DÜZCE ÜNİVERSİTESİ

KONURALP TIP DERGİSİ

MART/**MARCH** 2025

CİLT/**VOL**: 17 SAYI/**ISSUE**: 1

KONURALP MEDICAL JOURNAL

ISSN: 1309-3878

Emerging Sources Citation Index **Web of Science Core Collection** Tübitak Ulakbim Türk Tıp Dizini **ERIH PLUS** CAS Source Index (CASSI) **EMBASE,** BASE - Bielefeld Academic Search Engine **HINARI®WHO,** CAB Abstracts, **Abstracts on Hygiene and Communicable Diseases,** Nutrition Abstracts and Reviews Series A: Human and Experimental, **Protozoological Abstracts,** Sugar Industry Abstracts, **Review of Medical and Veterinary Mycology,** Review of Medical and Veterinary Entomology, **Tropical Diseases Bulletin,** CINAHL, **Index Copernicus,** DOAJ, **Socol@r,** JournalTOCs, **TurkMedline,** Türkiye Atıf Dizini

KONURALP MEDICAL JOURNAL

Vol. 17, Issue 1, 2025 / Cilt 17, Sayı:1, 2025 e-ISSN: 1309-3878

Bilimsel Kurul / Scientific Committee

Organization, Preperation and Correspondence / Organizasyon, Hazırlık ve Yazışma Adresi

- Düzce Üniversitesi, Tıp Fakültesi, Aile Hekimliği AD, Konuralp/ Düzce, Türkiye- Düzce University, Faculty of Medicine, Department of Family Medicine Konuralp/Düzce, TÜRKİYE

Date of Issue / Yayın Tarihi: 27.03.2025

- **Konuralp Medical Journal** is an international peer-reviewed journal and is published three times a year. The responsibility of the articles published belongs to the authors.

- Dergimiz uluslararası hakemli bir dergi olup dört ayda bir yayımlanmaktadır. Yayımlanan makalelerin sorumluluğu yazarlara aittir.

- **Konuralp Medical Journal** is indexed / abstracted in Emerging Sources Citation Index, Web of Science Core Collection, Tübitak Ulakbim Türk Tıp Dizini, ERIH PLUSCAS, Source Index (CASSI), EMBASE, BASE - Bielefeld Academic Search Engine, HINARI®WHO, CAB Abstracts, Abstracts on Hygiene and Communicable Diseases, Nutrition Abstracts and Reviews Series A: Human and Experimental, Protozoological Abstracts, Sugar Industry Abstracts, Review of Medical and Veterinary Mycology, Review of Medical and Veterinary Entomology, Tropical Diseases Bulletin, CINAHL, Index Copernicus, DOAJ, Socol@r, JournalTOCs, TurkMedline, Türkiye Atıf Dizini.
- **Konuralp Tıp Dergisi** Emerging Sources Citation Index, Web of Science Core Collection, Tübitak Ulakbim Türk Tıp Dizini, ERIH PLUSCAS, Source Index (CASSI), EMBASE, BASE - Bielefeld Academic Search Engine, HINARI®WHO, CAB Abstracts, Abstracts on Hygiene and Communicable Diseases, Nutrition Abstracts and Reviews Series A: Human and Experimental, Protozoological Abstracts, Sugar Industry Abstracts, Review of Medical and Veterinary Mycology, Review of Medical and Veterinary Entomology, Tropical Diseases Bulletin, CINAHL, Index Copernicus, DOAJ, Socol@r, JournalTOCs, TurkMedline, Türkiye Atıf Dizini tarafından endekslenmektedir.

Contents / İçindekiler

Research Articles / Araştırma Makaleleri

RESEARCH ARTICLE

Merve Pozlu Cifci[1]
Ozden Gokdemir[2]
Olgu Aygün[3]
Ismail Cifci[4]
A. Dilek Guldal[5]

[1] Ege University Faculty of Medicine, Izmir, Türkiye
[2] Izmir University of Economics, Faculty of Medicine, Department of Family Medicine, Izmir, Türkiye
[3] İzmir Provıncıal Dırectorate of Health-Bozyaka Educatıon and Research Hospıtal Department of Family Medicine, Izmir, Türkiye
[4] Esenyalı Family Health Center, İzmir, Türkiye
[5] Dokuz Eylül University, Department of Family Medicine, Izmir, Türkiye

Corresponding Author:
Ozden Gokdemir
mail: gokdemirozden@gmail.com

Received: 03.10.2023
Acceptance: 29.11.2024
DOI: 10.18521/ktd.1370882

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

The Frequency of Gastroesophageal Reflux Disease (GERD) in Migraineurs and the Impact of GERD Treatment on Migraine Attacks

ABSTRACT

Objective: To determine the frequency of gastroesophageal reflux disease (GERD) in migraine patients and investigate the impact of adherence to gastroesophageal reflux treatment on migraine attack frequency.

Method: A total of 757 people aged 18-45 years who applied to the Dokuz Eylul University (DEU) Neurology-Headache Clinic and the DEU Family Health Centers and met the inclusion criteria were included in the study. Exclusion criteria were pregnancy, cancer or alarming findings, and any mental disability that would prevent understanding of the questionnaire. The Migraine Diagnostic Questionnaire, including International Headache Society (IHS) criteria, was used to diagnose migraine, the Gastroesophageal Reflux Disease Questionnaire (GERDQ) to diagnose GERD, and the Modified Morisky Scale to assess medication compliance. SPSS 22 package was used for statistical analysis. Chi-square, t-test and ANOVA were used for statistical evaluation of the data, and $p<0.05$ was considered significant.

Results: Of the 757 people who participated in the study, 428 patients were diagnosed with migraine and followed up in the neurology outpatient clinic. Of the 329 patients who applied for family health centers, 122 were diagnosed with migraine and 108 with non-migraine headaches. 99 did not complain of headache. When the GERDQ scores of the patients participating in the study were evaluated, there were 183 people (33.3%) with a GERDQ score of 8 and above among the migraineurs, 19 people (17.6%) with non-migraine headaches and 8 people (8.1%) without headaches. The GERDQ reflux score of migraineurs was found to be higher than in the other groups and this level was statistically significant ($p<0.001$). When the migraine attack frequency of migraine patients treated for GERD was evaluated, no significant relationship was found between treatment adherence and migraine attack frequency.

Conclusions: The incidence of GERD in patients with migraine was found to be higher than in patients without migraine, and the compliance of these patients with treatment was found to be lower. It may be useful to ask patients with migraine about GERD when they come to the outpatient clinic and to support their motivation for treatment.

Keywords: GERD, Migraine, Medication Adherence.

Migren Hastalarında Gastroözofageal Reflü Hastalığı (GÖRH) Sıklığı ve GÖRH Tedavisinin Migren Atakları Üzerindeki Etkisi

ÖZET

Amaç: Migren hastalarında gastroözofageal reflü hastalığı (GÖRH) sıklığını belirlemek ve GÖRH tedavisine uyumun migren atak sıklığı üzerindeki etkisini araştırmak.

Yöntem: Dokuz Eylül Üniversitesi (DEÜ) Nöroloji-Baş Ağrısı Kliniği ve DEÜ Aile Sağlığı Merkezlerine başvuran ve dahil etme kriterlerini karşılayan 18-45 yaş arası toplam 757 kişi çalışmaya dahil edildi. Dışlama kriterleri gebelik, kanser, alarm bulguları ve anketin anlaşılmasını engelleyecek herhangi bir zihinsel engeldi. Migreni teşhis etmek için Uluslararası Baş Ağrısı Derneği (IHS) kriterlerini içeren Migren Tanı Anketi, GÖRH' yi teşhis etmek için Gastroözofageal Reflü Hastalığı Anketi (GERDQ) ve ilaç uyumunu değerlendirmek için Modifiye Morisky Ölçeği kullanıldı. İstatistiksel analiz için SPSS 22 paketi kullanıldı. Verilerin istatistiksel değerlendirilmesinde ki-kare, t-testi ve ANOVA kullanıldı ve $p<0,05$ anlamlı kabul edildi.

Bulgular: Çalışmaya katılan 757 kişiden 428'i migren tanısı almış ve nöroloji polikliniğinde takip ediliyordu. Aile sağlığı merkezlerine başvuran 329 hastadan 122'si migren tanısı almış ve 108'i migren dışı baş ağrısı tanısı almıştı. 99'unun baş ağrısı şikâyeti yoktu. Çalışmaya katılan hastaların GERDQ skorları değerlendirildiğinde migren tanısı olanlarda GERDQ skoru 8 ve üzeri olan 183 kişi (%33,3), migren dışı baş ağrısı olan 19 kişi (%17,6) ve baş ağrısı olmayan 8 kişi (%8,1) vardı. Migren tanısı olanlarda GERDQ reflü skorunun diğer gruplara göre yüksek olduğu ve bu düzeyin istatistiksel olarak anlamlı olduğu bulundu ($p<0,001$). GÖRH tedavisi alan migren hastalarında tedaviye uyumun migren atak sıklığı ile anlamlı bir ilişkisinin olmadığı görüldü.

Sonuç: Migren tanısı olan hastalarda GÖRH insidansı migreni olmayan hastalara göre daha yüksek bulunmuş ve bu hastaların tedaviye uyumunun daha düşük olduğu görülmüştür. Migrenli hastalara poliklinik başvurularında GÖRH varlığı araştırılması ve tedavi motivasyonlarının desteklenmesi faydalı olabilir.

Anahtar Kelimeler: GÖRH, Migren, İlaç Uyumu.

INTRODUCTION

Migraine is described as a neurological disorder characterized by hyperexcitability of brain networks, sometimes due to metabolic imbalances (like magnesium deficiency) or genetic predispositions. It often involves central and peripheral nervous system dysfunction, and its comorbidities can include various metabolic, cardiovascular, and gastrointestinal disorders, such as GERD. GERD is a condition where stomach acid frequently flows back into the esophagus, causing irritation. It can be exacerbated by increased nitric oxide levels, which may also play a role in migraine pathophysiology by relaxing the lower esophageal sphincter, making reflux more likely. When examining the pathophysiology of both migraine and gastroesophageal reflux disease, some studies have found that migraines in patients are associated with brain hyperexcitability (due to from magnesium deficiency) and ion channel abnormalities due to chromosome 18 abnormalities. In addition, some studies have reported hypomagnesemia following the use of proton pump inhibitors in patients with gastroesophageal reflux patients. Plasma nitrate, nitrite, and total nitrite levels have been found to be high in both periods (interictal and attack period) in both aura-free and aura-present migraineurs. Nitric oxide (NO) at low concentrations is effective in maintaining the mucosal integrity of the stomach. Increased NO levels relax the lower esophageal sphincter. Impairment of the lower esophageal integrity also plays a role in the pathogenesis of reflux (1-5).

Although both of these conditions are frequently encountered in clinical practice, there is limited research in the literature on this topic. One study compared migraine patients, tension-type headache patients, and a control group to evaluate the incidence of gastroesophageal reflux disease, finding a significantly higher incidence of gastroesophageal reflux in migraine patients (6-9).,

The aim of this research is to determine the incidence of gastroesophageal reflux disease in migraineurs and to investigate the effect of adherence to gastroesophageal reflux treatment on migraine attack frequency.

MATERIAL AND METHODS

The study was designed as cross-sectional descriptive research. The necessary permissions were obtained from the Dokuz Eylul University Ethics Committee. It was conducted among individuals aged 18-45 years who presented to the Department of Family Medicine, the Department of Neurology Headache Clinic, and the Family Health Centre.

Individuals aged between 18 and 45 were included in the study, as the most common age range for migraine is reported to be 30 to 50 years in international studies and 30 to 39 years in Turkey.

The sample size was calculated to be minimum 632, with a minimum of 316 people in each group, assuming a 10% difference between the two groups, a 5% margin of error, 80% power, and a 95% confidence level.

The study was completed with a total of 757 individuals who applied to the Headache Clinic and Family Health Centers and who met the inclusion criteria, which were defined as being between 18 and 45 years of age, willing to participate in the study, and having no physical or mental problems that would interfere with understanding and answering the questionnaire. Exclusion criteria were pregnancy, dysphagia, odynophagia, iron deficiency anemia, weight loss, hematemesis or any condition that would make it difficult to understand and respond to the questionnaire.

Participants were verbally informed about the study before enrolment. The researcher then took anthropometric measurements (height and weight) and, using a face-to-face interview technique, completed a sociodemographic questionnaire (including age, education, alcohol consumption, smoking habits, and exercise status) and a health-related information form.

Participants completed the Migraine Diagnosis Questionnaire, which includes the criteria of the International Headache Society (IHS) criteria for migraines, the GERDQ questionnaire, and the Modified Morisky Scale for medication adherence.

Data Collection Tools

Sociodemographic questionnaire: The researcher prepared a questionnaire that included the following information about the participant: age, sex, weight, height, education level, exercise status in the past six months, and questions about smoking and alcohol consumption.

Migraine diagnosis questionnaire: A 15-item questionnaire was administered based on the criteria for migraine diagnosis according to the International Classification of Headache Disorders (ICHD-III) by the International Headache Society. The questionnaire items cover a range of topics including the age of onset, frequency, duration, headache characteristics, location, intensity, triggers, and associated symptoms (10).

GERDQ: Gastroesophageal Reflux Disease Questionnaire: The GERDQ is a patient-centered self-administered questionnaire used for the diagnosis and management of gastroesophageal reflux disease (GERD). It aims to differentiate between patients with frequent and occasional symptoms, and to guide treatment accordingly. It also allows the effects of treatment on patients' symptoms and daily life to be monitored.

The GERDQ questionnaire scores the frequency of six items experienced in the past seven days (heartburn, regurgitation, dyspepsia, nausea, need for non-prescription medication, and sleep

disturbance). Using a four-point scale ranging from zero to three, zero indicates zero days in a week, one indicates one day in a week, two indicates two days in a week, and three indicates 4-7 days in a week. Two items of the GERDQ questionnaire assess the impact of symptoms on the patient's daily life (need for non-prescription medication and sleep disturbance). The remaining four items (heartburn, regurgitation, need for non-prescription medication, and sleep disturbance) are used to monitor and evaluate the response to treatment.

During the administration of the questionnaire, a score of 2 or 3 on any item indicates the need to reassess the treatment (11). In the GERDQ questionnaire, complaints experienced in the past week are assessed, and scores are assigned for each item, which are then summed to obtain a total score [0-18]. Additionally, the sum of scores from the 5th and 6th questions provides an impact score [0-6]. An impact score of 3 or higher indicates a significant impact of reflux. The validity and reliability studies for the GERDQ were conducted by Hançerlioğlu and Bor, and it is recognized as a practical tool for assessing reflux symptoms in the Turkish population (12).

