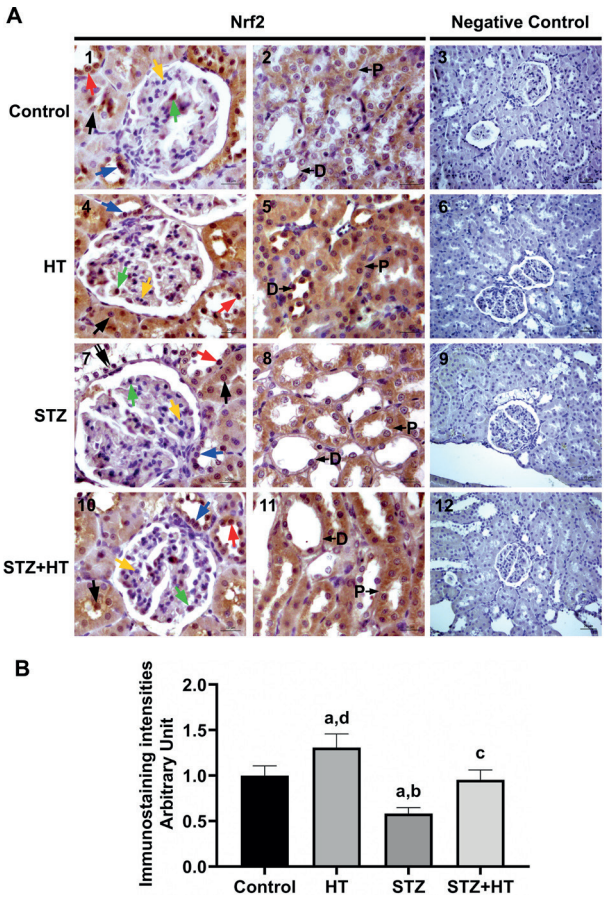


**Figure 1. A.** Representative micrographs of immunohistochemical analysis results of Nrf2. **B.** Quantification of immunohistochemical staining. The data were expressed as mean±SD. Black arrow; proximal convoluted tubule cell, red arrow; distal convoluted tubule cells, blue arrow; macula densa cell, green arrow; podocyte cell, yellow arrow; mesangial cell, double arrow; vacuoilized cells, P; proximal straight tubule cell, D; distal straight tubule cell. Statistical significance ($p$<0.05) compared with [a]control and [b]HT, [c]STZ and [d]STZ+HT. HT; hydroxytyrosol, STZ; streptozotocin. The lens magnification of micrograph 1, 2, 4, 5, 7, 8, 10, and 11 is 100x. The lens magnification of micrograph 3, 6, 9, and 12 is 40x.

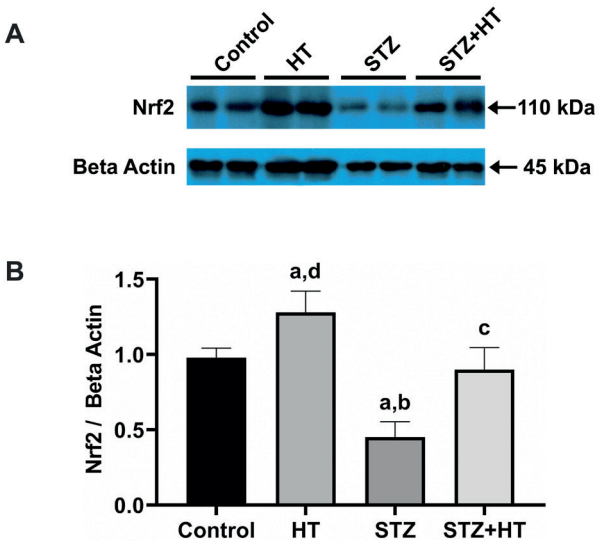


**Figure 2. A.** Representative bands of Western blot analysis results in kidney tissue extracts of Nrf2 protein. **B.** Quantification of Western blot Nrf2 protein level. The data were expressed as mean±SD. Statistical significance ($p$<0.05) compared with [a]control and [b]HT, [c]STZ and [d]STZ+HT. HT; hydroxytyrosol, STZ; streptozotocin.

### Nrf2 Western blot results

In all groups, Nrf2 Western blot results were compatible with immunohistochemistry results. In the HT group, Nrf2 protein was significantly increased compared to the control and STZ+HT groups ($p$<0.001) (Figure 2). STZ group Nrf2 protein level was significantly decreased compared to the control and HT groups ($p$<0.001) (Figure 2). STZ+HT group Nrf2 protein was significantly increased compared to the STZ group. However, the Nrf2 protein level in the STZ+HT group was similar to that in the control group ($p$=0.545) (Figure 2).

## DISCUSSION

In this study, we aimed to investigate the effects of hydroxytyrosol, which is an anti-oxidative polyphenolic compound with known protective effects on the kidney and the basis of the Mediterranean diet, on Nrf2 expression in type 1 diabetic rats. As a result of the study, we determined that Nrf2 expression increased in the HT group treated with hydroxytyrosol compared to the control group, while it decreased in the STZ group treated with streptozotocin compared to the control group. However, we found that in the STZ+HT group, which received hydroxytyrosol together with streptozotocin, Nrf2 expression was at the same level as the control.

In this study, immunohistochemistry and Western blot results of Nrf2 were found to be similar. We found that Nrf2 expression was increased in the HT group that was administered hydroxytyrosol compared to the control group. We found that this increase occurred mostly in proximal and distal convoluted tubules and proximal and distal straight tubules. It has been previously reported that hydroxytyrosol activates Nrf2 in many different tissues and cells (31-33). In the present study, as in previous studies (31-33), hydroxytyrosol may have increased the intensity of Nrf2 expression by activating Nrf2 in many kidney cells, compared to the control group. We found that the streptozotocin, which was given to induce diabetes, reduced Nrf2 expression in the STZ group compared to the control and HT groups, and this decrease was observed in all cells that showed expression. Previous studies have found that streptozotocin decreased renal Nrf2 expression at both the mRNA and protein levels (19,34). Therefore, it can be understood that the reason for decreased Nrf2 expression in the STZ group results directly from the effects of streptozotocin. In the STZ+HT group, which was administered hydroxytyrosol by inducing diabetes with streptozotocin, Nrf2 expression was found to be increased compared to the STZ group. This increase was observed in all cells expressing Nrf2. In diabetic rats, hydroxytyrosol reduces proteinuria by 67-73% and glomerular volume and glomerulosclerosis index by 20-30% (24). Hydroxytyrosol relieves renal hypertrophy index by improving renal dysfunction parameters including creatinine, blood urea nitrogen, serum and urine albumin in serum and urine, and activities of tissue oxidative stress markers and inflammatory cytokines. In addition, hydroxytyrosol increases *Nrf2* mRNA expression and decreases *Keap1* mRNA expression in diabetic rat kidneys (35). In many studies conducted on diabetic kidneys, Nrf2 activated with different agents reduces oxidative stress, inflammation, and fibrosis (13-15). Activated Nrf2 reduces malondialdehyde levels and increases SOD activity, thereby reducing renal damage and extracellular vesicle accumulation (16). Nrf2 activation reduces kidney damage and apoptosis by reducing inflammation and oxidative stress through HO-1 mediation (17,18). In addition, HO-1 induced by Nrf2 relaxes DN by inhibiting apoptosis, angiogenesis, inflammation, and oxidative stress (19). Nrf2 activation is protective for albuminuria and glomerular remodeling observed in DN (15). When these studies are evaluated together, it can be understood that Nrf2 activated by different agents relaxes diabetic nephropathy by reducing oxidative stress, apoptosis, inflammation, fibrosis, and angiogenesis. In addition, hydroxytyrosol has been reported to activate Nrf2 in many different tissues and cells (31-33). It has also been reported that hydroxytyrosol activates *Nrf2* mRNA expression (35). In this study, it is predicted that the Nrf2 protein, which was suppressed in diabetic kidneys with streptozotocin, is activated with hydroxytyrosol and the activated Nrf2 can relax diabetic nephropathy by reducing oxidative stress, apoptosis, inflammation, fibrosis, and angiogenesis.

In conclusion, this study has shown for the first time that hydroxytyrosol, a polyphenolic compound abundant in olive oil and the basis of the Mediterranean diet,

activates the expression of Nrf2 in diabetic kidneys, which protects against kidney damage by reducing oxidative stress, inflammation and fibrosis caused by diabetes. In addition, this study reported in detail the effects of hydroxytyrosol in diabetic kidneys on Nrf2 at the cellular level. Since this study was conducted with an experimental model, the effects of hydroxytyrosol on diabetic nephropathy patients are predictive. Therefore, the course of diabetic nephropathy can be evaluated in future studies in regions where olive and olive oil, which are abundant in hydroxytyrosol, are consumed a lot. In addition, using hydroxytyrosol as an Nrf2 activating agent in diabetes patients can reduce diabetes-related kidney damage. There is certainly a need for new clinical studies to use this on patients.

### Ethical approval

This study has been approved by the Düzce University Animal Experiments Local Ethics Committee (approval date 21/12/2022, number 2022/12/05). Written informed consent was obtained from the participants.