The Modified Morisky Medication Adherence Scale: The Modified Morisky Medication Adherence Scale is a 6-item scale with response options of "yes" and "no." In the participant's responses, in the 2nd and 5th questions, a score of 1 is assigned for "yes", and 0 for "no." In the 1st, 3rd, 4th, and 6th questions, a score of 0 is given for "yes," and 1 for "no." The total score obtained from the 1st, 2nd, and 6th questions indicates low motivation if 0 or 1, and high motivation if >1. The total score obtained from the 3rd, 4th, and 5th questions indicates low knowledge if 0 or 1, and high knowledge if >1.

The Turkish validity and reliability of the Modified Morisky Medication Adherence Scale were conducted by Bekir Vural and colleagues (13,14).

RESULTS

A total of 757 people were included in the study, consisting of whom 481 (63.5%) were women and 276 (36.5%) were men. The age range of the participants was between 18 and 45 years, with a mean age of 32.67 (std=6.61) for females and 32.48 (std=6.22) for males. Regarding the participants' educational status of the participants, 33.8% had completed high school education, while 29.2% had completed university or higher education (Table 1).

Regarding health care utilization, after being diagnosed with migraine, 43.5% of the patients went to the family health center (FHC) and 56.5% to the headache clinic (Table 1).

Among our participants: 35.7% smoked and 22.5% consumed alcohol, while 31.3% reported regular exercise in the previous six months. The mean body mass index (BMI) was 24.96 kg/m2.

Table 1. Demographic characteristics of the participants

Demographic characteristics of the participants	**Total (n=757)**	
	Number (n)	Percentage (%)
Age Groups		
18-20	23	3.0
21-25	93	12.3
26-30	158	20.9
31-35	227	30.0
36-40	161	21.3
41-45	95	12.5
Sex		
Women	481	63.5
Men	276	36.5
Education Status		
Illiterate	7	0.9
Literate	20	2.6
Primary School	89	11.8
Secondary School	164	21.7
High School	256	33.8
University and higher	221	29.2
Application places of patients		
Family Health Center	329	43.5
Headache treatment clinic	428	56.5

50.6% of individuals were classified as underweight or normal weight, while 9.6% were classified as obese or morbidly obese. Of the 757 participants, 193 (29.3%) reported having a good/regular sleep pattern and 124 (18.8%) reported early morning awakening/tiredness (Table 2).

Table 2. Typical behaviors of the participants

Typical behaviors of the participants	**Total (n=757)**	
	Number (n)	%
Tobacco usage		
Yes	270	35.7
No	487	64.3
Alcohol usage		
Yes	170	22.5
No	587	77.5
Exercise		
Yes	237	31.3
No	520	68.7
Classification according to body mass index		
Underweight and Normal Weight	383	50.6
Overweight	301	39.8
Obese and Morbid Obese	73	9.6
Sleep behaviors		
Good/regular	193	29.3
Insomnia/difficulty falling asleep	105	16.0
Frequent waking / difficulty falling back asleep	114	17.3
Early morning waking / exhaustion after waking	124	18.8
Difficulty waking up in the morning/ excessive sleepiness	122	18.5

The most commonly reported chronic conditions were as follows 4.8% had hypothyroidism, 3% had hypertension, 2.6% had diabetes mellitus, 2.5% had asthma and 0.9% had allergies (Table 3).

Table 3. The chronic disease status of the participants

The chronic disease status of the participants	**Total (n=757)**	
	Number (n)	%
Chronic diseases		
+	157	20.7
-	600	79.3
Top 5 Chronic Diseases		
Hypothyroid	37	4.8
Hypertension	23	3.0
Diabetes Mellitus	20	2.6
Asthma	19	2.5
Allergy	7	0.9

Of the 757 participants in the study, 428 were diagnosed with migraine and were patients in the neurological headache clinic. Of the patients attending the FHC, 122 were diagnosed with migraine and 108 with non-migraine headaches. 99 had no headache complaints. Of the 658 respondents with headache complaints, 404 (61.4%) had sought medical advice. The most common diagnoses were migraine in 274 (41.6%) and tension-type headache in 27 (4.1%) (Table 4).

When asked about the frequency of headache episodes, 193 people (29.3%) reported having headaches once a month, 192 (29.2%) 1-3 days a week and 183 (27.8%) every other week (Table 4).

Regarding the type of headache onset, 212 (32.2%) reported sudden onset and 446 (67.8%) reported gradual onset and progression. Regarding the characteristics of the headache, 596 individuals (90.6%) reported throbbing pain, 114 individuals (17.3%) reported dull pain and 213 (32.4%) reported a feeling of heaviness associated with the headache (Table 4).

The most common pattern of headache onset was irregular, reported by 298 respondents (45.3%). When asked about the duration of the headache, 369 (56.1%) reported that it lasted from 4 to 12 hours and 112 (17%) that it lasted from 12 to 24 hours. The intensity was severe in 321 people (48.8%) and moderate in 243 people (36.9%) (Table 4).

Aura symptoms were present in 293 people (44.5%) with headaches. The most common accompanying symptoms of headache were sensitivity to sound, reported by 617 individuals (93.8%), sensitivity to light, by 596 individuals (90.6%), and nausea, by 559 individuals (85%) (Table 4).

The location of the headache was unilateral in 532 patients (80.9%). The most common headache onset was in the afternoon, reported by 295 patients (44.8%). Regarding the use of medication for headache, 470 patients (71.4%) reported the use of analgesics and 172 (26.1%) reported the use of headache prophylaxis (Table 4).

Of the patients with headaches, 293 had aura symptoms. These symptoms began before the headache in 150 (51.2%), were visual in 233 (79.5%) and lasted between 5 and 20 minutes in 173 (59%). Of the 431 female headache sufferers, 223 (51.7%) reported an association between their headache and menstruation. When the relationship between gender and headache type was evaluated, the number of women in the headache groups was statistically significantly higher than in the non-headache group ($p=0.13$). Looking at mean age, the mean age of migraineurs was 33.68 years, non-migraineurs 29.28 years and non-headaches 30.25 years ($p<0.001$).

The proportion of participants in each category with a GERDQ score of 8 or higher was as follows: 183 (33.3%) in migraineurs, 19 (17.6%) in non-migraineurs and 8 (8.1%) in non-headaches. Migraineurs had higher GERDQ reflux scores, and this increase was statistically significant compared with both migraineurs and non-headaches ($p<0.001$) (Table 5).

Table 4. Headache characteristics of the participating patients

Headache characteristics of the participants	Total (n=658)	
	Number (n)	Percentage (%)
Age of headache onset		
5-15	212	32.2
16-25	340	51.7
26-35	96	14.6
36-45	10	1.5
Headache-related medical consultations		
Yes	404	61.4
No	254	38.6
Frequency of headaches		
Everyday	17	2.6
4-6 day/week	55	8.4
1-3 day/week	192	29.2
Once every two weeks	183	27.8
Once a month	193	29.3
Once every two months	17	2.6
A few days per year	1	0.2
Onset pattern of headaches		
Suddenly	212	32.2
Slow progressing	446	67.8
Characteristics of headaches		
Throbbing	596	90.6
Dull	114	17.3
Exploding	69	10.5
Heaviness	213	32.4
Pulsating	95	14.4
Tingling, burning, numbness	49	7.4
Features of headaches		
Always	187	28.4
Episodic (coming in attacks)	173	26.3
Intermittent (occurring at irregular intervals)	298	45.3
Duration of headache		
Less than 1 hour	33	5.0
1-3,59 hours	60	9.1
4-11,59 hours	369	56.1
12-23,59 hours	112	17.0
More than 24 hours	84	12.8
Severity of headache		
Mild	22	3.3
Moderate	243	36.9
Severe	321	48.8
Very severe	72	10.9
Headache with aura		
Yes	293	44.5
No	365	55.5
Symptoms accompanying headache		
Nausea	559	85.0
Vomiting	237	36.0
Light sensitivity	596	90.6
Sound sensitivity	617	93.8
Odor sensitivity	410	62.3
Loss of appetite	442	67.2
Increase in pain with physical activity	550	83.6
The site of the headache		
Unilateral	532	80.9
Bilateral	71	10.8
Sometimes unilateral, sometimes bilateral	47	7.1
Others	8	1.2
Onset time of headache during the day		
Morning	146	22.2
Afternoon	295	44.8
Evening	198	30.1
During sleeping	19	2.9
Use of painkillers for headache		
Yes	470	71.4
No	188	28.6
Prophylactic treatment for headache		
Yes	172	26.1
No	486	73.9

Headache patients were assessed using the GERDQ and scored according to their impact score. An evaluation of those who scored 8 or more on the GERDQ showed that 31 (16.9%) of the migraineurs were significantly affected by reflux disease, 4 (21.1%) of the non-migraineurs and 2 (25%) of the non-headaches. However, this difference was not statistically significant ($p = 0.774$).

The headache patients were asked about their use of medication for stomach complaints. The Modified Morisky Treatment Adherence Scale (MMTAS) was used to assess the use of proton pump inhibitors for gastric symptoms in 226 patients. Among patients with migraine headaches, 69.5% (n=132) were found to be poorly motivated to adhere to treatment, a significantly higher proportion than both migraine-free patients and those without headaches ($p=0.017$). Among those with non-migraine headaches, 50% (n=18) had low motivation to adhere to treatment (Table 6).

Table 5. The relationship between headache and GERDQ

The relationship between headache and GERDQ	Evaluation based on GERDQ score (n=757)				p value
	0-7 point		≥8 points		
	n	%	n	%	
Headache type					
Migraine headache	367	66.7	183	33.3	
Non-migraine headache	89	82.4	19	17.6	**<0.001**
No headache	91	91.9	8	8.1	

GERDQ: Gastroesophageal Reflux Disease Questionnaire

Table 6. Evaluation of those receiving treatment for GERD according to the MMMAS

Headache type	Evaluation according to The Modified Morisky Medication Adherence Scale				p value
	Poor medication adherence		High medication adherence		
	n	%	n	%	
Migraine headache	132	69.5	58	30.5	
Non-migraine headache	18	50	18	50	**0.017**
No headache	5	41.7	7	58.3	

MMMAS: Morisky Medication Adherence Scale

DISCUSSION

According to the literature, migraine is described as a recurrent primary headache disorder characterized by unilateral, pulsating pain of moderate to severe intensity, with pathophysiological mechanisms that are not fully understood. In our study, when examining the pain characteristics of patients, we found them to be consistent with the literature. There is no definitive laboratory test for diagnosing migraines. The diagnosis is based on the criteria established by the International Headache Society (IHS), which we also adhered to in our study (10,15).

Epidemiological studies conducted in our country have found that the prevalence of migraine is 16.4%. In general, 21.8% of women and 10.9% of men are affected. Migraines can occur at any age, but they are most seen between the ages of 30 and 50 (16,19). The prevalence of migraine varies by age and sex, with higher rates observed in males before adolescence, but a marked increase in females after puberty. The prevalence continues to rise until the age of 40 and then begins to decline. The most common onset of migraines is in the second and third decades of life. People who experience their first migraine attack after the age of 50 represent only about 2% of cases. All studies show a higher incidence of migraine in women, with a male-to-female ratio of 1:2-3. While the exact reason for this is unknown, it is thought to be related to female sex hormones. The higher proportion of female participants in our study may be because migraines tend to be more severe in women, leading to a higher rate of hospital admissions. Possible reasons for this imbalance include the effects of sex hormones, genetic factors, differential exposure to environmental stressors, and differences in pain perception and response to stress (20,21).

In one study, the incidence of gastroesophageal reflux disease (GERD) was reported to be about 27% in the migraine group and 10% in the control group. Gastric ulcers were also found to be more common in the migraine and tension-type headache groups compared to the control group (11.9% in the migraine group, 11.9% in the tension-type headache group, and 5.65% in the control group) (22). In our study, the prevalence of GERD was found to be 33.3% in migraine patients, 17.6% in the non-migraine group, and 8.1% in the group without headaches. Another clinic-based study found no significant difference in the prevalence of gastritis and peptic ulcers between migraineurs and non-migraineurs. However, like our study, they reported a higher frequency of gastroesophageal reflux disease (GERD) in migraineurs compared with those without migraines (42% in migraineurs and 18% in those without migraines) (23). While the exact mechanism underlying the increased incidence of GERD in migraine patients is not fully understood, it is known that these two conditions share common pathophysiological mechanisms. Autonomic nervous system dysfunction has been associated with both types of headaches, particularly migraine, and with gastrointestinal disorders (24-26). Therefore, it is likely that autonomic nervous system dysfunction plays a role in the pathogenesis of these disorders and may explain the co-occurrence of gastroesophageal reflux disease

(GERD) and migraine. Additionally, patients with dyspeptic symptoms have been reported to have abnormal visceral mechanics, sensory functions, and vagal function, and migraine is also associated with visceral nerve dysfunction (27).

Both epidemiological data and pathophysiological evaluations have reported an association between migraine and gastrointestinal disorders. These studies suggest that gastrointestinal symptoms in migraine patients may be a consequence of migraine attacks. The cross-sectional design of this study, however, does not allow us to determine whether migraine attacks cause gastrointestinal symptoms or if other common risk factors contribute to the relationship between these two conditions. Gastrointestinal symptoms may also be a side effect of medications used to treat migraines. Opioid analgesics can cause constipation and nausea, and various gastrointestinal complaints are known to be common side effects of non-steroidal anti-inflammatory drugs (NSAIDs). Additionally, psychological factors may have a common basis for both conditions, as both are strongly associated with anxiety and depression (28). In our study, no significant association was found between the use of analgesics and GERD. One possible explanation for this is that some patients using NSAIDs also use proton pump inhibitors (PPIs), which may delay the onset of NSAID-induced gastric erosion or suppress symptoms. This may explain the lack of significance in the incidence of reflux disease among patients using NSAIDs in our study.

A review of the current literature reveals a lack of studies specifically investigating the impact of gastroesophageal reflux disease (GERD) treatment on the frequency of migraine attacks. In our study, no significant effect of GERD treatment on migraine attack frequency was observed. A potential explanation for this finding could be that proton pump inhibitor (PPI) therapy in GERD patients provides symptomatic relief rather than a definitive cure in many cases. Additionally, a significant proportion of patients may use PPIs intermittently, primarily during symptom exacerbation, rather than following a consistent and prescribed treatment regimen. Further research is required to elucidate this issue. In analyzing other data, we found that according to the Modified Morisky Medication Adherence Scale, medication adherence motivation in the migraine group was significantly lower compared to the group without headaches. To our knowledge, no other study has examined the relationship between treatment adherence in gastroesophageal reflux disease and the frequency of migraine attacks. While previous studies have explored the relationship between reflux and migraines, which is also included in our study, this study's strength lies in its evaluation of treatment adherence (8,29,30). Another strength is that the data were collected through face-to-face interviews conducted by a physician.

However, several limitations must be considered. The number of individuals with non-migraine headaches or without headache symptoms was lower than that of migraineurs, which might have contributed to the differing observed rates of reflux disease. Additionally, the inclusion of patients undergoing long-term PPI therapy, who experienced a reduction or disappearance of symptoms, could have influenced the reported prevalence of reflux disease. There may also have been some data loss due to inconsistent PPI usage, as some patients may not have been able to provide clear information. When designing the study, it might have been more appropriate to include patients in the migraine group, the other headache group, and a control group without headaches, provided that they had not taken proton pump inhibitors or similar reflux treatments for at least the last 6-8 weeks. Especially in the control group, treatments for conditions like gastritis or peptic ulcers may have masked GERD symptoms, potentially reducing the prevalence in this group.

CONCLUSION

Our study results indicate that gastroesophageal reflux disease (GERD) is significantly more prevalent among migraineurs compared to those with non-migraine headaches or without headache symptoms. Consistent with the literature, migraine prevalence was significantly higher among women than men in our participant group.

When assessing the impact of headaches and the results of the GERDQ questionnaire regarding the influence of GERD, no significant differences were found in impact scores between migraineurs, individuals with non-migraine headaches, and those without headache symptoms. Among GERD-treated patients, a significant association was observed between poor medication adherence and the presence of migraine headaches. However, no significant link was found between medication knowledge and migraine. Although these findings are intriguing, they require further research for validation.

No significant association was found between medication adherence and migraine attack frequency in GERD-treated patients. These findings provide valuable insights into the relationship between migraine, GERD, medication adherence, and the impact of GERD on migraine sufferers. However, more research is necessary to confirm and expand upon these findings. To better understand the relationship between migraine and GERD, future studies should explore the impact of the duration and severity of PPI treatment on migraine attack frequency. Additionally, intervention studies aimed at improving medication adherence and awareness could be beneficial.

It would also be useful to investigate the effectiveness of different treatment strategies in managing migraine attacks during GERD therapy.

Acknowledgement: All the authors would like to thank Simon Edward Mumford, MSc. TESOL, Writing Centre Advisor at the Izmir University of Economics for his great contribution in editing the language of the manuscript.

REFERENCES

1. Altamura C, Corbelli I, de Tommaso M, Di Lorenzo C, Di Lorenzo G, Di Renzo A, et al. Pathophysiological bases of comorbidity in migraine. Front Hum Neurosci. 2021;15:1-12.
2. Domitrz I, Cegielska J. Magnesium as an important factor in the pathogenesis and treatment of migraine-From theory to practice. Nutrients. 2022;14(5):1-16.
3. Gallai V, Sarchielli P, Albeni A, Floridi A. Nitrites, prostaglandins, and trigeminal vasoactive peptides in internal jugular venous blood in migraine patients during spontaneous attacks. Neurol Sci. 2000;21.
4. Goadsby PJ, Karsan N. Molecular mechanisms of migraine: Nitric oxide synthase and neuropeptides. Int J Mol Sci. 2023;24(15):11993.
5. Mauskop A, Varughese J. Why all migraine patients should be treated with magnesium. J Neural Transm. 2012;119(5):575-9.
6. Cámara-Lemarroy CR, Rodriguez-Gutierrez R, Monreal-Robles R, Marfil-Rivera A. Gastrointestinal disorders associated with migraine: A comprehensive review. World J Gastroenterol. 2016;22(36):8149-60.
7. Ponce J, Garrigues V, Agréus L, Tabaglio E, Gschwantler M, Guallar E, et al. Structured management strategy based on the Gastro-oesophageal Reflux Disease (GERD) Questionnaire (GerdQ) vs. usual primary care for GERD: Pooled analysis of five cluster-randomised European studies. Int J Clin Pract. 2012;66(9):897-905.
8. Shen Z, Bian Y, Huang Y, Zhou W, Chen H, Zhou X, et al. Migraine and gastroesophageal reflux disease: disentangling the complex connection with depression as a mediator. PLOS ONE. 2024;19(7)
9. Hormati A, Bian Y, Huang Y, Zhou W, Chen H, Zhou X, et al. Higher risk of migraines among patients with GERD. PLOS ONE. 2023;18(5).
10. Headache Classification Committee of the International Headache Society (IHS). The international classification of headache disorders, 3rd edition (beta version). Cephalalgia. 2013;33(9):629-808.
11. Jones R, Junghard O, Dent J, Wiklund I. Development of the GerdQ, a tool for the diagnosis and management of gastro-oesophageal reflux disease in primary care. Aliment Pharmacol Ther. 2009;30(10):1030-8.
12. Hançerlioğlu S, Bor S. Turkish validation of the reflux disease questionnaire. J Gastrointest Liver Dis. 2021;30(2):193-7.
13. Vural UB, Özgür U, Acar T, Topsever DP, Filiz DTM. Modifiye Morisky Ölçeğinin Türkçe Geçerlilik Güvenilirlik Çalışması. Turkish Fam Physician. 2012;4:17-20.
14. Morisky DE, Ang A, Krousel-Wood M, Ward HJ. Predictive validity of a medication adherence measure in an outpatient setting. J Clin Hypertens (Greenwich). 2008;10(5):348-54.
15. Pescador Ruschel MA, De Jesus O. Migraine headache. NCBI Bookshelf [Internet]. National Library of Medicine; 2023. [cited:2024 Nov 13] Available from: https://www.ncbi.nlm.nih.gov/books/NBK582491/
16. Bıçakçı Ş, Bozdemir N, Over F, Saatçi E, Sarıca Y. Prevalence of migraine diagnosis using ID Migraine among university students in southern Turkey. J Headache Pain. 2008;9:159-63.
17. Wacogne C, Lacoste JP, Guillibert E, Hugues FC, Le Jeunne C. Stress, anxiety, depression and migraine. J Headache Pain. 2003;4:451-5.
18. Ertas M, Baykan B, Orhan EK, Zarifoglu M, Karli N, Saip S, et al. One-year prevalence and the impact of migraine and tension-type headache in Turkey: A nationwide home-based study in adults. J Headache Pain. 2012;13:147-57.
19. Aamodt AH, Stovner LJ, Hagen K, Zwart JA. Comorbidity of headache and gastrointestinal complaints. The Head-HUNT Study. Cephalalgia. 2008;28(2):144-51.
20. Al-Hassany L, Haas J, Piccininni M, Kurth T, Maassen Van Den Brink A, Rohmann JL. Giving researchers a headache – Sex and gender differences in migraine. Front Neurol. 2020;11:1-16.
21. Chalmer MA, Kogelman LJA, Callesen I, Christensen CG, Techlo TR, Møller PL, et al. Sex differences in clinical characteristics of migraine and its burden: A population-based study. Eur J Neurol. 2023;30(7):1774-84.
22. Lee SH, Lee JJ, Kwon Y, Kim JH, Sohn JH. Clinical implications of associations between headache and gastrointestinal disorders: A study using the Hallym smart clinical data warehouse. Front Neurol. 2017;8:1-8.
23. Peşkersoy C, Peker Ş, Kaya A, Ünalp A, Gökay N. Evaluation of the relationship between migraine disorder and oral comorbidities: Multicenter randomized clinical trial. Turk J Med Sci. 2016;46(3):712-8.
24. Peroutka SJ. What turns on a migraine? A systematic review of migraine precipitating factors. Curr Pain Headache Rep. 2014;18(10):454.
25. Heitkemper M, Jarrett M, Cain KC, Burr R, Levy RL, Feld A, et al. Autonomic nervous system function in women with irritable bowel syndrome. Dig Dis Sci. 2001;46(6):1276-84.

26. Shechter A, Stewart WF, Silberstein SD, Lipton RB. Migraine and autonomic nervous system function: A population-based, case-control study. Neurology. 2002;58(3):422-7.
27. Kurth T, Holtmann G, Neufang-Hüber J, Gerken G, Diener HC. Prevalence of unexplained upper abdominal symptoms in patients with migraine. Cephalalgia. 2006;26(5):506-10.
28. Craske MG, Wolitzky-Taylor KB, Labus J, Wu S, Frese M, Mayer EA, et al. A cognitive-behavioral treatment for irritable bowel syndrome using interoceptive exposure to visceral sensations. Behav Res Ther. 2011;49(6-7):413-21.
29. Katić BJ, Golden W, Cady RK, Hu XH. GERD prevalence in migraine patients and the implication for acute migraine treatment. J Headache Pain. 2009;10(1):35-43.
30. Basavannaiah S. A comparative study of laryngopharyngeal reflux disease and migraine: Insights from a hospital-based analysis. Tr-ENT. 2024;34(1):13-7.

RESEARCH ARTICLE

Metin Deniz Karakoc[1]

[1] Pamukkale University, Faculty of Medicine, Department of Medical Pharmacology, Denizli, Türkiye

Corresponding Author:
Metin Deniz Karakoc
mail: mdkarakoc@pau.edu.tr

Received: 05.05.2024
Acceptance: 20.11.2024
DOI:10.18521/ktd.1478663

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Retrospective Analysis of Pregnant Cases in Terms of Drug-Drug Interactions and Teratogenic Risks

ABSTRACT

Objective: While teratogenic risks in pregnant women are frequently discussed, polypharmacy and drug-drug interactions (DDI) are topics with little known information. The aim of this study is to determine the polypharmacy status, DDI, and teratogenic risk profile during pregnancy.

Method: A retrospective cohort study was conducted covering the year 2023 on pregnant women who were referred for pharmacology consultation due to a history of drug use. Investigation of DDI was performed through the Micromedex and Medscape online query modules.

Results: It was found that 113 pregnant women used a total of 71 different active ingredient drugs from 24 diverse pharmacological groups. The average number of drugs used per individual was 2.97. Analgesics, antibiotics, and gastric acid inhibitors were the most used medications, respectively. 11.6% of the women had a comorbidity, and cardiovascular diseases were the most common. It was determined that 28.3% of women had a serious or moderate DDI. The rate of drugs in categories D and X, which are particularly risky in terms of teratogenicity, was found to be 40.8%.

Conclusions: In addition to teratogenic effects, polypharmacy and DDI are also significant risk factors in pregnant women. There is still a crucial need for evidence on the medications prescribed in pregnancy, how it specifically affects women with comorbidities, and related benefits and harms.

Keywords: Polypharmacy, Teratogenicity, Drug Interactions, Comorbidities, Pregnancy.

Gebe Olguların İlaç-İlaç Etkileşimleri ve Teratojenik Riskler Açısından Retrospektif Analizi

ÖZET

Amaç: Gebelerde teratojenik riskler sıkça tartışılan bir konudur ancak polifarmasi ve ilaç-ilaç etkileşimleri (İİE) hakkında çok az bilgi mevcuttur. Çalışmanın amacı, gebelik sırasında meydana gelen polifarmasi durumunu, İİE ve teratojenik risk profillerini belirlemektir.

Yöntem: 2023 yılı süresince gebelikte ilaç kullanım öyküsü nedeniyle farmakoloji konsültasyonuna yönlendirilen kadınları kapsayan retrospektif bir kohort çalışması yapıldı. İİE araştırması Micromedex ve Medscape online sorgu modülleri kullanılarak gerçekleştirildi.

Bulgular: 113 gebenin toplamda 24 farklı farmakolojik gruptan 71 farklı etken madde içeren ilaç kullandığı bulundu. Birey başına kullanılan ilaçların ortalamasının 2,97 olduğu saptandı. Sırasıyla ağrı kesiciler, antibiyotikler ve mide asidi inhibitörlerinin en çok kullanılan ilaçlar olduğu belirlendi. Kadınların %11,6'sında bir komorbidite olduğu ve en sık kardiyovasküler hastalıklara sahip oldukları belirlendi. Gebelerin %28,3'ünde ciddi veya orta derecede İİE olduğu tespit edildi. Teratojenik açıdan özellikle riskli olan D ve X kategorisindeki ilaç oranının %40,8 olduğu saptandı.

Sonuç: Teratojenik etkilerin yanı sıra, polifarmasi ve İİE de gebelerde önemli risk faktörleridir. Gebelikte reçete edilen ilaçlar, özellikle komorbiditeleri olan kadınlar üzerindeki fayda ve zararları konusunda kanıtlara halen kritik derecede ihtiyaç bulunmaktadır.

Anahtar Kelimeler: Polifarmasi, Teratojenite, İlaç Etkileşimleri, Komorbiditeler, Gebelik.

INTRODUCTION

Pregnancy is a lengthy physiological process that lasts approximately 40 weeks, ideally culminating in a healthy birth. Although women generally prefer to minimize medication use during this sensitive period, they often require drug treatment for various reasons related to chronic illnesses, infections, or pregnancy-related conditions (e.g., anemia, emesis). Additionally, it is common for women to use medications for acute or chronic diseases before recognizing their pregnancy. For pregnant women with various comorbidities, medication use becomes unavoidable, despite the potential adverse effects on both the expectant mother and the fetus (1). Avoiding treatment for certain conditions throughout pregnancy to mitigate the risks associated with medication use can negatively impact both maternal and fetal health (2). However, it is possible to reduce these risks by implementing rational precautions. The frequency of medication use during pregnancy can vary between countries due to differences in educational, cultural, and socioeconomic conditions, such as access to healthcare providers and medications. Various studies conducted in different populations indicate that between 60% and 84.7% of women use at least one medication during their pregnancies (3,4). The teratogenic risks and potential drug-drug interactions (DDIs) that may arise for individuals requiring multiple medications necessitate careful consideration, given their potential to create serious complications for maternal and fetal health.

Developing a new drug requires years of meticulous research and substantial financial resources. However, due to social and ethical considerations, the opportunity to conduct clinical trials on pregnant women is highly restricted during this process. Consequently, teratogenicity data for the majority of drugs are primarily derived from experimental animal studies. Pregnant women gain access to medications only after the drug has been licensed. From that point onward, the teratogenic risk profile of the drug can be revealed over time through clinical observations, retrospective studies, and adverse event reports submitted by healthcare professionals. There remains insufficient information regarding the teratogenic effects of most drugs available on the market. Therefore, decisions about medication use during pregnancy must sometimes be made by physicians without adequate evidence regarding the effectiveness and safety of the treatment.