### Author contribution

Concept: HS; Design: HS; Data Collection or Processing: HS, KK; Analysis or Interpretation: HS; Literature Search: KK; Writing: HS. All authors reviewed the results and approved the final version of the article.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

# The relationship between infection parameters and urine volume in acute kidney injury

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## ABSTRACT

**Aim:** Acute kidney injury (AKI) is a clinical syndrome that can cause disturbances in fluid-electrolyte and acid-base balance, resulting in the accumulation of nitrogen and uremic toxins along with the loss of kidney functions within hours or days. In this study, it was aimed to retrospectively examine patients with acute kidney injury to determine whether there is a relationship between infection parameters and urine volume.

**Materials and Methods:** The study included a total of 144 patients with (n=74) and without infection (n=70) out of 294 patients with AKI who received treatment between 1 January 2020 and 31 December 2021 in the nephrology clinic of a tertiary university hospital.

**Results:** The mean age was 66.4±15.7 (range:19-95) in patients with infection and 63.8±15.2 (range:36- 93) in non-infected patients. 51.4% (n=38) of those with infection and 52.9% (n=37) of those without infection were women. There was no difference between the individuals with and without infection in terms of age and gender (p>0.05). Infection was present in 51.4% (n=74) of the patients included in the study. Urinary tract (31.3%) and respiratory tract infections (13.2%) were the most common in those with infection. A moderate negative correlation was observed between admission CRP and discharge creatinine level in patients with infection. There was no correlation between PCT and sedimentation rate, urine volume and admission/discharge creatinine level. Moderate positive correlations were found between admission/discharge PCT and admission/discharge urine volume in patients without infection. In addition, moderate negative correlations were found between admission/discharge sedimentation rate and admission urine volume.

**Conclusions:** No correlation was found between PCT and sediment (incoming/exit) and outflow urine volume in patients with infection.

**Keywords:** acute kidney injury, CRP, PCT, Sedim, urine volume

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## INTRODUCTION

Acute kidney injury (AKI) is a clinical syndrome that results in the accumulation of nitrogen and uremic toxins within hours or days of loss of renal function and may lead to disturbance in fluid–electrolyte and acid–base balance (1). AKI is currently a major cause of morbidity and mortality. Its frequency, etiology, prognosis, and mortality vary according to many characteristics of the patients such as age, gender, race, comorbidities, damage to other organs, the stage at the time of diagnosis, and the period in which the studies were conducted (2).

In addition to being a predictor of chronic kidney disease (CKD), AKI is a disease that increases the consumption of health resources (3). While the prevalence of AKI in the general population is below 1%, it rises to 2%–5% in inpatients and 25%–30% in patients in intensive care units (4). Although AKI often manifests as a complex multifactorial syndrome, sepsis, and septic shock are common causes of the disease (5). Inflammation markers are frequently monitored in the diagnosis of infection and treatment management of AKI.

Albumin and prealbumin are negative acute phase reactants synthesized by the liver. In recent years, acute phase reactants such as C-reactive protein (CRP) and procalcitonin (PCT) have been suggested to be potential markers for early diagnosis of infection (6). CRP is a marker synthesized by the liver and its level increases within hours. It is often used because it is cheap and easily accessible. Apart from infections, its levels can be elevated in the presence of cancer, autoimmune diseases, surgery, and trauma (7). PCT, the calcitonin propeptide composed of 116 amino acids with a long half-life in the blood and released from thyroid C cells, has proven to be an early, sensitive, and accurate marker in identifying bacterial infection and assessing the severity of infections and sepsis. PCT concentrations may be elevated in burns, the early stage of trauma, and various non-sepsis conditions, such as invasive surgical interventions (8).

Mehanic et al. reported that PCT is a reliable marker for early diagnosis and evaluation of the prognosis of bacterial infections. However, damage to the kidney or liver may alter the PCT elimination rate and clearance (9).

The aim of this study was to evaluate whether there is a relationship between infection parameters and urine volume in patients with AKI. We think that the results obtained in this study will help clinicians to determine the areas of intervention and priority in preventing the deterioration of AKI.

## MATERIALS AND METHODS

This was a single-center retrospective descriptive correlation study. A total of 144 patients with (n=74) and 144 patients without (n=70) infections were included in the study among 294 patients with AKI treated at the nephrology department of a tertiary university hospital between 1 January 2020 and 31 December 2021.

AKI was diagnosed according to Kidney Disease Improving Global Outcomes classification. Patients were evaluated according to the decrease in the amount of urine volume in the first 6 h after hospitalization and the increase in creatinine (Cr) level in 48 h (10).

To determine the source of infection in patients diagnosed with AKI, complete urinalysis and urine culture were performed for urinary tract infections; stool culture was performed for gastrointestinal tract infections; clinical and laboratory tests as well as chest X-rays were used for respiratory tract infections. Clinical evaluation of the patients for the diagnosis of infection was performed daily. The definitive diagnosis of the infection was made by two independent experts based on the clinical and laboratory results.

Patients diagnosed with chronic kidney damage and failure, using drugs, such as angiotensin converting enzyme inhibitors, angiotensin receptor blockers, non-steroidal anti-inflammatory drugs, patients with a history of surgery and trauma in the past week, history of cancer and autoimmune disease, with contrast exposure, hospital stay <48 h, catheter infection, no test results in the last month, <18 years of age, transferred to other clinics, patients who died or discontinued treatment, and those non-compliant with urine collection were not included in the study.

The parameters evaluated in the study were age, gender, length of hospitalization, hemodialysis status, laboratory test results during hospitalization (creatinine, urea, electrolytes, total protein, albumin, hemoglobin, arterial blood pH, and bicarbonate), daily urine volume, and infection parameters (CRP, PCT, and sedimentation rate). Ethics committee approval and institutional permission were obtained (No: 2022/141), and the study was conducted in accordance with the Declaration of Helsinki.

### Laboratory analysis

Hemogram tests were performed using Sysmex XN-1000 and biochemistry tests were performed using the Beckman Coulter AU 5800 device. CRP concentrations were measured with immunoturbidimetric method using Beckman Coulter AU 5800. Sedimentation was measured with the Western green method using the Vision C (YHLO, Biotech) device. Procalcitonin was measured with an immunoassay method adapted to the AQT90 Flex analyzer.

### Statistical analysis

Data analysis was performed using SPSS version 24.0 statistical software. Descriptive statistics for the variables of the study were presented as numbers (n) and percentages (%). The normality assumption of continuous variables was evaluated using the Kolmogorov-Smirnov test. Continuous variables were presented as mean ($\bar{X}$) ± standard deviation (SD) and median (min-max). Since parametric assumptions were not met, the Mann–Whitney U test was used to compare the means of the two independent groups. Group means were expressed as ($\bar{X}$) ± SD. The correlation between two variables was evaluated using Spearman's rank correlation analysis (coefficient [r]). In all analyses, a p-value <0.05 was considered statistically significant. If there was a significant correlation, the coefficient was evaluated as weak for r = 0-0.24, moderate for r = 0.25–0.49, strong for r = 0.50–0.74, and very strong for r = 0.75-1.00 with a minus (-) sign indicating a negative and plus (+) sign indicating a positive correlation.

## RESULTS

The study included 144 patients. The mean age was 66.4 ± 15.7 (min–max: 19-95) in patients with infection and 63.8 ± 15.2 (min-max: 36-93) in patients without infection. Females accounted for 52.9% (n=37) of patients with an infection and 51.4% (n=38) of patients with an infection. There was no difference between patients with and without infection in terms of age and gender (p>0.05). Infection and urine volume parameters of patients with and without infection are presented in Table 1.

Infections were present in 51.4% (n=74) of the patients included in the study. The most common infections were urinary tract (31.3%) and respiratory tract (13.2%) infections (Table 2).

In patients with infections, a moderate negative correlation was observed between admission CRP and discharge creatinine. No correlation was found between PCT and sedimentation (admission/discharge) and discharge urine volume and admission/discharge creatinine (Table 3).

**Table 1.** Infection and urine volume parameters of patients with and without infection.

| | **Without infection (n = 70)** | | **With infection (n = 74)** | |
|---|---|---|---|---|
| | **$\bar{X}$ ± sd** | | **$\bar{X}$ ± sd** | |
| | Admission | Discharge | Admission | Discharge |
| CRP (mg/dL) | 0.4±0.1 | 0.3±0.2 | 9.0±7.6 | 1.8±2.1 |
| Sedimentation (mm/h) | 44.0±15.9 | 36.5±15.9 | 33.7±16.4 | 18.8±11.2 |
| Procalcitonin (ng/mL) | 0.1±0.0 | 0.1±0.0 | 2.4±1.0 | 1.1±0.7 |
| Creatinine (mg/dL) | 1.7±0.5 | 1.0±0.3 | 4.2±2.5 | 1.9±1.2 |
| Urine (cc) | 1080±269.5 | 1379.3±286.5 | 612.8±252.0 | 1257.6±430.2 |

**Table 2.** Infection status of patients.

| | N | % |
|---|---|---|
| No | 70 | 48.6 |
| Yes | 74 | 51.4 |
| Urinary tract infection | 45 | 31.3 |
| Respiratory tract infection | 19 | 13.2 |
| Soft tissue infection | 5 | 3.5 |
| Bile tract infection | 3 | 2.1 |
| Endocarditis | 2 | 1.4 |

Moderate positive correlations were found between admission/discharge PCT and admission/discharge urine in patients without infection. Furthermore, moderate negative correlations were observed between admission/discharge sedimentation and admission urine (Table 4).

## DISCUSSION

In this study, we evaluated whether there was a change in urine volume as a result of improvement in infection parameters due to the treatment of AKI. No correlation was found between PCT and sedimentation (admission/discharge) and discharge urine volume in patients with infection. In patients without infection, moderate positive correlations were found between admission/discharge PCT and admission/discharge urine volume. In addition, moderate negative correlations were observed between admission/discharge sedimentation and admission urine volume.

Many studies have been conducted on infection parameters such as neutrophil gelatinase-associated lipocalin (NGAL), presepsin, CRP, and PCT in patients with AKI (7,8,11,12). However, there are no studies in the literature evaluating the relationship between infection parameters and urine volume.

In a study conducted by Nakamura et al. evaluating several parameters between sepsis and non-sepsis groups, there was a moderate positive correlation between presepsin and creatinine and also a moderate negative correlation between presepsin and eGFR (13). Another study by Nakamura et al. showed that the diagnostic accuracy of the PCT level was significantly lower in patients with AKI than that of the PCT level in patients without AKI. In addition, there was a significant positive correlation between PCT and creatinine and a negative correlation between PCT and eGFR among patients. These results suggest that the kidneys are one of the organs responsible for eliminating PCT from the blood (14). Meisner et al. reported that renal elimination of PCT is not a major mechanism for the

**Table 3.** The relationship between infection and urinary parameters in patients with infection.

| | | $\bar{X}$ ± SD | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Creatinine (admission) | 4.2±2.5 mg/dL | 1 | | | | | | | | |
| 2 | Creatinine (discharge) | 1.9±1.2 mg/dL | 0.769** | 1 | | | | | | | |
| 3 | Urine (admission) | 612.8±252.0cc | -0.011 | -0.13 | 1 | | | | | | |
| 4 | Urine (discharge) | 1257.6±430.2cc | -0.099 | -0.242* | 0.96** | 1 | | | | | |
| 5 | CRP (admission) | 9.0±7.6 mg/dL | -0.213 | -0.343** | -0.016 | 0.11 | 1 | | | | |
| 6 | CRP (discharge) | 1.8±2.1 mg/dL | -0.192 | -0.203 | -0.057 | 0.06 | 0.465** | 1 | | | |
| 7 | Sedimentation (admission) | 33.7±16.4 mm/hour | 0.062 | -0.049 | 0.036 | 0.047 | 0.217 | 0.187 | 1 | | |
| 8 | Sedimentation (discharge) | 18.8±11.2 mm/hour | 0.081 | 0.029 | 0.242* | 0.158 | 0.181 | 0.227 | 0.620** | 1 | |
| 9 | Procalcitonin (admission) | 2.4±1.0 ng/mL | 0.227 | -0.082 | 0.226 | 0.154 | 0.103 | -0.101 | 0.049 | 0.143 | 1 |
| 10 | Procalcitonin (discharge) | 1.1±0.7 ng/mL | 0.11 | 0.066 | 0.107 | -0.002 | 0.107 | -0.082 | 0.067 | 0.146 | 0.593** |

** Correlation is significant at $p < 0.01$.

**Table 4.** The relationship between infection and urinary parameters in patients without infection.

| | | $\bar{X}$ ± SD | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Creatinine (admission) | 1.7±0.5 mg/dL | 1 | | | | | | | | |
| 2 | Creatinine (discharge) | 1.0±0.3 mg/dL | 0.577** | 1 | | | | | | | |
| 3 | Urine (admission) | 1080±269.5cc | -0.06 | -0.004 | 1 | | | | | | |
| 4 | Urine (discharge) | 1379.3±286.5cc | 0.061 | -0.153 | 0.462** | 1 | | | | | |
| 5 | CRP (admission) | 0.4±0.1 mg/dL | 0.206 | 0.14 | 0.23 | 0.093 | 1 | | | | |
| 6 | CRP (discharge) | 0.3±0.2 mg/dL | 0.137 | 0.049 | 0.119 | 0.12 | 0.674** | 1 | | | |
| 7 | Sedimentation (admission) | 44.0±15.9 mm/hour | 0.281* | 0.192 | -0.325** | -0.062 | 0.092 | 0.167 | 1 | | |
| 8 | Sedimentation (discharge) | 36.5±15.9 mm/hour | 0.286* | 0.179 | -0.328** | -0.093 | -0.014 | 0.173 | 0.866** | 1 | |
| 9 | Procalcitonin (admission) | 0.1±0.0 ng/mL | -0.017 | -0.051 | 0.267* | 0.261* | 0.084 | 0.176 | -0.094 | -0.082 | 1 |
| 10 | Procalcitonin (discharge) | 0.1±0.0 ng/mL | -0.009 | -0.034 | 0.272* | 0.336** | 0.062 | 0.142 | -0.123 | -0.081 | 0.944** |

** Correlation is significant at $p < 0.01$.

removal of PCT from plasma. The plasma elimination rate may be prolonged up to 30%–50% in some patients with renal dysfunction; since PCT elimination may not be severely affected by this moderate prolongation, the authors concluded that PCT can be used diagnostically in patients with normal renal function as well as in patients with renal failure (15). Nie et al. found that AKI formation, serum creatinine, and cystatin C levels showed a positive correlation with PCT levels (8). In a study by Takahashi et al. investigating the diagnostic accuracy of procalcitonin and presepsin in patients with AKI and infection, higher PCT and presepsin levels were found in patients with infection, and the increase in PCT and presepsin levels was associated with the severity of infection and renal dysfunction. The diagnostic accuracy of PCT and presepsin for infection was not lower in patients with AKI than that in patients without AKI (16). In the present study, no correlation was found between PCT (admission/discharge) and creatinine (admission/discharge) in patients with infection.

Xie et al. found a significant increase in serum CRP levels and a decrease in prealbumin levels in patients with AKI and infection compared to those without infection (17). In the present study, admission CRP level was higher in patients with AKI and infection than that in patients without infection. This result was consistent with the literature.

Since there is no other study in the literature investigating the relationship between PCT, sedimentation, CRP, and urine volume in patients with and without infection, it was not possible to compare the results of the present study with the results of another study.

There are certain limitations to our study. First, this retrospective single-center study included only a small sample size. Second, the effect of the cellular immune status of patients (such as immunosuppressed patients receiving chemotherapy or steroid therapy) on PCT, CRP, and sedimentation levels was not taken into account. Third, other markers of infection (Interleukin-6, myeloid cells-1, Presepsin, and NGAL) were not analyzed due to difficulties obtaining them. In addition, patients with missing data on admission for PCT determination were excluded from the study. Despite these limitations, the results obtained in the present study are important in terms of examining the relationship between infection parameters and urine volume.

As a result, no correlation was found between PCT and sedimentation (admission/discharge) parameters and

urine volume (discharge) in patients with infection. However, a small sample of patients with AKI was evaluated in the present study. We believe that our study will be a pioneer for future multicenter and large-scale studies with large patient groups.

### Ethical approval

This study has been approved by the Clinical Research Medical Ethics Committee Dicle University (approval date 12.05.2022, number 2022/141). Written informed consent was obtained from the participants.

### Author contribution

Concept: İO, HS, YY; Design: Hİ, YY, EA; Data Collection or Processing: EA, FYA, ZY; Analysis or Interpretation: İO, Hİ, AKK; Literature Search: İO, HS, YY; Writing: İO, HS, EE. All authors reviewed the results and approved the final version of the article.

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RESEARCH ARTICLE

# Prevalence of dry eye in patients using topical antiglaucoma medications

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## ABSTRACT

**Aim:** Topical antiglaucoma drugs may have adverse effects on the ocular surface. In this study, our aim was to report the frequency of dry eye and the use of artificial tear drops in patients diagnosed with glaucoma and using topical antiglaucoma drugs. We also evaluated factors affecting this association.

**Methods:** Based on the medical records, we selected patients admitted to the ophthalmology department between 2020 and 2021 who had been diagnosed with glaucoma. In this study, we included patients who were using topical antiglaucoma medications and were older than 40 years of age. Age, gender, type, and number of glaucoma medications used, dry eye diagnosis, and use of artificial tear drops and/or topical cyclosporine were recorded.

**Results:** We found that 346 (27%) of the 1,274 patients using topical antiglaucoma drugs had dry eyes and were using artificial tear drops. Gender (female) and the number of antiglaucoma medications used were associated with an increased risk of dry eye in these patients, while increasing age was not associated with dry eye.

**Conclusion:** Dry eye is common in patients using topical antiglaucoma medications and should be considered in the treatment of glaucoma.

**Keywords:** antiglaucoma medications, artificial tear drops, dry eye, glaucoma, ocular surface disease

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## INTRODUCTION

Glaucoma is a major cause of irreversible blindness worldwide (1, 2). The global prevalence is reported to be 3.54% in people aged 40-80 years (3). Topical antiglaucoma drugs are the most common choice for the initial treatment of glaucoma (4). There are several drug options available to glaucoma patients, but many patients need more than one drug to control intraocular pressure (IOP) (5). Studies often report a high prevalence of dry eye and ocular surface disease (OSD) in glaucoma patients using topical antiglaucoma medications (6-8). Both preservatives and active drug molecules can affect the ocular surface. OSD has been reported to occur in more than half of the glaucoma patients who use topical antiglaucoma medications (9). OSD can cause burning, itching, foreign body sensation, blurred vision, and poor quality of life in glaucoma patients (10). OSD may reduce patient compliance and adherence, resulting in the failure of the treatment (11). It may also affect the success of the subsequent glaucoma filtration surgery (9). Artificial tear drops and lubricants are frequently used in the treatment of dry eye and OSD in glaucoma patients who use topical antiglaucoma medications (12). Most studies investigating dry eye disease in glaucoma patients have been conducted on a relatively low number of patients. The aim of this study was to investigate the frequency of dry eye, and the use of artificial tear drops in glaucoma patients who use topical antiglaucoma medications. We also evaluated the risk factors associated with dry eye disease in a large group of glaucoma patients.