The development of standardized and comprehensible systems for assessing the teratogenic risk of drugs enables physicians, along with other healthcare professionals, patients, and their families, to make more informed decisions regarding potential risks. Various systematic approaches, differing in classification, are employed worldwide for evaluating drug-induced teratogenic risks. In our country, the Turkish Drug and Medical Devices Agency (TITCK), the authority responsible for medicines, uses a classification system that categorizes teratogenic risks into five groups: A, B, C, D, and X. Category A is deemed the safest, while category X is strictly contraindicated during pregnancy, providing essential therapeutic guidance to clinicians (5). The teratogenicity classification system used in our country aligns with the system established by the US Food and Drug Administration (FDA) in 1979. Although this organization proposed a novel method in 2015, the five-letter classification system remains widely preferred within the healthcare community due to its established and easily comprehensible language (6).

Given these considerations, our study aimed to contribute to the literature by analyzing the drug use profiles, potential DDIs, and teratogenic risks in pregnant individuals referred for pharmacology consultation by gynecologists over a one-year period, based on the TITCK's five-letter classification system.

MATERIAL AND METHODS

Ethical Approval: The research is a retrospective cohort study covering the year 2023. Approval for the research was initially obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (permission number: E.462180), and it was conducted in accordance with the principles of the Declaration of Helsinki.

Study Design and Subjects: The study population comprises pregnant individuals referred by gynecologists from our university hospital and four other private and public hospitals in the Denizli province for pharmacological evaluation. A report on drug use and teratogenic risk is prepared based on the anamnesis obtained from these individuals. Informed consent was obtained from all participants before the study commenced. The anamneses were collected by recording responses to standard questions found in the teratogenicity information form, which is available in printed format in our department.

In addition to identity and contact information, the form includes detailed questions regarding age, education status, number of pregnancies, gestational weeks, number of live births/stillbirths, number of normal births/cesarean sections, history of miscarriage, intentional abortion, or birth anomalies in previous pregnancies, presence of allergies or chronic diseases, and usage status of tobacco, alcohol, or other addictive substances. The form also captures details of medication use, including duration, dosage, and reasons for initiation (e.g., prescribed by a physician, heard from the media, or purchased over-the-counter from a pharmacy). All forms were

completed face-to-face under the supervision of the same pharmacologist, who also prepared the evaluation reports.

Data Processing Procedure: During the study period, counseling services were provided to a total of 113 pregnant women, all of whom had their forms completed in full, and a report was prepared for each individual. All participants were included in the study. The drugs were classified according to both the TITCK teratogenic risk classification and the degree of interaction. The TITCK pregnancy categories used in the risk classification of drugs are summarized in Table 1.

Table 1. TITCK* pregnancy categories used in risk classification of drugs

Category	Description
A	Adequate and well-controlled studies have failed to demonstrate a risk to the fetus in the first trimester of pregnancy and there is no evidence of risk in later trimesters.
B	Animal reproduction studies have failed to demonstrate a risk to the fetus, and there are no adequate and well-controlled studies in pregnant women, or animal studies have shown an adverse effect that was not confirmed in controlled studies in women in the first trimester and there is no evidence of risk in later trimesters.
C	Animal reproduction studies have shown an adverse effect on the fetus, and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.
D	There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.
X	Studies in animals or humans have demonstrated fetal abnormalities, and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits.

*TITCK: Turkish Drug and Medical Devices Agency

Potential DDI were analyzed using the IBM Micromedex DRUGDEX® online database (https://www.micromedexsolutions.com) and Medscape Drug Interaction Checker online query modules (https://reference.medscape.com/drug-interactionchecker). DDIs were classified into three categories: 'serious,' which should be avoided or for which an alternative treatment is preferred; 'moderate,' which should be used with caution and closely monitored; and 'minor,' where the clinical impact is insignificant or unknown, as described in the Medscape Drug Interaction Checker online query module.

Statistical Analysis: Statistical analysis of the data was conducted using the IBM Statistical Package for the Social Sciences (SPSS) version 29.0. Descriptive statistics, including frequency, mean ($\bar{x}$) ± standard error, and percentage (%), were employed to present the data. The chi-square (χ^2) test was utilized to assess the relationship between categorical variables, such as smoking and alcohol history, as well as abortion and stillbirth history. For the analysis of relationships between continuous variables, such as age, number of pregnancies, and time of physician consultation, the suitability of parameters for normal distribution was assessed using the Shapiro-Wilk test, and the homogeneity of variances was checked with Levene's test. Since the parameters met the criteria for normal distribution, the variables were analyzed using the independent sample t-test. One-way analysis of variance (ANOVA) was applied for multiple groups, including education level, number of medications used, and reasons for medication use, as parametric conditions were satisfied. Post-hoc pairwise comparisons were conducted using the Bonferroni-corrected Mann-Whitney U test, with $p < 0.05$ considered statistically significant.

RESULTS

Demographic and Clinical Characteristics of the Participants: It was determined that 7% of the study group (n = 8) continued to use substances with addictive and teratogenic effects until they learned about their pregnancy (2 smokers, 2 alcohol users, and 4 individuals who used both tobacco and alcohol). No significant relationship was found between the individuals' educational status and their smoking or alcohol use ($p = 0.757$). Additionally, those who used addictive substances sought pharmacology consultation later than those who did not (9.5 ± 1.77 vs. 7.8 ± 2.62, respectively; $p = 0.032$).

It was determined that the mean age of the study group was 26.4 ± 4.7 (minimum: 19, maximum: 37). It was detected that all individuals had at least basic literacy levels, with the majority being high school graduates (50.4%, n= 57) and university graduates (38.9%, n= 44). It was determined that pregnant women referred for risk assessment sought consultation at an average gestational age of 8 weeks (7.92 ± 2.60 weeks), with a minimum of 4 weeks and a maximum of 16 weeks. It was found that there is no significant difference between educational level and the duration of seeking medical consultation for teratogenic risk assessment (p= 0.830). The distribution of the pregnant women in the study according to some obstetric characteristics is presented in Table 2.

Table 2. Distribution of pregnant women according to some obstetric characteristics at the time of application

Parameters	n	%
Pregnancy period		
1st Trimester	107	94.7
2nd Trimester	6	5.3
3rd Trimester	0	0
Miscarriage or stillbirth		
No	99	87.6
Yes	14	12.4
Number of previous live births		
Primigravida	69	61.1
1	38	33.6
2	4	3.5
3	2	1.8
Anomalies in previous births*		
No	39	88.6
Yes	5	11.4

*Evaluated among 44 individuals who had one or more pregnancies before their current pregnancy.

The Results of Subgroup Analysis: It was found that 11.6% of the individuals (n = 13) had a chronic disease diagnosed at least one year prior to pregnancy, requiring continuous medication. Additionally, 7% of the participants (n = 8) reported allergies to various substances. The average number of active substances used by pregnant women with chronic diseases (2.77 ± 1.48) was slightly lower than that of those without chronic diseases (3.00 ± 1.23); however, this difference was not statistically significant (p = 0.599). The distribution of the study group according to comorbidities and drug use status is presented in Table 3.

It was found that there is no significant relationship between individuals who use addictive substances and those who do not regarding a history of stillbirth or abortion (p = 0.665). Furthermore, no significant differences were observed between individuals who have experienced stillbirth or abortion in previous pregnancies and those with a chronic illness, compared to other pregnant women, in terms of the timing of seeking medical consultation for teratogenic risk assessment (p = 0.539 and p = 0.457, respectively).

Table 3. Distribution of pregnant women according to comorbidities and drug usage status

Parameters	n	%
Distribution of chronic diseases		
Cardiovascular system	4	3.5
Respiratory system	3	2.7
Endocrine system	3	2.7
Musculoskeletal system	1	0.9
Gastrointestinal system	1	0.9
Dermatological	1	0.9
Number of active substance used*		
1	13	11.5
2	30	26.5
3	34	30.1
4	22	19.5
5	7	6.2
6	4	3.5
7	3	2.7
Reason for starting the drug therapy		
Prescription drugs	103	91.2
Self medication	4	3.5
Advice of a friend/relative	4	3.5
Over-the-counter from a pharmacy	2	1.8

* Except for prenatal vitamins, minerals and antiemetic drugs routinely used during pregnancy

In our study, it was found that pregnant women used an average of 2.97 ± 1.26 active substances, with the majority of these drugs prescribed by other physicians (91.2%). The participants utilized a total of 71 different active ingredient drugs from 24 diverse pharmacological groups. The distribution of active substances used by pregnant women according to pharmacological groups is presented in Table 4.

Table 4. Distribution of active substances used by pregnant women according to pharmacological groups

Pharmacological group	Number of active substances	Number of pregnant women using*	Percentage of pregnant women using (%) *
Analgesics (NSAIDs)	6	49	43.4
Antibiotics	4	37	32.7
Proton pump inhibitors & H_2 receptor antagonists	6	36	31.9
Antidepressants	5	33	29.2
Cold remedies (combined)	3	29	25.7
Muscle relaxants	3	26	23.0
Hormone derivatives & corticosteroids	6	25	22.1
Ionic and non-ionic contrast agents	5	22	19.5
Antipsychotics	2	12	10.6
Laxatives & Purgatives	3	11	9.7
Antihistaminics	2	8	7.1
Vitamins & Minerals**	3	7	6.2
Antianemics	2	5	4.4
Antiepileptics	2	5	4.4
Antihypertensives	3	4	3.5
Antivirals	2	4	3.5
Respiratory system drugs	2	4	3.5
Antiarrhythmics	2	3	2.7
Antidiarrheals	2	3	2.7
Antifungals	2	3	2.7
Others	6	10	9.0
Total	**71**	**336**	

*The majority of women use more than one **active substances**. **Prenatal vitamin & minerals were excluded; NSAIDs: Non-steroidal anti-inflammatory drugs; H_2 receptor antagonists: Histamine 2 receptor antagonists

DDI in Pregnant Women: In the study, it was found that there were 14 "serious" DDIs among 18 active substances, which required treatment discontinuation or modification. Additionally, 23 "moderate" DDIs were identified among 33 different drugs that necessitated close monitoring. No pregnant woman was observed with the simultaneous presence of two or more serious DDIs. However, it was determined that five pregnant women had one serious and one moderate DDI simultaneously. Consequently, the rate of pregnant women with identified DDIs that should be considered was calculated to be 28.3% (n= 32). Furthermore, 89 "minor" DDIs with no clinical significance and requiring no special precautions were also identified. Among the detected DDIs, the types categorized as "serious" are presented in Table 5, while "moderate" interactions are detailed in Table 6.

Table 5. Serious drug-drug interactions and interaction mechanisms detected in pregnant women

Mechanism of interaction	Interacting drug pairs
Accelerating the metabolism of the other by inducing the CYP450 enzyme*	carbamazepine - esomeprazole carbamazepine - tetracycline
Increasing serum levels of each other or potentiating their effects by affecting the p-gp and CYP450 systems	clarithromycin - colchicine diltiazem - colchicine
Potentializing each other's effects by increasing the QTc interval	clarithromycin - escitalopram clarithromycin - formoterol clarithromycin - sertraline
Reducing the absorption of the other by reducing gastrointestinal absorption*	ferric maltol - tetracycline magnesium sulfate - tetracycline
Increasing the level of the other by decreasing renal clearance*	flurbiprofen - methotrexate
Increasing the level and effects of the other through CYP450 enzyme inhibition*	isoniazid - omeprazole
Physiological antagonism	perindopril - flurbiprofen perindopril - ibuprofen
Potentializing each other's toxic effects	perindopril - pregabalin

*The first drug causes changes in the metabolism of the second drug.

Table 6. Moderate drug - drug interactions and interaction mechanisms detected in pregnant women

Mechanism of interaction	Interacting drug pairs
Increasing the serum level and effects of the other by CYP450 enzyme inhibition	bupropion - metoprolol clarithromycin - dexamethasone clarithromycin - prednisolone esomeprazole - escitalopram methylprednisolone - alprazolam metronidazole - alprazolam miconazole vaginal - dexamethasone omeprazole - escitalopram sertraline - metoprolol
Effect on increasing serum potassium level	drospirenone -diclofenac propranolol - ibuprofen
Increased toxic effects due to functional synergism	chlorpheniramine - quetiapine escitalopram - ibuprofen pregabalin - chlorpheniramine tizanidine - escitalopram
Increase the serum level and effects of the other drug by causing an increase in gastric pH	famotidine - methylphenidate
Increased toxic effect with functional synergism	gabapentin - alprazolam
Reducing the expected therapeutic effect by physiological antagonism	metoprolol - albuterol
Potentializing each other's toxic effects	perindopril - flurbiprofen valsartan - diclofenac
Increase in expected therapeutic effect with physiological synergism	perindopril - insulin aspart tizanidine - valsartan
Additive interaction on increasing the QTc interval	albuterol - loperamide

Teratogenic Drug Use in Pregnant Women: In our study, it was determined that 43.7% of the drugs used by pregnant women are in category 'C', 26.7% in category 'D', 14.1% in category 'X', 12.7% in category 'B', and 2.8% in category 'A'. The rate of drugs in categories D and X, which are particularly risky in terms of teratogenicity, was found to be 40.8% and listed in Table 7. The distribution of the number of drugs used according to TITCK classification is presented in Figure 1.

Table 7. Distribution of fetotoxic D and X category drugs

Category D active substances	Number and percentage (%) of individuals	Category X active substances	Number and percentage (%) of individuals
Amitriptyline	3 (2.65)	Ethinyl estradiol	3 (2.65)
Paroxetine	3 (2.65)	Drospirenone	3 (2.65)
Tetracycline	3 (2.65)	Isotreonine	2 (1.77)
Alprazolam	2 (1.77)	Levonorgestrel	2 (1.77)
Carbamazepine	2(1.77)*	Atorvastatin	1 (0.88)
Imipramine	2 (1.77)	Methotrexate	1 (0.88)
Topiramate	2 (1.77)	Misoprostol	1 (0.88)
Valproic Acid	2(1.77)*	Norgestrel	1 (0.88)
Candesartan	1 (0.88)	Simvastatin	1 (0.88)
Clonazepam	1 (0.88)	Warfarin	1 (0.88)
Irbesartan	1 (0.88)		
Lisinopril	1 (0.88)		
Lithium	1 (0.88)		
Losartan	1 (0.88)		
Perindopril	1 (0.88)		
Phenobarbital	1 (0.88)		
Phenytoin	1 (0.88)		
Ramipril	1 (0.88)		
Valsartan	1 (0.88)		
	30 (%26.6)		**16 (%14.2)**

The classification system is based on the pregnancy categories used by the Turkish Drug and Medical Devices Agency (TITCK) for the risk classification of drugs in pregnancy. *1 individual used valproic acid and carbamazepine simultaneously. Abbreviations: DDI, drug-drug interactions; e.g, for example; NSAIDs: non-steroidal anti-inflammatory drugs.