## MATERIAL AND METHODS

In this retrospective chart review of consecutive patients, we included glaucoma patients who were referred to our outpatient clinic between 2020 and 2021. From this group of patients, only those who were older than 40 years of age and on topical antiglaucoma medications were included in the study. Patients' medical records were evaluated retrospectively. Age, gender, type, and number of glaucoma medications used, dry eye diagnosis, and use of artificial tear drops and/or topical cyclosporine were recorded. The two-year period was chosen because the glaucoma medications are fully reimbursed for two years if a patient has glaucoma certified by an ophthalmologist. Certification examinations included a complete ophthalmic examination and applanation tonometry. Visual field testing, corneal pachymetry, and retinal nerve fiber layer thickness measurements using optical coherence tomography are also performed as necessary. Both Schirmer's test and tear break-up time measurements are performed in patients who report the typical symptoms of dry eye disease. Patients are diagnosed with glaucoma and/or dry eye based on the results of the examination and tests. All of the aforementioned procedures adhered to the principles of the Declaration of Helsinki, and the study received ethical approval from the local ethics committee (Protocol No: 2020/24 Date: 09.07.2020).

### Statistical analysis

Statistical analysis was performed using SPSS version 20.0 (IBM Corporation, Armonk, NY). Quantitative data were expressed as means ± standard deviations, and qualitative data were expressed as proportions (%). The chi-square test was used to compare groups by gender. Independent-samples t-test was used to compare the gender groups for the presence of dry eye. Logistic regression analysis was also performed to determine the risk factors for dry eye in patients using topical antiglaucoma drugs.

## RESULTS

A total of 1,274 patients were diagnosed with glaucoma in this period. The characteristics of these patients are summarized in Table 1. The mean age was 68.3±11.3 years. Of these patients, 638 (50.1%) were female and 636 (49.9%) were male. A total of 758 (59.5%) patients were using one medication for glaucoma, 340 (26.7%) patients were using two medications, and 176 (13.8%) patients were using three or more medications (Table 1). Of the 758 patients using one medication for glaucoma, 289 (38.2%) patients were on monotherapy, and 469 (61.8%) patients were using fixed combinations. Of the 1,274 patients using glaucoma medications, 346 (27.2%) had been diagnosed with dry eye and were using artificial tear drops and/or cyclosporine, and 928 (72.8%) did not have dry eye. Dry eye was more common in female patients (31.8%

| **Table 1.** Characteristics of glaucoma patients. | |
|---|---|
| | **n (%)** |
| **Age groups** | |
| 40-60 years | 280 (22) |
| 60-80 years | 774 (60.8) |
| >80 years | 219 (17.2) |
| **Gender** | |
| Male | 636 (49.9) |
| Female | 638 (50.1) |
| **Number of antiglaucoma medications** | |
| 1 | 758 (59,5) |
| 2 | 340 (26.7) |
| 3 or more | 176 (13.8) |
| Total | 1274 (100) |

n: number

vs. 22.5% p<0.001). Logistic regression analysis using age groups, gender, and number of medications showed that female patients were 1.6 times more likely to have dry eye disease (OR: 1.6 p<0.001). Increasing the number of medications from one to two or three was associated with an increased likelihood of having dry eye (OR: 1.8 p: 0.008 and OR: 2.1 p: 0.001, respectively). Increasing age was not associated with dry eye (p: 0.560).

## DISCUSSION

Medical therapy using topical antiglaucoma drugs is the most common initial treatment for glaucoma (4). OSD and dry eye are often observed in patients using topical antiglaucoma drugs (9). An important factor in this association is that the prevalence of both diseases increases with age (13,14). On the other hand, both glaucoma drugs and preservatives in these medications were reported to affect the ocular surface and cause dry eye and OSD (9). Fechtner et al. evaluated ocular surface disease index (OSDI) scores of patients using topical antiglaucoma drugs and reported that 48.4% of these patients have some degree of OSD (15). Erb et al. reported that 52.6% of glaucoma patients using antiglaucoma drugs had dry eye in their study (6). In our study, 346 (27.2%) of 1,274 glaucoma patients were diagnosed with dry eye and used artificial tear drops, which is lower than the corresponding rates in the previous studies. This value is also higher than the prevalence of dry eye in the general population of similar age groups, which is about 14% (14). Previous studies investigating OSD in glaucoma often used symptom questionnaires to diagnose dry eye and included all the patients with mild, moderate, and severe symptoms. Our results indicate the proportion of patients who were diagnosed with dry eye and used artificial tear drops, which may be the reason for the difference in the prevalence between previous studies and the present study. It may be more appropriate to compare the frequency of moderate and severe symptoms with the corresponding frequencies in our study. In the study by Fechtner et al., the proportion of patients with moderate and severe OSD was 27,1% which is similar to our results (15).

Dry eye disease is known to be more prevalent in women and hence female gender is a risk factor for dry eye (14,16). We also found that dry eye is more common in the female patients compared to the male patients in our glaucoma patient group. In the logistic regression analysis, the female gender was also associated with an increased likelihood of having dry eye in glaucoma patients. Erb et al. reported that dry eye is more prevalent in female glaucoma patients (6). Costa et al. also reported in their study that female gender is a risk factor for the use of artificial tear drops (8).

The preferred initial treatment for glaucoma is monotherapy with one medication to control the IOP (4). However, monotherapy is insufficient to control IOP in a large group of patients, and at least 50% of patients require two or more drugs to achieve the target IOP at the follow-up (17). About 40% of the patients in our study were using two or more antiglaucoma drugs, and an increased number of glaucoma medications was associated with an increased risk of dry eye. This finding was also reported in the previous studies (6,8,15). Erb et al. reported that dry eye was found more frequently when three or more IOP-lowering medications were used to treat glaucoma (6). Fechtner et al. also reported that OSDI scores in glaucoma patients increased significantly as the number of antiglaucoma medications increased (15). The number of antiglaucoma medications was also found to be a

risk factor for the use of artificial tear drops in another study (8). Preservatives in antiglaucoma drugs were frequently reported to be involved in the pathogenesis of dry eye in glaucoma patients (12,18). However, some studies reported that there isn't enough evidence to show a significant difference in the efficacy and safety between preservative-containing and preservative-free drugs (19,20). All participants in the present study were using glaucoma drugs with preservatives, either Benzalkonium chloride (BAK) or Purite. We couldn't draw a direct conclusion on the effect of preservatives in this study, but the finding of increased risk of dry eye associated with an increased number of glaucoma drugs may suggest an association between dry eye and preservatives because patients on multiple drugs are exposed to a higher amount of preservatives than patients on monotherapy.

We did not find an association between age and dry eye in glaucoma patients using antiglaucoma drugs. Fechtner et al. also reported that OSDI scores did not change significantly with age in glaucoma patients using topical antiglaucoma drugs (15). Another study reported a positive correlation between age and dry eye in glaucoma patients, but this study included patients under 40 years of age, who have a lower prevalence of dry eye (6). This difference between this study and ours may be due to the fact that our study included only patients over the age of 40. A high proportion of patients in our study (60%) were relatively old, between 60 and 80 years. This may be the reason for the findings related to age and dry eye in our study. A study including patients of all ages using topical antiglaucoma medications may be more effective in showing the effect of age, but this is complicated by the lower prevalence of glaucoma in people under the age of 40.

There are some limitations of this study. Firstly, this is a retrospective study, which may limit data analysis and lead to possible biases. We did not include patients younger than 40 years, who have a lower prevalence of glaucoma and dry eye. Also, all patients in this study were using glaucoma drugs containing preservatives, and the findings represent patients using glaucoma drugs containing preservatives.

In conclusion, we found that 27% of the patients using topical antiglaucoma drugs had dry eye and used artificial tear drops in this study, which included a large group of glaucoma patients older than 40 years. This prevalence is lower than previously reported values. Gender (female) and the number of antiglaucoma medications used were associated with an increased risk of dry eye disease in glaucoma patients, while age was not associated with the risk of dry eye. Prevention and appropriate treatment of this condition are necessary to maintain patient compliance.

### Ethical approval

This study has been approved by the Niğde Ömer Halisdemir University Non-invasive Clinical Research Ethics Committee (approval date 09.07.2020, number 2020/24). Written informed consent was obtained from the participants.

### Author contribution

Concept: EK, KRZ; Design: EK, KRZ; Data Collection or Processing: MÇK, GYB; Analysis or Interpretation: EK, KRZ; Literature Search: MÇK, GYB; Writing: EK. All authors reviewed the results and approved the final version of the article.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

# Analysis of an anatomy laboratory for microbiological contamination

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## ABSTRACT

**Aim:** In order to prevent any microbiological contamination in laboratories, it is vital to determine both routine microbiological screening and the appropriate protocol. This study was based on this hypothesis and discussed the microbiological contamination and prevention procedures in an anatomy laboratory.