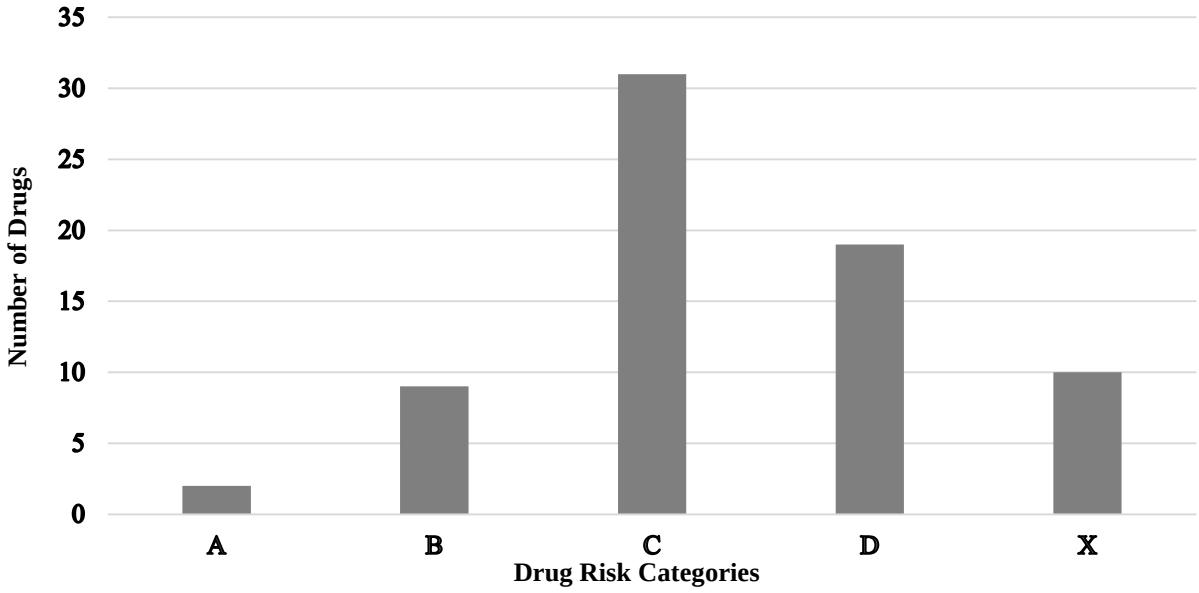

Figure 1. The distribution of the number of drugs used according to TITCK classification

DISCUSSION

A healthy pregnancy encompasses various factors that directly influence both maternal and fetal health. Many women may resort to drug therapy to address various health issues that arise during pregnancy and in the period before they become aware of their pregnancies. However, the teratogenic risks associated with drug use, along with potential DDIs, raise serious concerns for maternal and fetal health. The use of addictive

substances such as tobacco and alcohol increases the risk of miscarriage, causes premature births, and results in teratogenic effects (7). In our study, it was found that individuals using addictive substances did not differ from other pregnant women in terms of education level. However, these individuals tended to seek consultation later than their counterparts. This delay is likely associated with lifestyle choices and a lack of awareness. The data obtained highlight the importance of raising awareness about the risks of addictive substances during pregnancy for all individuals in society, regardless of gender, starting from adolescence.

The individuals in our study were primarily young women, with an average age of approximately 27 years, the majority of whom were in the first trimester of their first pregnancy (around 8 weeks). Despite this young average age, a notable 11.6% of participants had comorbidities requiring continuous drug use, which poses various risks for a healthy pregnancy. Cardiovascular, respiratory, and endocrine system diseases were the most frequently encountered comorbidities. Cardiovascular diseases, in particular, warrant attention due to their association with an increased risk of stroke, myocardial infarction, and cardiomyopathy during the peripartum period (8). The severity of these conditions necessitates the ongoing use of one or more medications, despite potential fetal risks.

In our study, the average number of drugs used by patients with comorbidities was 2.77, while the overall average for the entire study group was 2.97 (with a range from 1 to 7), suggesting that medication use may not always be rational. Analysis of the most commonly used medications revealed that Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), antibiotics, and gastric acid inhibitors were among the most frequently prescribed. Such irrational drug use during pregnancy is likely to result in permanent health problems for both the expectant mother and the fetus.

Polypharmacy, defined as the concurrent use of multiple drugs, is a critical concern for pregnant individuals due to the associated risks, such as DDIs, increased side effects, and treatment failures (9). Our study found that 88.5% (n= 100) of participants were using two or more active substances (excluding prenatal vitamins, minerals, and antiemetic medications used during pregnancy). A recently published study on polypharmacy among pregnant women in Indonesia reported that 39.1% of participants used two or more active substances, with NSAIDs and gastric acid inhibitors being the most commonly used, primarily prescribed by physicians (10). In Türkiye, it was reported that 65% of pregnant women used two or more active substances, with an average of 2.6 active substances per participant; the most frequently used drugs were antibiotics and pain relievers (6). A meta-analysis encompassing studies conducted between 2013 and 2019 found that pregnant women in Ethiopia used an average of 1.7 medications per pregnancy, with 86.9% of these being prescription drugs. This study highlighted the widespread use of medications with high teratogenic risks, identifying antianemic agents, antibiotics, NSAIDs, and gastric acid inhibitors as the most commonly used drugs (11). A recently published study covering a 20-year period in England, which examined approximately 1.5 million pregnant women, found a polypharmacy prevalence of 58.7%. This study also reported that pregnant women with multimorbidity, obesity, a history of smoking, and those outside the 25-34 age range were at a higher risk of polypharmacy than their peers. It noted that the most commonly used medications included antibiotics, gastric acid inhibitors, antifungals, and analgesics (12). As evidenced by the studies described above, the commonly used drug groups among pregnant women may vary from country to country, influenced by socio-economic conditions and lifestyle. Nevertheless, it is generally observed that pregnant women commonly use medications such as NSAIDs, antibiotics, and gastric acid inhibitors. All these studies conducted in diverse regions underscore that polypharmacy is prevalent among pregnant women (excluding prenatal vitamins, minerals, and antiemetic drugs), with most medications being prescription drugs. In this context, our research aligns with the findings of these studies.

DDI is defined as the alteration of expected therapeutic and toxic effects that occurs when two or more drugs interact with each other. These interactions can occur at pharmacokinetic levels (such as absorption, distribution, metabolism, and elimination) and/or pharmacodynamic levels (involving receptor agonism or antagonism) (5). DDI is commonly encountered, particularly among the elderly, due to the prevalence of various chronic diseases and organ failures (14). Conversely, pregnant women are not typically considered a population where DDIs are prevalent, largely due to their younger average age and the tendency to minimize medication use during this sensitive period. However, our study found that pregnant women used an average of three active substances, influenced by comorbidities and irrational drug use, leading to exposure to significant DDIs. Notably, 28.3% of pregnant participants were affected by severe or moderate DDIs, which poses substantial risks to both maternal and fetal health. In a study involving pregnant and breastfeeding women, it was reported that 91% of prescriptions contained DDIs, with 1.4% involving drugs that were contraindicated for concurrent use (15). Another study assessing DDIs in pregnant women receiving inpatient treatment in a maternal intensive care unit revealed that 95.1% were exposed to at least one

moderate or severe DDI. The primary drugs associated with serious interactions in that study included magnesium sulfate, metoclopramide, propranolol, and diazepam (16). In contrast, our research identified carbamazepine, clarithromycin, and perindopril as the main drugs responsible for serious interactions. It is important to note that our study did not account for clinically insignificant or unknown minor drug interactions, which may contribute to discrepancies in total DDI rates across different studies. While variations exist regarding the most common interacting drugs and the rates of serious interactions, a consistent theme across all studies is the highlighted risk of polypharmacy and DDIs in medication use during pregnancy, alongside the inherent teratogenic risks.

In our study, the majority of pregnant women (94.7%) were in their first trimester, while the remainder were in the second trimester. During this early stage of pregnancy, which is particularly vulnerable to teratogenic effects, the participants used a total of 71 different active substances. Among these, 19 drugs were classified in category "D", indicating potential risks, while 10 were in category "X", which contains definitive evidence of teratogenic risk. Notably, many participants began their medication regimens before becoming aware of their pregnancies, and some were required to continue using medications due to chronic health conditions. The high percentage (40.8%) of individuals using fetotoxic drugs in our study may be attributed to the specific nature of our study group, which consisted of pregnant women referred for pharmacologist consultation by gynecologists due to their history of medication use, rather than representing a general pregnant population. As detailed in Table 7, the most commonly identified drugs included oral contraceptives, anti-acne preparations, antidepressants, antibiotics, and antiepileptics. In a study examining 18,575 prescriptions over a 3-year period in Canada, it was reported that 9.1% of pregnant women were exposed to category D or group X drugs (17). In another study conducted in Taiwan, it was reported that 1.1% of the 217,226 prescriptions written for 14,125 pregnant women over a period of three years were identified as category D or X drugs. It was noted that hormonal preparations used for birth control were the most commonly encountered medications in the first trimester (18). A study conducted in Italy, which examined 33,343 prescriptions written within one year, was reported that approximately 1% of pregnant women were exposed to category X drugs (19). While the ranking of the most commonly exposed drugs in these studies shows slight variations, the findings are generally consistent with those of our study. However, the higher rate observed in our research can be attributed to the focus on a specialized population referred by gynecologists for risky drug use, rather than a general pregnant population. In our study, it was determined that not only category D drugs, but also category X drugs such as warfarin, isotretinoin, misoprostol, atorvastatin, simvastatin, methotrexate, and various hormone preparations were administered to women who were not yet aware of their pregnancy. The most common teratogenic effects resulting from the use of these drugs are stillbirth, low birth weight babies, developmental disorders, and organ failure (20). Warfarin has teratogenic effects, leading to embryopathy, Angiotensin-Converting Enzyme (ACE) inhibitors cause cranial malformations, statins lead to skeletal system anomalies, and tetracyclines cause bone and dental abnormalities as well as neural tube defects (21, 22). Antiepileptic drugs such as valproic acid, carbamazepine, phenytoin, phenobarbital and topiramate have specific teratogenic effects such as craniofacial anomalies, orofacial clefts, mental retardation and neurodevelopmental disorders (23). In our study, we also found that a significant number of women used various ionic and non-ionic contrast agents for radiological imaging before recognizing their pregnancy. Although radiological contrast agents are not directly teratogenic, exposure to ionizing radiation during the first trimester can have direct teratogenic and carcinogenic effects on the fetus (24). Therefore, physicians should thoroughly question women about their pregnancy status and only perform such radiological imaging once pregnancy is confirmed.

Furthermore, our study determined that a vast majority of the drugs used by these women (91.2%) were initiated through a physician's prescription. This underscores the necessity for physicians to inquire about potential pregnancy status or plans for pregnancy when prescribing medications to women of childbearing age. In a study conducted in Canada, which examined the prescriptions of approximately 110,000 pregnant women, it was reported that 6.3% of women used at least one medication posing a risk to the fetus (25). Similarly, a study in the Netherlands indicated that 95.5% of women used at least one drug during pregnancy (excluding prenatal vitamins, minerals, and anti-emetics), with 6.5% of these substances classified as teratogenic, and roughly one-third having suspected pharmacological effects on the fetus (26). A study in Brazil found that 26% of the prescribed drugs were classified as category C, 1.5% as category D, and 1.5% as category X (27). Additionally, a retrospective study evaluating a five-year period in Canada determined that drugs in category D were prescribed to 5.5% of pregnant women, while drugs in category X were prescribed to 2.5%. During the first trimester, benzodiazepines and antidepressants were the most commonly prescribed drugs in category D, whereas oral contraceptives and ovulation stimulants were the most frequently prescribed in category X (28). Our research focused on a participant group referred for

pharmacological consultation due to the use of risky drugs, rather than on a general pregnant population. Consequently, the rates of risky drug usage in our study were higher compared to the studies mentioned above. However, the findings from our study, along with those from others, highlight that the use of risky drugs classified in categories D and X is a common issue on a global scale.

In our study, it was determined that the most commonly used active substances (43.7%) were classified as category C drugs. Compounds in this category have demonstrated fetotoxic effects in pregnant experimental animals; however, due to insufficient studies in humans, their use should be carefully considered based on the benefit-to-harm ratio. This indicates that the use of category C drugs during pregnancy is not entirely safe (5). Notably, many frequently used medications in our study, including pain relievers, antidepressants, proton pump inhibitors, cold remedies, and muscle relaxants, fall into this category. In a separate study, it was reported that two out of every three pregnant women in Israel were prescribed an antimicrobial drug during pregnancy (29). Another study indicated that antibiotic treatment during pregnancy is also prevalent in Western countries, accounting for 80% of the drugs prescribed to pregnant women (30). Furthermore, a review noted that one in four women received antibiotics during their pregnancy. The review stated that antibiotics such as beta-lactams, vancomycin, nitrofurantoin, metronidazole, clindamycin, and fosfomycin are relatively safe and effective during pregnancy, while fluoroquinolones and tetracyclines should be avoided. Additionally, antibiotic exposure during pregnancy has been associated with adverse effects in newborns, including congenital anomalies, asthma, and atopic dermatitis (31). Consistent with these findings, antibiotics emerged as one of the most frequently prescribed drug groups in our research. However, our study identified that, in addition to other antibiotics, clarithromycin, tetracycline, and levofloxacin were also prescribed to pregnant women, which contradicts the recommendations from the aforementioned studies. Furthermore, serious DDIs were detected, particularly among pregnant women using clarithromycin, due to the concurrent use of various antidepressants and asthma medications.

The use of NSAIDs and antidepressants during pregnancy, much like antibiotic use, poses significant risks. NSAIDs can lead to various complications, including miscarriage, premature birth, low birth weight, and organ failure in newborns, primarily due to the inhibition of prostaglandin synthesis as these drugs cross the placenta. A recently published study evaluating six years of data corroborates our findings, reporting that NSAIDs are the most commonly used drug class among pregnant women (32). Therefore, during pregnancy, even if pregnancy is suspected, painkillers should be avoided as much as possible, or they should be used in the lowest possible effective dose and for the shortest possible time. In our study, among the frequently used antidepressants, the most commonly prescribed were selective serotonin reuptake inhibitors (SSRIs), including fluoxetine, sertraline, and escitalopram. A meta-analysis reported that the prevalence of antidepressant use during pregnancy was approximately 1.5% in Europe and Australia, while in North America, this rate was 5.5%, with SSRIs being the most commonly utilized class (33). The use of antidepressants during pregnancy has been linked to an increased risk of gestational diabetes mellitus, spontaneous abortion, and premature birth (34, 35). Additionally, children exposed to antidepressants in utero may experience delays in psychomotor, cognitive, and language development, as well as an increased incidence of attention deficit hyperactivity and autism spectrum disorders (36, 37). In cases where patients are on antipsychotic medications or when individuals with major depression cannot postpone treatment, it is crucial to emphasize the importance of contraception to both patients and their partners. In instances of an unintended pregnancy, considering the risks of discontinuing treatment, the option of abortion may need to be discussed. Conversely, due to the toxic effects and significant risk of drug interactions associated with antidepressants, redirecting pregnant individuals using these medications for indications like social anxiety toward psychological consultation instead of pharmacological therapy may be a prudent approach. Our study found a high prevalence of antidepressant use among pregnant women, aligning with the previously discussed studies. Additionally, the identification of various DDIs associated with these medications underscores the seriousness of the situation.