**Methods:** The study was carried out on 34 different spots in an anatomy laboratory. Swab samples taken from these points were examined for contamination and contamination was detected. The samples were taken from various locations, including the head, upper and lower extremities of both male and female cadavers, the door handle, the floor in front of the door, the faucet, the head, body, and foot parts of the dissection table, the dissection tool, the trailer, the inner and outer coating of the cadaver pool, the sink, the floor in front of the window, the stool, the living room wall, the formaldehyde liquid in the cadaver pool, the window handle, the instrument table, the morgue unit, the exterior surfaces of three different organ storage boxes, the inner surface of an organ storage box, the medical waste container, the handle of the organ storage cabinet, a training model, the lower surface of the dissection table, the medical waste storage box for dissection, and the blackboard.

**Results:** Bacillus subtilis was found in 16 out of 34 different spots and mold fungus was found in 2 of them. No contamination was detected in the remaining 16 spots. 69% of the spots were directly related to the cadaver.

**Conclusion:** As a result of our study, the importance of scanning anatomy laboratories in terms of microbiological contamination was highlighted and an appropriate protocol was determined.

**Keywords:** contamination, anatomy laboratory, microbiological analysis

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## INTRODUCTION

The areas where students are taught together are of great importance for the spread of infectious agents and maximum attention should be paid to the disinfection of these areas (1,2). Due to the easy spread of bacteria, bacterial contamination in research laboratories where students are taught is of critical importance (3-5).

Taking precautions against biological agents and substances that pose a threat to humans is called biosafety (6). Several biosafety measures should be taken to minimize the spread in research laboratories. These measures can be listed as eliminating routes of transmission, providing educational information, controlling the risk factors, reducing exposure, scanning both individuals and laboratories at regular intervals, drafting a biosafety manual, posting warnings for contamination, establishing a routine cleaning procedure, providing training for waste management, providing, and controlling protective materials (3,6,7). Basically, these measures can be examined in two categories. The first category is the safety of the laboratory and the individuals in the laboratory, while the second one is the safety of the external environment of the laboratory (8).

Biological threats in research laboratories have been classified into four different groups. The first group is biological threats that do not cause any diseases in humans, the second group is biological threats that cause diseases in humans but do not spread. The third group is biological threats with a risk of spread that cause serious human disease, but for which there is an effective treatment. The fourth group is biological threats that cause serious diseases in humans, with a risk of spread, but for which there is no effective treatment (8).

Research laboratories are classified into four different biosafety levels: basic laboratories with a biosafety level of 1 (BSL-1) and biosafety level of 2 (BSL-2), isolation laboratories with a biosafety level of 3 (BSL-3) and high containment laboratories with a biosafety level of 4 (BSL-4) (9). BSL-1 research laboratories are laboratories that can be run with substances or agents that have a minimal impact on the environment and individuals. BSL-2 are laboratories that can be operated with substances or agents of moderate impact. BSL-3 are laboratories that can be operated with substances or agents that may have a high impact. BSL-4 are those that can be run with substances or agents that may have a high-level impact and for which the route of transmission has not been fully determined (8). Anatomy laboratories are in the BSL-1 category.

Cadavers are the main teaching material in the anatomy laboratory. Infectious agents that may be found in cadavers during the dissection of cadavers for educational purposes are a serious source of contamination, especially for anatomists, students, and doctors. Many clinical conditions such as meningitis, phlebitis, peritonitis, pleuritis, and death have occurred due to this contamination. Prevention of cadaveric contamination has been the subject of research for many years and various procedures have been developed. However, the results of these studies are still inconclusive. For example, Burton Tabaac's study on cadaver fixation reported that bacterial growth occurred after fixation (10-12).

The aim of the present study is to review the state of the Karabük University, Faculty of Medicine Anatomy Laboratory in terms of microbiological contamination and to provide guidance to other laboratories on the precautions that can be taken.

## MATERIALS AND METHODS

The study was carried out in the practice laboratory of Karabuk University Faculty of Medicine, Department of Anatomy. The study was carried out with the approval of the non-interventional local ethics committee of Karabük University, dated 20.01.2022 and number 2022/785.

Cotton swabs were used to collect samples from 34 different areas, starting from the area closest to the laboratory door and including the furthest point, to determine the microbiological contamination in the anatomy laboratory. The samples were taken from various locations, including the head, upper and lower extremities of both male and female cadavers, the door handle, the floor in front of the door, the faucet, the head, body, and foot parts of the dissection table,

the dissection tool, the trailer, the inner and outer coating of the cadaver pool, the sink, the floor in front of the window, the stool, the living room wall, the formaldehyde liquid in the cadaver pool, the window handle, the instrument table, the morgue unit, the exterior surfaces of three different organ storage boxes, the inner surface of an organ storage box, the medical waste container, the handle of the organ storage cabinet, a training model, the lower surface of the dissection table, the medical waste storage box for dissection, and the blackboard (Figure 1).

The collected samples from the surfaces were also categorized into two groups: those directly associated with the cadaver and those that were not directly associated with the cadaver (Table 1).


**Figure 1.** Sample collection phase (a: Door handle, b: Faucet, c: Body of the dissection table, d: Outer coating of the cadaver table).

**Table 1.** Areas directly associated and not associated with the cadaver.

| Areas directly associated with the cadaver | Areas not directly associated with the cadaver |
|---|---|
| Head, body and foot parts of the dissection table | Door handle |
| Dissection tool | The floor in front of the door |
| Trailer | Faucet |
| Inner and outer coating of the cadaver pool | Window handle |
| Formaldehyde liquid in the cadaver pool | Handle of the organ storage cabinet |
| Head, upper and lower extremities of a male cadaver | A training model |
| Head, upper and lower extremities of a female cadaver | Sink |
| Instrument table | The floor in front of the window |
| Morgue unit | Stool |
| Exterior surface of the three different organ storage boxes | Living room wall |
| Inner surface of an organ storage box | Blackboard |
| Medical waste container | |
| The floor of the dissection table | |
| Medical waste storage box for dissection | |

The samples were transported to the microbiology laboratory at Karabük University Training and Research Hospital under cold chain conditions. Following the study protocol, the samples were incubated and bacterial identification was performed by a specialized microbiologist using BD Phoenix M50 (Canada).

A sterile standard transport medium was used to transport the collected samples to the laboratory. The samples were then inoculated onto blood agar, EMB, and chocolate agar. After inoculation, the samples were incubated for 24 hours at 35-37 °C in an incubator. The next day, bacteria grown on Petri plates were included in the identification process. Gram staining was performed by taking samples from bacterial colonies that fell into a single colony on the Petri dish. Bacterial identification was started according to the results. In our study, samples were taken from colonies with Gram-positive bacilli and added to Phoenix ID Broth. A range of 0.5-0.6 was accepted within McFarland. The bacteria were then introduced into the BD Phoenix M50 instrument and identified.

The BD Phoenix M50 device is a bacterial identification and susceptibility testing system with a detailed identification function. The device can identify bacteria and yeasts at the genus and species level with the chromogenic and fluorogenic substrates it contains.

## RESULTS

In the present study, *Bacillus subtilis* was found in 16 of the 34 spots sampled, while mold fungus was found in two and no contamination was found in the remaining 16 spots (Figure 2). *Bacillus subtilis* was found on the dissection tool, formaldehyde liquid in the cadaver pool, the head, upper and lower extremities of both male and female cadavers, the morgue unit, the exterior surfaces of two different organ storage boxes, a medical waste container, a training model, sink, floor in front of the window, stool, the medical waste storage box for dissection, and the blackboard. Mold fungus was found on the floor of the laboratory door and on the instrument table. In the study, only *Bacillus* subtyping was performed, no fungal subtyping was performed.

It was found that 11 of the detected *Bacillus subtilis* contaminations were in areas directly associated with the cadaver, while five were in areas not directly associated with the cadaver (Figure 3). The fact that the contaminated spots are directly associated

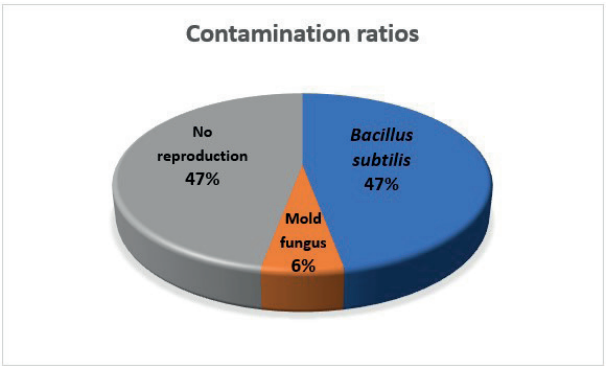


**Figure 2.** Contamination detected on the surfaces.

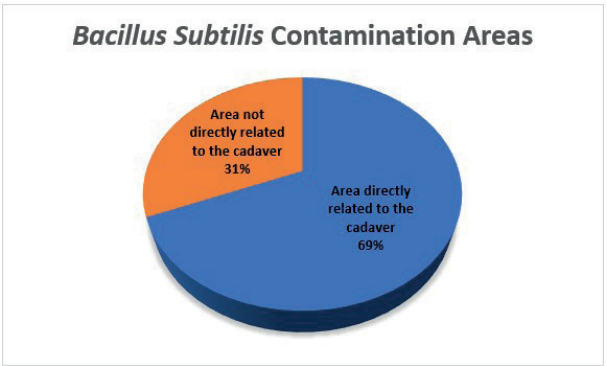


**Figure 3.** Bacillus subtilis contamination areas.

with the cadaver indicates that the contamination in the anatomy laboratory is related to the cadaver. Contamination was found in 69% of the 14 spots directly related to cadavers (the head, upper and lower extremities of cadavers, etc.). Further studies are planned to determine whether contamination in the anatomy laboratory is also observed among the personnel working in the laboratory.

Bacterial diseases have not been isolated in our laboratory so far. We attribute this to the strict precautionary procedures applied in the laboratory. Any disruption in these precautionary procedures may cause illness in laboratory staff and students.

## DISCUSSION

Samples were collected from 34 different areas in the study. While *Bacillus subtilis* was isolated from 16 (47%) of these, mold fungus was isolated from 2 (6%). It was found that 69% of the samples with *Bacillus subtilis* were from areas directly associated with cadavers.

Numerous investigations have been carried out on the contamination of research laboratories, as well as on the staff and students trained in these facilities, revealing significant findings. In a study examining 96 gowns belonging to medical faculty personnel for contamination, Koç et al. (13) found bacterial contamination in 25% of the gowns. It was determined that 62.5% of the contamination was caused by coagulase-negative staphylococci. Özkeser et al. (14), in a study assessing contamination levels in 20 research and student practice laboratories, found fungal and bacterial contamination in 30%. In a study investigating the contamination of mobile phones used by staff in intensive care units and operating rooms, Güldaş et al. (15) reported a contamination rate of 90.6%, with coagulase-negative staphylococci accounting for 57% of the contamination, and the presence of *Bacillus subtilis*. Alpay et al. (16) conducted a study examining the mobile phones of healthcare professionals and found contamination in 17.7% of 45 samples. In a study investigating the risk of microbial contamination in chemistry and microbiology laboratories, Farnsworth et al. (17) took 165 samples from laboratory surfaces and personnel and found contamination in 30% of them. In another study investigating viral contamination in a clinical microbiology laboratory, Wang et al. (18) found contamination in gloves, fume hoods, and clothing. Wurtz et al. (19) included 119 BSL-3 and BSL-4 laboratories in their contamination analysis study and found contamination in 23 of the laboratories. They found 15 different laboratory-acquired infectious agents in 4 of the 23 laboratories where contamination was detected. These studies show that there is always a risk of contamination in research laboratories. In our study, the anatomy laboratory in the BSL-1 category was used, and *Bacillus subtilis* was found in 16 of 34 spots and mold fungus in 2 of them. The fact that 11 of them are directly related to the cadaver in the areas where contamination is detected suggests that the cadaver is a major source of contamination. When we look at the literature, it is clear that laboratories and laboratory equipment used for different purposes in different categories are at risk of contamination. This can be true even for laboratories where some protective and sterilizing

substances are used. One of the best examples of this is the presence of contamination in cadaver pools and cadavers despite the use of formaldehyde.

Cadavers are the primary source of contamination in anatomical laboratories. Examination of historical accounts reveals instances in the literature where physicians, particularly anatomists who provide cadaver training, have succumbed to cadaver contamination. For instance, Anatomist Dr. Marie-Francois Xavier Bichat, renowned as the father of hematology, and the contamination-related deaths of students and anatomists in William Hewson's Department of Anatomy in Philadelphia serve as striking examples of this phenomenon. Many diseases such as Hepatitis B-C, HIV, prion diseases, Tuberculosis, and Creutzfeldt-Jakob disease have been reported in the literature due to cadaver contamination. The widespread use of cadaver embalming and fixation solutions and the more frequent application of appropriate procedures have reduced cadaveric contamination (11,20-22).

Biosafety procedures are indispensable factors in preventing this contamination. According to biosafety procedures, research laboratories should be cleaned at least once a day and appropriate disinfection or sterilization methods should be used in laboratories, the management scheme of research laboratories should be clear and management personnel should inspect both laboratories and personnel on a daily basis, warning signs should be displayed in areas or on substances susceptible to contamination and the personnel in research laboratories should be trained regularly. In addition to these, procedures should be established for the disposal or recycling of waste materials, ensuring a consistent supply of appropriate protective materials to meet the needs of research laboratories and implementing regular ventilation procedures for these laboratories. In addition, transmission routes in research laboratories should be determined and precautions should be taken, the physical infrastructure of research laboratories should be improved, and maximum safety conditions should be established (2,3,6-8,11,17).

The results of this study show the potential risk of contamination in anatomy laboratories and emphasize the utmost importance of following biosafety protocols. The risk of contamination is evident even if these procedures are suspended for a short period of time. In summary, the recommended precautions include regular training, consistent sterilization practices, and periodic audits of precautionary procedures. We believe that this study will contribute to the literature by providing a reminder of contamination risks and precautions in the anatomy laboratory.

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### Ethical approval

This study has been approved by the Ethics Committee of Karabük University (approval date 20.01.2022, number 2022/785). Written informed consent was obtained from the participants.

### Author contribution

Concept: YS, ŞT, HS, EB; Design: YS, ŞT, HS, EB; Data Collection or Processing: YS, ŞT, HS, EB; Analysis or Interpretation: YS, ŞT, HS, EB; Literature Search: YS, ŞT, HS, EB; Writing: YS, ŞT, HS, EB. All authors reviewed the results and approved the final version of the article.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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RESEARCH ARTICLE

# Evaluation of patient satisfaction following oral glucose tolerance test

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## ABSTRACT

**Aim:** Diabetes mellitus (DM) is a common metabolic disease. Early diagnosis of diabetes prevents the increase in mortality and morbidity due to complications. The oral glucose tolerance test (OGTT) is a test used in the diagnosis of DM and in the determination of impaired glucose tolerance (IGT). In our study, it was aimed to evaluate the satisfaction of patients who underwent OGTT.

**Methods:** A 25-question questionnaire was applied to 300 patients who underwent OGTT, aiming to evaluate their satisfaction. The physical and psychological status of the patients was examined before, during, and after the test.

**Results:** Patients who were informed about the test before the test experienced less nausea during fluid intake (p=0.005). Approximately 58.7% of the participants agreed to repeat the test. Those who felt nauseous or hungry during the test were statistically less likely to accept retesting. The retest acceptance rates were statistically higher (p<0.05) among individuals who did not feel uncomfortable with the blood draw and inactivity and who did not vomit or feel uneasy during the test. 70% of the participants answered 'yes' to the suggestion of using an alternative diagnostic method.

**Conclusion:** OGTT is the gold standard for the diagnosis of IGT and DM, despite tests such as HbA1c and fasting plasma glucose, which are more easily performed and practical. It would be beneficial to develop another method that is easy to apply, better tolerated by patients, easy to repeat, and can be standardized instead of OGTT.

**Keywords:** diabetes mellitus, oral glucose tolerance test, satisfaction

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## INTRODUCTION

According to the International Diabetes Federation, the prevalence of diabetes mellitus (DM) is projected to increase from 463 million in 2019 to an estimated 578 million by 2030 and 700 million by 2045 (1). Simultaneously, the prevalence of individuals with prediabetes is also expected to rise. This substantial increase in the number of prediabetic patients is evolving into a significant public health problem of the 21st century (2). Diabetes mellitus, a chronic disease characterized by irregular glycemic states, leads to both microvascular and macrovascular complications and is associated with high morbidity and mortality (3).

Prediabetes is a risk factor for progression to both diabetes and cardiovascular disease. The term 'prediabetes' is used to describe individuals whose glucose levels do not meet the criteria for diabetes, but who have abnormal carbohydrate metabolism. Prediabetes is typically characterized by impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) and/or the presence of an A1C level ranging between 5.7–6.4% (39–47 mmol/mol). IFG is defined as fasting plasma glucose (FPG), levels falling between 100 and 125 mg/dL, while IGT is indicated by 2-hour post-meal glucose (2-h PG) levels during the 75-gram OGTT ranging from 140 to 199 mg/dL (4). Studies have revealed that approximately 70% of individuals with IGT or IFG are at risk of developing type 2 diabetes, with 20-30% of them progressing to diabetes within 5-10 years (1). Prediabetes is often associated with obesity, particularly abdominal or visceral obesity, dyslipidemia characterized by high triglycerides and/or low HDL cholesterol, as well as hypertension (4).

Identifying IGT is crucial for implementing type 2 DM prevention strategies in high-risk individuals. The oral glucose tolerance test (OGTT) has been widely utilized in clinical settings to diagnose impaired glucose tolerance (IGT) and/or type 2 diabetes mellitus (5). In addition to the OGTT, diabetes diagnosis also involves fasting plasma glucose and A1C measurements (4). The prevalence of DM calculated according to plasma fasting glucose was found to be 40% lower than that calculated according to the oral glucose tolerance test. Measuring fasting plasma glucose level alone causes the person with 70-80% impaired glucose tolerance to be overlooked (6,7). Similarly, relying solely on A1C measurements results in overlooking more than half of the diabetes cases detected by OGTT. Despite its effectiveness, OGTT has limitations; it is challenging to repeat, time-consuming, laboratory-dependent, laborious, and difficult for patients to tolerate. Patients often report discomforts such as nausea and vomiting during the test (8-11). Nevertheless, due to its higher sensitivity and specificity compared to other methods, OGTT remains the most appropriate diagnostic tool for diabetes (5).