In the study, significant data were obtained regarding polypharmacy and DDIs, topics for which there is limited information concerning pregnant women. However, because the study was conducted at a single center with a limited number of patients from a specific population, the results cannot be generalized. These were the limitation of the study.

CONCLUSION

Our study revealed that approximately 84% of the medications used by pregnant women pose uncertain safety profiles or potential teratogenic risks. Additionally, we found a high prevalence of polypharmacy and significant drug-drug interactions (DDIs) among this population. Therefore, it is essential for physicians to conduct thorough inquiries with patients who are planning or suspecting pregnancy and to clearly explain the associated risks before prescribing medications.

Active substances should be selected from low-risk categories whenever possible. Furthermore, educating pregnant women about rational drug use and highlighting the risks linked to polypharmacy is crucial for safeguarding both maternal and fetal health. In cases of potentially harmful drug use during pregnancy, it is crucial to seek immediate guidance from a pharmacologist or a teratogenicity service. The pregnant woman and her physician should be thoroughly informed about the potential consequences of medication use during pregnancy, including options for discontinuation, modification, postponement of treatment, or even considering pregnancy termination. Our study highlights that drug use during pregnancy represents a significant public health concern, necessitating a multidisciplinary approach to address it effectively. Nevertheless, further multicenter and large-scale studies are required to gain a more comprehensive understanding of this issue.

REFERENCES

1. Webster WS, Freeman JA. Prescription drugs and pregnancy. Expert Opin Pharmacother. 2003;4(6):949-61.
2. Andrade SE, Raebel MA, Morse AN, Davis RL, Chan KA., Finkelstein JA, et al. Use of prescription medications with a potential for fetal harm among pregnant women. Pharmacoepidemiol Drug Saf. 2006;15(8):546-54.
3. Lupattelli A, Spigset O, Twigg MJ, Zagorodnikova K, Mårdby AC, Moretti ME, et al. Medication use in pregnancy: a cross-sectional, multinational web-based study. BMJ Open. 2014;4(2):e004365.
4. Costa DB, Coelho HL, Santos DB. Use of medicines before and during pregnancy: prevalence and associated factors. Cad Saude Publica. 2017;33(2):e00126215.
5. Addis A, Sharabi S, Bonati M. Risk classification systems for drug use during pregnancy: are they a reliable source of information?. Drug Saf. 2000;23(3):245-53.
6. Ulusoy KG. Evaluation of pregnant women who applied to a university hospital for drug use during pregnancy: case series. Sakarya Med J. 2020;10(3):459-66.
7. Yuan S, Liu J, Larsson SC. Smoking, alcohol and coffee consumption and pregnancy loss: a Mendelian randomization investigation. Fertil Steril. 2021;116(4):1061-67.
8. Wu P, Chew-Graham CA, Maas AH, Chappell LC, Potts JE, Gulati M, et al. Temporal changes in hypertensive disorders of pregnancy and impact on cardiovascular and obstetric outcomes. Am J Cardiol. 2020;125(10):1508-16.
9. Thunbo MØ, Vendelbo JH, Volqvartz T, Witte DR, Larsen A, Pedersen LH. Polypharmacy in polymorbid pregnancies and the risk of congenital malformations-a systematic review. Basic Clin Pharmacol Toxicol. 2022;130(3):394-414.
10. Judistiani RTD, Pratiwi AE, Wahyudi K, Gunawan A, Rahmawati A, Ruslami R. Medication use and associated factors among Indonesian pregnant women: a cross-sectional study. J Multidiscip Healthc. 2023;16:4173-79.
11. Ayele Y, Mekuria AN, Tola A, Mishore KM, Geleto FB. Prescription drugs use during pregnancy in Ethiopia: a systematic review and meta-analysis. SAGE Open Med. 2020;8:2050312120935471.
12. Subramanian A, Azcoaga-Lorenzo A, Anand A, Phillips K, Lee SI, Cockburn N. et al. Polypharmacy during pregnancy and associated risk factors: a retrospective analysis of 577 medication exposures among 1.5 million pregnancies in the UK, 2000-2019. BMC Med. 2023;21(1):21.
13. Cascorbi I. Drug interactions-principles, examples and clinical consequences. Dtsch Arztebl Int. 2012;109(33-34):546-56.
14. Gnjidic D, Johnell K. Clinical implications from drug-drug and drug-disease interactions in older people. Clin Exp Pharmacol Physiol. 2013;40(5):320-25.
15. Ferracini AC, Rodrigues AT, Visacri MB, Stahlschmidt R, Silva NMOD, Surita FG, et al. Potential drug interactions and drug risk during pregnancy and breastfeeding: an observational study in a women's health intensive care unit. Rev Bras Ginecol Obstet. 2017;39(6):258-64.
16. Pessoa TL, Clemente Junior WS, Costa TXD, Bezerra PKDV, Martins RR. Drug interactions in maternal intensive care: prevalence, risk factors, and potential risk medications. Einstein (Sao Paulo). 2019;17(3):eAO4521.
17. Yang T, Walker MC, Krewski D, et al. Maternal characteristics associated with pregnancy exposure to FDA category C, D, and X drugs in a Canadian population. Pharmacoepidemiol Drug Saf. 2008;17(3):270-7.
18. Kao LT, Chen YH, Lin HC, Chung SD. Prescriptions for category D and X drugs during pregnancy in Taiwan: a population-based study. Pharmacoepidemiol Drug Saf. 2014;23(10):1029-34.
19. Gagne JJ, Maio V, Berghella V, Louis DZ, Gonnella JS. Prescription drug use during pregnancy: a population-based study in Regione Emilia-Romagna, Italy. Eur J Clin Pharmacol. 2008;64(11):1125-32.
20. van Gelder MM, van Rooij IA, Miller RK, Zielhuis GA, de Jong-van den Berg LT, Roeleveld N. Teratogenic mechanisms of medical drugs. Hum Reprod Update. 2010;16(4):378-94.
21. Pham A, Polic A, Nguyen L, Thompson JL. Statins in Pregnancy: Can We Justify Early Treatment of Reproductive Aged Women?. Curr Atheroscler Rep. 2022;24(8):663-70.

22. Abadie RB, Keller CL, Jones NT, Mayeux EL, Klapper RJ, Anderson L, et al. Review of Teratogenic Effects of Leflunomide, Accutane, Thalidomide, Warfarin, Tetracycline, and Angiotensin-Converting Enzyme Inhibitors. Cureus. 2023;15(12):e50465.
23. Tomson T, Battino D, Perucca E. Teratogenicity of antiepileptic drugs. Curr Opin Neurol. 2019;32(2):246-52.
24. International Commission on Radiological Protection. Pregnancy and medical radiation. Ann ICRP. 2000;30(1):iii-43.
25. Kulaga S, Zargarzadeh AH, Bérard A. Prescriptions filled during pregnancy for drugs with the potential of fetal harm. BJOG. 2010;117(3):373.
26. de Waard M, Blomjous BS, Hol MLF, Sie SD, Corpeleijn WE, van Goudoever JHB, et al. Medication use during pregnancy and lactation in a Dutch population. J Hum Lact. 2019;35(1):154-64.
27. Carmo TA, Nitrini SM. Drug prescription for pregnant women: a pharmacoepidemiological study. Cad Saude Publica. 2004;20(4):1004-13.
28. Adam MP, Polifka JE, Friedman JM. Evolving knowledge of the teratogenicity of medications in human pregnancy. Am J Med Genet C Semin Med Genet. 2011;157C(3):175-82.
29. Lurie Y, Bar M, Levdov IA, Tkachenko D, Bentur Y, Kurnik D. Adherence with prescription drugs in pregnant and breastfeeding women consulting with the Israel Poison Information Center Teratology Service. Clin Toxicol (Phila). 2021;59(6):457-63.
30. Kuperman AA, Koren O. Antibiotic use during pregnancy: how bad is it?. BMC Med. 2016;14(1):91.
31. Bookstaver PB, Bland CM, Griffin B, Stover KR, Eiland LS, McLaughlin M. A Review of antibiotic use in pregnancy. Pharmacotherapy. 2015;35(11):1052-1062.
32. Ying XH, Bao DN, Jiang HY, Shi YD. Maternal non-steroidal anti-inflammatory drug exposure during pregnancy and risk of miscarriage: a systematic review and meta-analysis. Eur J Clin Pharmacol. 2022;78(2):171-80.
33. Molenaar NM, Bais B, Lambregtse-van den Berg MP, Mulder CL, Howell EA, Fox NS, et al. The international prevalence of antidepressant use before, during, and after pregnancy: A systematic review and meta-analysis of timing, type of prescriptions and geographical variability. J Affect Disord. 2020;264:82-89.
34. Eke AC, Saccone G, Berghella V. Selective serotonin reuptake inhibitor (SSRI) use during pregnancy and risk of preterm birth: a systematic review and meta-analysis. BJOG. 2016;123(12):1900-07.
35. Wang XY, Ying XH, Jiang HY. Antidepressant use during pregnancy and the risk for gestational diabetes: a systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2023;36(1):2162817.
36. Morales DR, Slattery J, Evans S, Kurz X. Antidepressant use during pregnancy and risk of autism spectrum disorder and attention deficit hyperactivity disorder: systematic review of observational studies and methodological considerations. BMC Med. 2018;16(1):6.
37. Tanguay N, Abdelouahab N, Simard MN, Séguin JR, Marc I, Herba CM, et al. Antidepressants use during pregnancy and child psychomotor, cognitive and language development at 2 years of age-results from the 3D cohort study. Front Pharmacol. 2023;14:1252251.

RESEARCH ARTICLE

Merve Uzkar[1]
Celal Kus[2]
Raziye Sule Gumustakim[2]
Zeynep Yasemin Tas[2]
Mustafa Emre Eryilmaz[3]

[1] Department of Family Medicine, Kahramanmaraş Afşin State Hospital, Kahramanmaras, Türkiye
[2] Department of Family Medicine, Kahramanmaras Sutcu Imam University School of Medicine, Kahramanmaras, Türkiye
[3] Department of Family Medicine, Kahramanmaras Andırın State Hospital, Kahramanmaras, Türkiye

Corresponding Author:
Zeynep Yasemin Tas
mail:zeynepyasemintas7@gmail.com

Received: 01.07.2024
Acceptance: 10.10.2024
DOI:10.18521/ktd.1507815

It was presented as a verbal statement at the AHEKON Online Family Medicine Congress on 20-22 November 2020 in Turkey. We received the 3rd prize for oral presentations at the congress. Also, this article is based on the specialty thesis in medicine.

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

The Relationship of Internet-Social Media Use with Cognitive Functions and Depression Level in Adults over 50 Years

ABSTRACT

Objective: This study was conducted to evaluate the effects of internet-social media use on depression and cognitive functions in individuals aged 50 and over.

Method: This research is a cross-sectional descriptive study. The sample of the research consisted of 398 people. Data were collected by face-to-face interview using a questionnaire, Geriatric Depression Scale-Short Form and Montreal Cognitive Assessment Scale.

Results: The average age of the participants in the study was determined as 59.8±7.8 years. It was determined that 40.7% of individuals use social media, 23.9% find social media useful, and 37.4% use social media for communication purposes. It was determined that independent variables such as age, education, income level, and whom one lives with at home significantly affected the depression level and cognitive dysfunction parameters ($p<0.001$). Cognitive dysfunctions were significantly lower in individuals who used the internet and social media than in those who did not ($p<0.001$). According to logistic regression analysis, it was determined that older age increased the probability of cognitive dysfunction by 1.06 times, and illiteracy increased by 178.10 times.

Conclusions: In our study, cognitive dysfunction and depression levels were determined to be significantly lower in middle-aged and elderly individuals who use the internet and social media. The risk of cognitive dysfunction is higher in elderly, illiterate, literate or primary school graduates who do not know how to use the internet and social media, compared to younger, university graduates and individuals who use the internet and social media.

Keywords: Cognitive Function, Depression, Elderly, Social Media, Internet.

50 Yaş Üstü Erişkinlerde İnternet-Sosyal Medya Kullanımının Bilişsel Fonksiyonlar ve Depresyon Düzeyi ile İlişkisi

ÖZET

Amaç: Bu çalışma, 50 yaş ve üzeri bireylerde internet-sosyal medya kullanımının depresyon ve bilişsel işlevler üzerindeki etkilerini değerlendirmek amacıyla yapılmıştır.

Yöntem: Bu araştırma kesitsel tanımlayıcı bir çalışmadır. Araştırmanın örneklemini 398 kişi oluşturdu. Veriler, anket, Geriatrik Depresyon Ölçeği-Kısa Form ve Montreal Bilişsel Değerlendirme Ölçeği kullanılarak yüz yüze görüşme yoluyla toplandı.

Bulgular: Araştırmaya katılanların yaş ortalaması 59,8±7,8 yıl olarak belirlendi. Bireylerin %40,7'sinin sosyal medyayı kullandığı, %23,9'unun sosyal medyayı faydalı bulduğu, %37,4'ünün sosyal medyayı iletişim amaçlı kullandığı belirlendi. Yaş, eğitim, gelir düzeyi, evde kiminle yaşadığı gibi bağımsız değişkenlerin depresyon düzeyi ve bilişsel işlev bozukluğu parametrelerini anlamlı düzeyde etkilediği belirlendi ($p<0,001$). İnternet ve sosyal medya kullanan bireylerde, kullanmayanlara göre bilişsel işlev bozuklukları anlamlı düzeyde daha düşüktü ($p<0,001$). Lojistik regresyon analizine göre ileri yaşın bilişsel işlev bozukluğu olasılığını 1,06 kat, okuma yazma bilmemenin ise 178,10 kat arttığı belirlendi.