Our study aimed to assess the satisfaction of patients who underwent OGTT.

## MATERIAL AND METHOD

In our study, we included 300 patients who presented to the general internal medicine and endocrinology outpatient clinics at our hospital and were given OGTT indications. Exclusion criteria encompassed factors such as drug use, presence of infection, and prolonged sedentary lifestyle, which could potentially influence the test result before the OGTT. The study protocol adhered to the guidelines outlined by the American Diabetes Association (ADA) for the 75-g OGTT. Prior to the OGTT, patients were instructed to maintain a diet containing at least 150 grams of carbohydrates for a minimum of 3 days. Additionally, they were advised to consume an evening meal containing 30-50 grams of carbohydrates and to engage in regular physical activity during this period. At the beginning of the test, a catheter was inserted into the subjects' forearm vein to facilitate frequent blood sampling. Following a fasting period of at least 8 hours, a 7 ml blood sample was drawn through this catheter into a tube containing fluoride oxalate. Subsequently, participants were administered a 300 cc glucose solution containing 75 grams of anhydrous glucose. Blood samples were obtained from the patients again at the end of the second hour for glucose level measurement. Importantly, patients were instructed not to smoke or engage in physical activity before or during the test to ensure the accuracy and reliability of the results (4,12,13).

After the final blood sample was taken, satisfaction with the test was assessed using visual analog scales

(12,14). A questionnaire consisting of 25 questions in 4 parts was applied to the patients, and their physical and psychological conditions were assessed before, during, and after the test. The first part consisted of 4 questions describing the socio-demographic characteristics of the patients. In the second part of the questionnaire, there were 7 'yes/no' questions (if yes, mild-moderate-severe) evaluating the physical and psychological conditions of the patients before the test. Physical factors included nausea, vomiting, cold sweats, tremors in the hands, palpitations, and feeling faint, which may be related to hypoglycemia during the fasting period, the complaints they may experience, and compliance with the appointment time. The psychological factors section provides information about the anxiety experienced before the test, the situations that may be experienced by the patient before the test, and the questioning of the discomfort, and degree of hunger that may be caused by the test. The third part of the questionnaire consists of yes/no questions that evaluate the physical and psychological state during the test. The physical factors included difficulty in drinking the given liquid, experiencing nausea and vomiting during drinking, immobilization during the test, and discomfort from two blood draws. The psychological factors included the feeling of distress experienced during the test or the ease of access to health personnel in case of any distress encountered, and the analytical approach of the personnel. The fourth part aimed to ask about post-test information, confirmation of acceptance in case of re-testing, and a request for another diagnostic method.

The average time required to complete the questionnaire was 30 minutes. Illiterate participants were provided with assistance from their relatives and filled out the questionnaire under the supervision of nurses. This support was explicitly mentioned at the bottom of the questionnaire to ensure transparency and accuracy in the data collection process.

### Statistical analysis

The data were presented as numerical values, percentages, means, and standard deviations. Statistical analysis was performed using the SPSS 18 computer statistics program. The questionnaire data were analyzed using the chi-square test. A p-value of less than 0.05 was considered significant, indicating a statistically significant difference between the groups.

## RESULTS

This study analyzed data from 300 patients who underwent OGTT. Table 1 presents the demographic characteristics of the patients. The analysis revealed no statistically significant differences between pre-test fasting times and complaints such as nausea, vomiting, sweating, and palpitation ($p>0.05$). Patients with symptoms did not experience hypoglycemia. The impact of pre-test information on the complaints experienced during the test is analyzed in Table 2. According to the chi-square analysis, patients who were informed before the test reported significantly lower complaints of nausea ($p=0.005$). The variables and their influence on the acceptance of a repeat OGTT are shown in Table 3. Approximately 58.7% of the participants agreed to repeat the test. The statistical analysis revealed that individuals who accepted the test-retest reported higher levels of staff courtesy and ease of access. Additionally, acceptance of repeating the test was significantly higher among individuals who did not feel discomfort during the blood draw, inactivity, or hunger, those who did not experience vomiting, and those who did not feel uneasy ($p<0.05$). Patients who experienced nausea and discomfort due to hunger were statistically less likely to accept the test

**Table 1.** Demographic characteristics.

| | **Variables** |
|---|---|
| **Gender** | |
| Woman (n) | 142 (%58.6) |
| Man (n) | 176 (%41.4) |
| **Age** | |
| Average (years) | 44.5 ±12.6 |
| Median | 47 |
| **Education status** | 10 (%3.3) |
| Illiterate (n) Primary school (n) | 83 (%27.7) |
| Middle school (n) | 54 (%18) |
| High school (n) | 94 (%31.3) |
| University (n) | 59 (%19.7) |

**Table 2.** Pre-test information and the complaints encountered during the test.

| | Information | | p |
|---|---|---|---|
| | Yes n (%) | No n (%) | |
| Uneasiness | | | |
| No (n) | 124 (57.6) | 39 (45.8) | 0.262 |
| Yes (n) | 91 (42.3) | 46 (54.1) | |
| Discomfort from the feeling of hunger | | | |
| No (n) | 97 (45.2) | 35 (41.1) | 0.536 |
| Yes (n) | 118 (54.8) | 50 (58.8) | |
| Difficulty drinking liquid | | | |
| No (n) | 79 (36.7) | 29 (34.1) | 0.669 |
| Yes (n) | 136 (63.2) | 56 (65.8) | |
| Nause during the test | | | |
| No (n) | 120 (55.8) | 32 (37.6) | 0.005 |
| Yes (n) | 95 (44.1) | 53 (62.3) | |
| Vomiting during the test | | | |
| No (n) | 176 (81.8) | 70 (82.3) | 0.920 |
| Yes (n) | 39 (18.2) | 15 (17.6) | |
| Feeling of boredom | | | |
| No (n) | 86 (40) | 27 (31.8) | 0.185 |
| Yes (n) | 129 (60) | 58 (68.2) | |
| Discomfort due to the blood draw | | | |
| No (n) | 164 (76.3) | 63 (74.2) | 0.694 |
| Yes (n) | 51(23.7) | 22 (25.8) | |

again. The impact of these variables on the request for an alternative diagnostic method is shown in Table 4. 70% of the participants answered "yes" to the request for another method. Patients who had difficulty drinking fluids, felt nauseated during the test, and were uncomfortable with inactivity were statistically more likely to request another method. Conversely, among those who did not seek an alternative method, there were statistically more individuals who did not feel discomfort during blood draws and did not vomit during the test ($p<0.05$). Additionally, a statistically significant difference was found when examining the relationship between the desire for an alternative method and educational status (Table 5), with a p-value of less than 0.05.

**Table 3.** Accepting the OGTT repetition and the variables.

| Variables | Accepting a repeat test | | p |
|---|---|---|---|
| | Yes n (%) | No n (%) | |
| Gender | | | |
| Woman (n) | 87 (54.1) | 74 (45.9) | |
| Man (n) | 89 (64) | 50 (36) | |
| Access to staff | | | |
| Yes (n) | 150 (93.2) | 115 (83.5) | 0.013 |
| No (n) | 11 (6.8) | 23 (16.5) | |
| Discomfort due to the blood draw | | | |
| Yes (n) | 20 (12.5) | 53 (38.2) | 0.00 |
| No (n) | 141 (87.5) | 86 (61.8) | |
| Difficulty drinking liquid | | | |
| Yes (n) | 152 (94) | 114 (82) | 0.001 |
| No (n) | 9 (6) | 25 (18) | |
| Nausea during the test | | | |
| Yes (n) | 65 (40.3) | 83 (59.8) | 0.00 |
| No (n) | 96 (59.7) | 56 (40.2) | |
| Vomiting during the test | | | |
| Yes (n) | 19 (11.8) | 35 (25.2) | 0.014 |
| No (n) | 142 (88.2) | 104 (74.8) | |
| Discomfort from the feeling of hunger | | | |
| Yes (n) | 78 (48.4) | 90 (64.7) | 0.01 |
| No (n) | 83 (51.6) | 49 (35.3) | |
| Uneasiness | | | |
| Yes (n) | 54 (33.5) | 83 (59.7) | 0.00 |
| No (n) | 107 (66.5) | 56 (40.3) | |
| Discomfort due to inactivity | | | |
| Yes (n) | 68 (42.3) | 86 (61.8) | 0.00 |
| No (n) | 93 (57.7) | 53 (38.1) | |
| Information | | | |
| Yes (n) | 142 (88.2) | 118 (84.9) | 0.501 |
| No (n) | 19 (11.8) | 21 (15.1) | |
| Staff courtesy | | | |
| Yes (n) | 156 (96.9) | 125 (90) | 0.013 |
| No (n) | 5 (3.1) | 14 (10) | |