Sonuç: Çalışmamızda internet ve sosyal medya kullanan orta yaşlı ve yaşlı bireylerde bilişsel işlev bozuklukları ve depresyon düzeylerinin anlamlı derecede düşük olduğu belirlendi. İnternet ve sosyal medyayı kullanmayı bilmeyen yaşlı, okuma-yazma bilmeyen, okuryazar veya ilkokul mezunu kişilerde, gençlere, üniversite mezunlarına, internet ve sosyal medya kullanan bireylere göre bilişsel işlev bozukluğu riski daha yüksektir.

Anahtar Kelimeler: Bilişsel İşlev, Depresyon, Yaşlı, Sosyal Medya, İnternet.

INTRODUCTION

People between the ages of 65-74 are classified as "young elderly", people between the ages of 75-84 are classified as "elderly", and people aged 85 and over are classified as "senile" (1). In addition to the progression of chronological age, physiological changes in organs and systems, decreases in physical and cognitive capacities, and the emergence of diseases are among the characteristics of old age (2).

As individuals get older, muscle weakness increases and accompanying chronic diseases make individuals even weaker. These reasons push the elderly to stay in their homes longer and make it difficult to go out. Depression is one of the most important issues that should be emphasized, which is most common in old age and negatively affects the life quality of the person. Death of relatives, decrease in social environment and feeling of loneliness, increase in chronic diseases, worsening of health, deterioration in cognitive functions, and economic concerns are the main causes of depression in old age (3, 4).

With the increase in the use of the internet and social media, it is possible to chat with friends and relatives who are far away from home, and meet new people. Thus, individuals feel less alone. Studies have also shown that internet use affects psychology in a good way, and the general health status of internet users is better (5).

The decline in cognitive functions that occur with advancing age and the increasing dementia make the life of the elderly quite difficult. Internet use activates various brain regions (6). The use of smart phones positively affects cognitive functions, prevents loneliness, and improves mental health (7).

The internet and social media can be useful in improving the quality of life of individuals by preventing cognitive dysfunction and depression. In our study, we examined the effects of internet and social media use on cognitive functions and depression in middle-aged and elderly individuals.

MATERIAL AND METHODS

Study Design and Population: This is a cross-sectional and descriptive study conducted to evaluate the effects of internet-social media use on depression and cognitive functions in adults aged 50 and over living in Kahramanmaraş province. The data was collected from the relatives of patients living in Kahramanmaraş who applied to Kahramanmaraş Medical Faculty Health Practice and Research Hospital between January and March 2020.

Sample Size Determination: The population of our study consists of a total of 232,233 people consisting 115,271 men and 116,962 women over the age of 50 and living in Kahramanmaraş. The sample size was based on 50% frequency for cases where prevalence was unknown; The sample size was determined as 403 people with a 5% margin of error and a 95% confidence interval. Since there were missing data in the survey forms, 5 participants were excluded from the study and the data of 398 people were included in the study.

A questionnaire was applied to volunteers aged 50 and over. Participants were informed before the administration of the questionnaire and were included in the study after providing their consent. The questionnaire prepared for individuals aged 50 and over who voluntarily participated in our study was applied by the researcher by face-to-face interview method. A standard 27-item questionnaire, the Geriatric Depression Scale (GDS-15)-Short Form to evaluate depression, and the Montreal Cognitive Assessment Scale (MoCA) to evaluate cognitive functions were used in the survey.

Data Collection Instruments: The volunteers participating in the study were first presented with a questionnaire consisting of 27 items. This questionnaire included questions about sociodemographic characteristics such as age, gender, occupation; about characteristics related to the social life of the participants, such as the people they live with; about internet-social media knowledge and use, purposes of internet-social media use, membership status on social media sites, opinions and preferences regarding internet-social media.

GDS-15 is a scale used for screening depression in the elderly and can be applied quickly and easily. Geriatric Depression Scale consisting of 30 questions was developed by Yesavage et al. in 1983 (8). In terms of ease of use, the validity and reliability of the 15-item short form was established by Burke et al. in 1991 (9). In Turkey, its validity and reliability were established by Durmaz et al. in 2018 (10).

(GDS-15) Short Form consists of 15 questions, and 5 questions (1, 5, 7, 11 and 13) are structured positively and the other questions are structured negatively. Answers of "no" to positive questions in the scale and "yes" to negative questions were accepted as 1 point. A score of 0-4 indicates no depression, a score of 5-8 indicates mild depression, a score of 9-11 indicates moderate depression, and a score of 12-15 indicates severe depression. The cut-off score was accepted 5 (9).

MoCA is used to distinguish normal healthy individuals from individuals with mild cognitive impairment. The scale was developed by Nasreddine et al. in 2005 (11). In 2010, it was adapted into Turkish by Selekler et al. The scale evaluates 8 cognitive functions including visuo-spatial skills, executive functions, attention and concentration, memory, abstract thinking, language, calculation and orientation. The lowest score that can be obtained from the scale is 0, and the highest score is 30. A score of 21 or more on the scale is considered normal (12).

Ethical Considerations: Ethics committee approval was obtained from the Kahramanmaraş Faculty of Medicine Non-Pharmaceutical Clinical Research Ethics Committee with the date 23.01.2020 and number 25.

Statistical Analysis: SPSS version 23.0 statistical package program was used in the analysis of the data. In the representation of the descriptive statistics of the study, mean ± standard deviation (SD) and median, interquartile range (IQR), minimum-maximum values were used for continuous numerical values, and number (n) and percentage (%) were used for categorical variables. Chi-square test was used to compare the categorical variables in the comparison of the groups. Variables found to be associated with cognitive dysfunction in univariate analyzes were included in Backward Conditional Logistic Regression Modeling, and multivariate analysis was performed with the last valid model after sequential models. The cut-off value of statistical significance was accepted as $p<0.05$.

RESULTS

This study was conducted with 398 middle-aged and elderly individuals between the ages of 50-82 in Kahramanmaraş province. While 163 (41.0%) of 398 individuals participating in the study were between the ages of 55-64, the mean age was 59.8±7.8 years. 207 (52.0%) of the participants were women and 319 (80.2%) were married. 245 (61.6%) middle-aged and elderly individuals stated that their income barely covers their expenses. 175 (44.0%) of the participants reported that they live with their spouses and children, and 144 (36.2%) reported that they only live with their spouses. Considering the chronic disease and regular drug use status of middle-aged and elderly individuals, 242 (60.8%) of the individuals have a diagnosis of a chronic disease, while 235 (59.0%) use drugs regularly.

While 40.7% of 398 individuals participating in our study know how to use the internet, 59.3% do not know how to use it.

Almost half of the participants are members of at least one social media platform. Among the social media sites, WhatsApp (45.7%) is the most subscribed application. This is followed by Facebook (20.1%), Instagram (13.8%) and Twitter (5.5%) (Table 1).

Table 1. The characteristics of the participants' internet and social media usage.

Characteristic		**n**	**%**
Knowledge on how to use internet	Knowing	162	40.7
	Unknowing	236	59.3
Internet usage purpose	Research-knowledge*	93	23.4
	E-mail*	42	10.6
	Staying up to date*	89	22.4
	Entertainment*	60	15.1
Meeting with family via the Internet	Available	214	53.8
	Not available	184	46.2
Knowledge on how to use social media	Knowing	162	40.7
	Unknowing	236	59.3
The meaning of social media for the elderly	Sharing*	98	24.6
	Communication*	162	40.7
	Entertainment*	86	21.6
	No idea	52	13.0
Social media membership	Available	196	49.2
	Not available	202	50.8
	Facebook*	80	20.1
	Twitter*	22	5.5
	Instagram*	55	13.8
	WhatsApp*	182	45.7
Total		**398**	**100.0**

* Participants marked more than one option.

When we look at the reasons for those who want to receive training to learn about the Internet, there are reasons such as being able to use online banking, being able to do e-shopping, and making reservations for a holiday (Table 2).

Table 2. The distribution of the participants' their internet usage preferences.

Characteristic		n	%
Preferences regarding internet use	I want to meet new people and make new friends	14	3.5
	I would like to communicate with my current friends, relatives, family	149	37.4
	I would like to call my old friends and reconnect	47	11.8
	I would like to play games	8	2.0
	I would like to use it for educational purposes	58	14.6
	I would like to share the objects I like	25	6.3
	Whatever maybe the reason, I prefer not to use	86	21.6
	I use it for my job	11	2.8
Total		**398**	**100.0**

When the depression levels of the participants were statistically compared according to their sociodemographic characteristics, the level of depression increased with advancing age **(p<0.001)**, while the level of depression in men was significantly higher than in women **(p=0.033).** Furthermore, while depression is less common in married people, moderate and severe depression is statistically significantly higher in divorced or widowed individuals **(p<0.001).** The risk of depression decreases as the education level increases. While moderate and severe depression is not observed at all in individuals whose income is more than their expenses, moderate and severe depression is observed statistically significantly more frequently in those whose expenses are less than their income compared to other groups **(p<0.001)** (Table 3).

When the depression levels of the participants were statistically compared according to the social life and clinical characteristics of the participants, it is seen that the incidence of depression is lower in those living with their spouses and children and in those living only with their spouses. Severe depression is statistically significantly higher, especially in those living alone or with their children **(p<0.001)** (Table 3).

Additionally, the level of depression is statistically significantly higher in individuals with chronic diseases **(p<0.001)** (Table 3).

When cognitive dysfunction is statistically compared according to the sociodemographic characteristics of the participants, the frequency of cognitive dysfunction increases significantly with advancing age. In addition, cognitive dysfunction is seen statistically significantly more frequently in middle-aged and elderly individuals who are divorced or widowed. On the other hand, the incidence of cognitive dysfunction decreases significantly as the education level and income level of the individuals increase.

When the cognitive dysfunction status of the participants was compared statistically according to the social life and clinical characteristics of the participants, middle-aged and elderly individuals living only with their children have statistically significantly more cognitive dysfunction **(p=0.001)** (Table 3).

Table 4 shows participants' depression levels and cognitive dysfunction according to their social media and internet usage. It was determined that the levels of cognitive dysfunction and depression were statistically significantly lower in individuals who knew how to use the internet and social media and used them on a daily basis **(p<0.001)** (Table 4). Statistical significance was determined in all parameters

It was found that the prevalence of cognitive dysfunction is statistically significantly higher as the depression severity levels of middle-aged and elderly individuals increases **(p<0.001)** (Table 5).

The results of the multivariate analysis of the effects of some characteristics of the participants on their cognitive dysfunction status are presented. In the first stage, characteristics such as age, marital status, educational status, income level, place of residence, other people living in the residence, regular exercise status, chronic disease presence, knowledge on internet use, daily internet use, knowledge on social media use, daily social media use, social media membership, which were significantly associated with cognitive dysfunction in univariate analyzes, were included to the logistic regression model. In 11th model prepared with the Backward Conditional method, the variables of age, education level, knowledge on internet use, daily internet use, knowledge on social media use and other people living in the residence were included. According to this model, all parameters were statistically significant (Table 6).

In logistic regression analysis, older age increased the risk of cognitive dysfunction by 1.06 times. Compared to being a university graduate, illiteracy increased the risk of cognitive dysfunction by 178.10 times. Compared to knowing how to use the Internet, not knowing increased the risk of cognitive dysfunction by 14.65 times (Table 6). At least 40% of the variance is explained by the variables in the model and the overall success rate of the model is 90%.

Table 3. Analysis of depression levels and cognitive dysfunctions according to participants' social lives and clinical characteristics

	Depression level								***p****	**Cognitive Dysfunctions**				***p****
	1		2		3		4			Not Available		Available		
	n	%	n	%	n	%	n	%		n	%	n	%	
Age									**<0.001**					**<0.001**
<55	80	63.0	22	17.3	17	13.4	8	6.3		78	61.4	49	38.6	
55-64	124	76.1	27	16.6	11	6.7	1	0.6		87	53.4	76	46.6	
65-74	29	33.0	31	35.2	15	17.0	13	14.8		25	28.4	63	71.6	
75-84	8	40.0	11	55.0	1	5.0	0	0		0	0	20	100	
Gender									**0.033**					0.076
Woman	121	58.5	43	20.8	32	15.5	11	5.3		90	43.5	117	56.5	
Man	120	62.8	48	25.1	12	6.3	11	5.8		100	52.4	91	47.6	
Marital Status									**<0.001**					**<0.001**
Married	216	67.7	64	20.1	29	9.1	10	3.1		164	51.4	155	48.6	
Never married	10	43.5	11	47.8	0	0	2	8.7		15	65.2	8	34.8	
Divorced	15	26.8	16	28.6	15	26.8	10	17.9		11	19.6	45	80.4	
Education Level									**<0.001**					**<0.001**
Illiterate	17	42.5	8	20.0	10	25.0	5	12.5		0	0	40	100	
Literate	9	50.0	6	33.3	3	16.7	0	0		2	11.1	16	88.9	
Income Rate									**<0.001**					**<0.001**
Income > Expense	64	90.1	7	9.9	0	0	0	0		51	71.8	20	28.2	
Income = Expense	135	55.1	61	24.9	33	13.5	16	6.5		113	46.1	132	53.9	
Income < Expense	42	51.2	23	28.0	11	13.4	6	7.3		26	31.7	56	68.3	
People Who Live With									**<0.001**					**0.001**
Spouse and Child	120	68.6	28	16.0	19	10.9	8	4.6		99	56.6	76	43.4	
Spouse	95	66.0	36	25.0	11	7.6	2	1.4		65	45.1	79	54.9	
Child	11	31.4	13	37.1	6	17.1	5	14.3		6	17.1	29	82.9	
Alone	14	36.8	9	23.7	8	21.1	7	18.4		17	44.7	21	55.3	
Other	1	16.7	5	83.3	0	0	0	0		3	50.0	3	50.0	
Chronic Disease									**<0.001**					**<0.001**
Yes	122	50.4	66	27.3	37	15.3	17	7.0		93	38.4	149	61.6	
No	119	76.3	25	16.0	7	4.5	5	3.2		97	62.5	59	37.8	
Total	241	60.6	91	22.9	44	11.1	22	5.5		190	47.7	208	52.3	

1 No Depression 2 Mild Depression 3 Moderate Depression 4 Severe Depression

Table 4. Depression levels and cognitive dysfunction status analysis of the participants according to their social media and internet usage

	Depression level								***p****	**Cognitive Dysfunctions**				***p****
	1		2		3		4			Not Available		Available		
	n	%	n	%	n	%	n	%		n	%	n	%	
Knowledge on how to use Internet									**<0.001**					**<0.001**
Knowing	114	70.4	35	21.6	9	5.6	4	2.5		137	84.6	25	15.4	
Unknowing	127	53.8	56	23.7	35	14.8	18	7.6		53	22.5	183	77.5	
Daily internet use									**<0.001**					**<0.001**
Using	110	70.5	34	21.8	8	5.1	4	2.6		131	84.0	25	16.0	
Not using	131	54.1	57	23.6	36	14.9	18	7.4		59	24.4	183	75.6	
Knowledge on how to use social media									**<0.001**					**<0.001**
Knowing	172	74.3	35	18.3	9	4.7	5	2.6		151	79.1	40	20.9	
Unknowing	99	47.8	56	27.1	35	16.9	17	8.2		39	18.8	168	81.2	
Daily social media use									**<0.001**					**<0.001**
Using	141	72.7	34	17.5	15	7.7	4	2.1		147	75.8	47	24.2	
Not using	100	49.0	57	27.9	29	14.2	18	8.8		43	21.1	161	78.9	
Social media membership									**<0.001**					**<0.001**
Have	143	73.0	34	17.3	15	7.7	4	2.0		149	76.0	47	24.0	
Does not have	98	48.5	57	28.2	29	14.4	18	8.9		41	20.3	161	79.7	
Total	241	60.6	91	22.9	44	11.1	22	5.5		190	47.7	208	52.3	

1 No Depression 2 Mild Depression 3 Moderate Depression 4 Severe Depression

Table 5. Cognitive dysfunction status of participants according to depression level

	Cognitive Function Disorder				*p**
	Not Available		Available		
	n	%	n	%	
Depression Level					**<0.001**
No depression	147	61.0	94	39.0	
Mild depression	28	30.8	63	69.2	
Moderate depression	11	25.0	33	75.0	
Severe depression	4	18.2	18	81.8	
Total	190	47.7	208	52.3	

Table 6. Results of multivariate analysis of the effects of some characteristics of the participants on cognitive dysfunction status.