**Table 4.** Request for another diagnostic method and the variables.

| Variables | Request for Another Method | | p |
|---|---|---|---|
| | Yes n (%) | No n (%) | |
| Gender | | | |
| Woman (n) | 126 (60) | 50 (55.6) | |
| Man (n) | 84 (40) | 40 (44.4) | |
| Access to staff | | | |
| Yes (n) | 187 (89.6) | 78 (86.7) | 0.630 |
| No (n) | 22 (10.4) | 12 (13.3) | |
| Discomfort due to the blood draw | | | |
| Yes (n) | 59 (28.1) | 14 (15.6) | 0.017 |
| No (n) | 151 (71.9) | 76 (84.4) | |
| Difficulty drinking liquid | | | |
| Yes (n) | 180 (85.5) | 86 (95.6) | 0.014 |
| No (n) | 30 (14.2) | 4 (4.4) | |
| Nausea during the test | | | |
| Yes (n) | 115 (71.4) | 33 (23.7) | 0.004 |
| No (n) | 95 (28.6) | 57 (76.3) | |
| Vomiting during the test | | | |
| Yes (n) | 19 (9) | 35 (38.8) | 0.003 |
| No (n) | 142 (91) | 104 (61.2) | |
| Uneasiness | | | |
| Yes (n) | 102 (48.5) | 35 (38.8) | 0.123 |
| No (n) | 108 (51.5) | 55 (61.2) | |
| Discomfort due to inactivity | | | |
| Yes (n) | 126 (60) | 28 (31.2) | 0.00 |
| No (n) | 84 (40) | 62 (68.8) | |
| Information | | | |
| Yes (n) | 147 (60.9) | 68 (84.2) | 0.328 |
| No (n) | 63 (39.1) | 22 (15.8) | |

**Table 5.** Requesting for another diagnostic method depending on the education level.

| | Other method requests | | p |
|---|---|---|---|
| | Yes n (%) | No n (%) | |
| Primary school | 45 (21.4) | 38 (42.2) | p<0.036 |
| Middle school | 42 (20) | 12 (13.3) | |
| High school | 71 (33.8) | 23 (25.5) | |
| University | 46 (21.9) | 13 (14.4) | |
| Illiterate | 6 (2.8) | 4 (1.9) | |

## DISCUSSION

The 75-g OGTT is widely recognized as the "gold standard" diagnostic test for diabetes and prediabetes (15). Previous studies have highlighted the limitations encountered during OGTT administration. To the best of our knowledge, our study is the first to assess not only the physical side effects but also the psychological impact of the OGTT on patients. It explores how these effects influence patients' decisions regarding retesting or recommendations for new diagnostic tests when necessary.

In our study, nausea was reported by 42% of the patients before the OGTT. Interestingly, no correlation was found between the severity of nausea and the duration of fasting. This observation suggests that despite the extension of the fasting period, there might not be a significant change in the intensity of nausea due to the sensitivity of the gastrointestinal system. Nausea may discourage individuals from initiating the test. Additionally, 25% of the patients experienced sweating and palpitations before the test. However, these symptoms were not associated with hypoglycemia. It was hypothesized that the test procedure itself, along with the anxiety and anticipation of the test results, might lead to increased sympathetic activity, potentially causing these symptoms.

Tolerating the fluid consumed during the OGTT can prove to be challenging. Among the participants, 49.3% experienced nausea, and 18% reported vomiting during liquid intake. Another study involving 36 participants found that drinking 75 grams of OGTT liquid led to gastric discomfort in 14%, belching in 19%, hunger in 24%, and nausea in 6% of the participants

(14). Similarly, in a study conducted by Harano et al., in which a 75-gram OGTT was administered to 19 patients, mild hypoglycemia was observed in 5 individuals, and discomfort such as nausea, vomiting, and heartburn was reported by 4 patients (16). These findings underscore the challenges associated with the tolerance for the liquid consumed during OGTT. The higher incidence of nausea in our study compared to similar studies in the literature can be attributed to our larger sample size of 300 participants, which significantly surpasses the numbers in other research. The fact that nearly half of our patients experienced nausea raises concerns about the OGTT, a commonly used diagnostic test that could adversely affect a significant portion of the population in larger studies. When accompanied by vomiting, it could lead to misinterpretation of the test results. Notably, 31.7 % of patients who were informed about the challenging nature of the liquid beforehand still experienced nausea. Despite psychological support and sufficient information, the taste of the liquid proved to be intense and unpleasant, making it difficult to tolerate and causing complaints among patients. This highlights the importance of reevaluating the methods and solutions used during OGTT to enhance patient comfort and minimize adverse effects.

Numerous studies have explored the possibility of using alternative fluids during OGTT, aiming to find a more tolerable option for patients than the standard 75-gram glucose solution. For instance, Harano et al. conducted a study involving 83 participants, where OGTT was performed using a standardized meal stimulation test. Interestingly, they found no metabolic differences in the results compared to the traditional 75-gram glucose test, and the patients found the alternative test more tolerable (16). In another study, the OGTT was performed using a standardized meal test containing 50 grams of glucose. The discomfort reported in this test was minimal, with stomach discomfort at 6%, belching at 7%, hunger at 14%, and no instances of nausea. Moreover, the 2nd-hour blood glucose results were comparable to those obtained from the standard 75-gram OGTT13. In a study involving 232 participants, OGTT was carried out using glucose and maltose solutions. The nausea rates were 2.3% for 50 grams of maltose, 4.2% for 100 grams of maltose, and 21% for 100 grams of glucose load (17). These studies suggest that using alternative substances or modifying the OGTT procedure might reduce the discomfort experienced by patients, making the test more tolerable while maintaining the accuracy of the diagnostic results.

Several studies have investigated different methods to improve the tolerability and acceptability of OGTT. In a study with 35 participants, the OGTT solution was diluted to 300 cc, 600 cc, and 900 cc. Surprisingly, the 600 cc solution was found to have the best taste and acceptability scores, while the 900 cc solution resulted in the fewest side effects (12). A recent study involving 399 pregnant women experimented with various glucose solutions for the 75g OGTT. Cold glucose solution and any-temperature glucose solution containing a tea bag resulted in slightly higher taste scores and lower degrees of nausea compared to the room-temperature water-based glucose solution (18). Another study with 30 participants tested a novel lemon-lime flavored beverage was tested during the OGTT. This alternative solution yielded similar biochemical results to the traditional OGTT, but significantly increased taste satisfaction and compliance rates among the participants (19). These studies demonstrate the ongoing efforts to enhance the OGTT experience for patients. Exploring different solutions, temperatures, and flavors not only improves patient comfort, but also ensures accurate test results, enhancing the overall effectiveness and acceptability of OGTT in various populations.

The provision of pre-test information did not alleviate the discomfort experienced by patients due to the double blood draw and inactivity during the test. Surprisingly, there is a lack of data in the existing literature concerning inactivity, which was a significant concern for half of the patients in our study. The high rates of easy access to personnel and their courteous approach might have contributed to the patients feeling safer. This sense of security may prevent glucose increases triggered by stress and anxiety. In our study, the primary metrics assessing patient satisfaction were their willingness to undergo the test again and their inclination toward alternative diagnostic methods. These aspects are pivotal in understanding the patient experience and can inform future improvements in the OGTT procedure to enhance patient comfort and

overall satisfaction. A significant correlation was observed between the decision to repeat the test and several factors, including feelings of hunger before the test, the occurrence of nausea and vomiting during the test, the accessibility and politeness of healthcare personnel, inactivity during the test, and discomfort or uneasiness related to the blood draws. Patients who experienced vomiting might fear a recurrence of the same or even more severe problems if the test were to be repeated. Remarkably, 70% of the participants expressed a preference for an alternative diagnostic method other than OGTT. Among those who wanted another method, 85.7% had difficulty drinking the liquid, indicating a substantial impact on their preference. Other factors influencing the demand for an alternative method included nausea, vomiting, discomfort of remaining still during the test, and discomfort associated with the blood draws. Interestingly, even positive interactions with healthcare professionals did not fully alleviate dissatisfaction stemming from OGTT. Furthermore, the increase in the educational level of the patients appeared to be associated with a preference for alternative diagnostic methods. This increased awareness might lead them to question whether there are other methods that are easier to tolerate, emphasizing the need for more patient-friendly testing approaches.

In clinical practice, A1c testing is often favored over OGTT to diagnose diabetes. A1c testing offers several advantages, such as not requiring fasting, the ingestion of a glucose solution, or prolonged waiting times for blood to be drawn. Additionally, A1c levels remain stable under stress and can be used to monitor individuals undergoing antihyperglycemic treatment. However, it's important to note that A1c testing can be relatively more expensive than other diagnostic tests, and its standardization is still an area of concern within the medical community (4).

## CONCLUSION

Despite the availability of alternative tests such as HbA1c and fasting plasma glucose, the 75g OGTT remains the gold standard for the diagnosis of impaired glucose tolerance (IGT) and diabetes mellitus (DM). However, the findings of our study suggest the need for the development of a new diagnostic method. Ideally, this method should be cost-effective, easy to administer, repeatable, well-tolerated by patients, and standardized. Such an approach could significantly improve the diagnostic experience for patients while ensuring accurate results, marking a potential breakthrough in diabetes diagnosis.

### Ethical approval

This study has been approved by the Atatürk University Faculty of Medicine Ethics Committee (approval date 15/02/2011). Written informed consent was obtained from the participants.

### Author contribution

Concept: PG, HB; Design: PG, HB, EMA; Data Collection or Processing: PG, ÇÖ, VG; Analysis or Interpretation: PG, EMA, GK; Literature Search: PG, VG, GK; Writing: PG, HB, GK. All authors reviewed the results and approved the final version of the article.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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