		Risk of cognitive dysfunction			*p**
		OR	95% GA		
Age		1.066	1.005	1.129	**0.032**
Education status	University	Reference			
	High school	-	-	-	-
	Secondary school	4.242	0.801	22.458	0.089
	Primary school	64.526	11.453	363.528	**<0.001**
	Literate	93.495	8.677	1007.417	**<0.001**
	Illiterate	178.109	32.535	975.026	**<0.001**
Knowledge on Internet	Knowing	Reference			
	Unknowing	14.655	3.062	70.135	**0.001**
Daily internet use	Using	Reference			
	Not using	4.983	0.965	25.730	0.055
Knowledge on social media	Knowing	Reference			
	Unknowing	2.945	1.151	7.535	**0.024**
Other people living in the residence	Spouse and child	Reference			
	Spouse	0.938	0.194	4.546	0.937
	Child	16.170	0.929	281.307	0.056
	Alone	2.150	0.261	17.742	0.477
	Other	0.574	0.121	2.717	0.484

DISCUSSION

When looking at the literature, there are many studies on the internet usage characteristics, depression status and cognitive functions of the elderly. However, there are very few studies in the world on the effects of social media and internet use on depression and cognitive functions. More than half of the 398 individuals who participated in our study did not know how to use the internet. Similar findings were obtained in studies in the literature. In the study conducted by Tekedere and Arpacı (13) with 106 middle-aged and elderly individuals living in nursing homes, it was determined that 41.5% of the participants knew how to use the internet, and 58.5% did not.

It was determined that those who know how to use the internet use the internet for research and information (23.4%), staying up to date (22.4%), entertainment (22.4%) and e-mail (10.6%). In the study of Tekedere and Arpacı (13), it was found that 70.5% of the elderly people used the internet for research and knowledge, 15.9% for e-mail, 9.1% for staying up to date and 4.5% for entertainment purposes. Loipha (14) revealed that the elderly use the internet for the purpose of obtaining information, following the news, social interaction and entertainment. In some studies, users mostly use the internet for social networking sites, following the news, and obtaining information (15). In many studies, similar results to our study were obtained.

In our study, WhatsApp was the most subscribed application, while various other studies have found that Facebook is the social media platform that the elderly are most subscribed to (16-19). In our study, it was seen that some elderly people wanted to learn how to use the internet for reasons such as being able to use internet banking, doing e-shopping, making holiday reservations.

This shows that some of the elderly are aware of the conveniences of technological life and they want to adapt to it and make their lives easier.

In our study, the level of depression was statistically significantly higher in individuals with chronic diseases. In a study conducted by Aksoy (20), it was reported that the presence of two or more chronic diseases increased the risk of depression. The effects and side effect profiles of drugs used for chronic diseases may also cause an increase in the frequency of depression.

In our study, marital status appears to have a statistically significant effect on depression. Aksoy (20) and Çınar (21) found that the depression levels of deceased and divorced elderly people were higher than married elderly people. Feelings of loneliness, loss of a spouse, and fulfilling the responsibilities of daily life alone may be factors that increase depression in single, widowed, or divorced elderly people.

In our study, the increase in the frequency of depression with decreasing education level was found to be statistically significant. Many studies have shown that the frequency of depression increases in individuals with low education level (22,23). The fact that people with education have better communication skills, learn to cope with problems better, and have less financial anxiety may explain why depression is less common.

When we look at the depression levels of the participants according to the income level, it is seen that the depression levels decrease as the income level increases, both in our study and in the study of Bingöl et al. (23) in people over the age of 65. Excessive anxiety, not eating healthy, low self-care may be the cause of this situation.

In our study, it was found that the level of depression decreases significantly in people who know and use the internet and social media, and there are studies in the literature that support this finding. In a study conducted with adults over 50 years of age in the USA, it was found that internet use contributes positively to the mental health of the elderly and reduces depression by 20-28% (19). In a study conducted in Korea, it was observed that adult internet users had better social relationships, lower levels of depression and fewer suicidal thoughts (24). According to a study conducted in China, depression levels also decrease significantly with internet use. According to this study conducted in China, internet use and online activities can reduce loneliness, increase and strengthen social relationships. Thus, it can reduce the level of depression in older adults (25). In the study conducted by Lin et al. (26) using GDO-15, as in our study, depressive symptoms were significantly higher in non-mobile phone users (27.2%) than in mobile phone users (10.6%).

In many studies, although the mechanism is unclear and controversial, it is seen that internet use has a positive effect on reducing depression levels in older adults and is similar to the findings in our study.

As the incidence of dementia is reported to be increasing in Turkey, protecting cognitive functions is becoming increasingly important (27). A study conducted in Singapore showed that more frequent (occasional or daily) use of a mobile phone caused a decrease in cognitive functions, attention and memory compared to those who never or rarely used a mobile phone (28). Lin et al.'s (26) study in 2020, using the MoCA scale as in our study, found that mild cognitive impairment was significantly higher in non-mobile phone users than in mobile phone users. The cohort study conducted by Almeida et al. (29) followed elderly men for 8.5 years and showed that the risk of dementia in elderly people who use computers is 30-40% lower than in those who do not use computers. Our study is similiar to other studies and our findings show that internet, social media and smartphone use can delay or prevent dementia by reducing the impairment in cognitive functions.

In our study, as the severity of depression increases, cognitive dysfunction increases statistically significantly. In the study of Salık et al. (30), it was stated that having depression is a risk factor for cognitive dysfunction, and this finding supports our study.

Study Limitations: The limitations of our study were as follows that illiterate people could not answer the first 3 questions in the Montreal Cognitive Assessment Scale, that there were few participants, especially in the 75-year-old group and those with a high level of education, and not questioning the start and duration of using social media and internet in our study, and not examining depression and laboratory parameters that may affect cognitive functions (such as vitamin D, vitamin B12, folic acid, thyroid hormones).

CONCLUSION

In our study, it was determined that the levels of cognitive dysfunction and depression were significantly lower in middle-aged and elderly individuals who use the internet and social media. Depression levels are higher in elderly individuals who do not know how to use the internet and social media, in individuals whose income is lower than their expenses, and in individuals with chronic diseases.

The risk of cognitive dysfunction is higher in older individuals who do not know how to use social media than in individuals who use social media.

A significant positive relationship was found between high levels of depression and cognitive dysfunction. For older individuals who do not want to use the internet and social media, practical applications can be developed to encourage their use and training programs can be offered so that they can learn to use it effectively.

REFERENCES

1. Dündar Ö, Alptekin V. Türkiye'de yaşlı nüfusun huzurevi tercihinin mekansal bağımlılığı. Journal of Economy Culture and Society. 2022;65:267-87.
2. Atkinson RL, Atkinson RC, Smith EE, Bem DJ, Hoeksema SN. Psikolojiye giriş. 5. Baskı, (Y. Alogan, Çev), Ankara: Arkadaş Yayınları; 2010.
3. Kaçan H, Değer VB. Yaşlının kendini ihmal etmesinde yalnızlık, depresyon ve ölüm kaygısının etkisi. Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi. 2024;13(1):231-42.
4. Polat F, Geçici F. Yaşlı bireylerin algıladığı yalnızlık düzeyi ile depresyon arasındaki ilişki. Journal of Inonu University Health Services Vocational School. 2020;8(1):72– 82.
5. Kandemir BH, Güre MDP. Yaşlılarda teknoloji kullanımının toplumsal hayata katılıma ve başarılı yaşlanmaya etkisinin incelenmesi. Tıbbi Sosyal Hizmet Dergisi. 2024;22:60-82.
6. Weinstein A, Lejoyeux M. Neurobiological mechanisms underlying internet gaming disorder. Dialogues Clin Neurosci. 2020;22(2):113-26.
7. Andrews JA, Brown LJ, Hawley MS, Astell AJ. Older adults' perspectives on using digital technology to maintain good mental health: Interactive group study. Journal of medical Internet research. 2019;21(2):e11694.
8. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, et al. Development and validation of a geriatric depression screening scale: a preliminary report. Journal of Psychiatric Research. 1982;17(1):37-9.
9. Burke WJ, Roccaforte WH, Wengel SP. The short form of the Geriatric Depression Scale: a comparison with the 30-item form. Journal of geriatric psychiatry and neurology. 1991;4(3):173–78.
10. Durmaz B, Soysal P, Ellidokuz H, Isik AT. Validity and reliability of geriatric depression scale-15 (short form) in Turkish older adults. Northern clinics of Istanbul. 2018;5(3):216–20.
11. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. Journal of the American Geriatrics Society. 2005;53(4):695–99.
12. Selekler K, Cangöz B, Uluc S. Montreal Bilişsel Değerlendirme Ölçeği ' nin hafif bilişsel bozukluk ve Alzheimer hastalarının ayırt edebilme gücünün incelenmesi. Turkish Journal of Geriatrics. 2010;13(3):166-71.
13. Tekedere H, Arpacı F. Orta yaş ve yaşlı bireylerin internet ve sosyal medyaya yönelik görüşleri. Türkiye Sosyal Araştırmalar Dergisi. 2016;20(2):377- 92.
14. Loipha S. Thai elderly behavior of internet use. Procedia-Social and Behavioral Sciences. 2014;147:104-10.
15. Abacı R, Kazaz N, Başa B. The internet addiction of Kosovo and Turkey elderly people. Procedia-Social and Behavioral Sciences. 2013;103:1104-117.
16. Bianchi C. Exploring how internet services can enhance elderly well-being. Journal of Services Marketing. 2021;35(5):585-03.
17. Becerikli SY. Kuşaklar arası iletişim açısından yeni iletişim teknolojilerinin kullanımı: İleri yaş grubu üzerine bir değerlendirme. İstanbul Üniversitesi İletişim Fakültesi Dergisi / İstanbul University Faculty of Communication Journal. 2013;44:19-31.
18. Hofer M, Hargittai E. Online social engagement, depression, and anxiety among older adults. New Media Society. 2024;26(1):113-30.
19. Cotten SR, Ford G, Ford S, Hale TM. Internet use and depression among older adults. Comput Hum Behav. 2012;28(2):496–99.
20. Aksoy ZB. Kronik hastalığı olan bireylerde psikolojik kırılganlık ve depresyon düzeylerinin belirlenmesi [doctoral dissertation]. Karabük Üniversitesi; 2024.
21. Özen Çınar İ, Kartal A. Yaşlılarda depresif belirtiler ve sosyodemografik özellikler ile ilişkisi. Preventive Medicine Bulletin. 2008;7(5):399-04.
22. Curran E, Rosato M, Ferry F, Leavey G. Prevalence and factors associated with anxiety and depression in older adults: Gender differences in psychosocial indicators. Journal of Affective Disorders. 2020;267:114-22.
23. Bingöl G, Demir A, Karabek R, Kepenek B, Yıldırım N. Bazı değişkenler açısından 65 yaş üstü bireylerin depresyon düzeylerinin incelenmesi. Göztepe Tıp Dergisi. 2010;25(4):169-76.
24. Jun HJ, Kim MY. What accounts for the relationship between internet use and suicidal ideation of Korean older adults? A mediation analysis. Journals of Gerontology. 2017;72(5):846–55.
25. Szabo A, Allen J, Stephens C, Alpass F. Longitudinal analysis of the relationship between purposes of internet use and well-being among older adults. The Gerontologist. 2019;59(1):58-8.
26. Lin L, Jing XC, Lv SJ, Liang HJ, Tian L, Li HL, et al. Mobile device use and the cognitive function and depressive symptoms of older adults living in residential care homes. BMC Geriatrics. 2020;20:1-8.
27. Akyol MA, Zehirlioğlu L, Erünal M, Mert H, Hatipoğlu NŞ, Küçükgüçlü Ö. Determining middle-aged and older adults' health beliefs to change lifestyle and health behavior for dementia risk reduction. American Journal of Alzheimer's Disease Other Dementias. 2020;35.
28. Ng TP, Lim ML, Niti M, Collinson S. Long-term digital mobile phone use and cognitive decline in the elderly. Bioelectromagnetics. 2012;33(2):176–85.

29. Almeida OP, Yeap BB, Alfonso H, Hankey GJ, Flicker L, Norman PE. Older men who use computers have lower risk of dementia. PloS one. 2012;7(8):e44239.
30. Salık S, Çakmak S, Uğuz Ş. Tedavi almamış major depresyon hastalarında erken dönemde bilişsel işlevler. Klinik Psikiyatri Dergisi. 2019;22:408-15.

RESEARCH ARTICLE

Arif Garbioglu[1]

[1] Zonguldak Bulent Ecevit University Faculty of Medicine Department of Forensic Medicine, Zonguldak, Türkiye

Corresponding Author:
Arif Garbioglu
mail: dr.arifgarbioglu@gmail.com

Received: 22.05.2024
Acceptance: 14.02.2025
DOI:10.18521/ktd.1488540

A part of this study was presented as an oral presentation at the 4th International 20th National Forensic Sciences Congress in Izmir, Turkey on 2-5 November 2023.

A Different Concept in Disability Assessment: Compulsory Individual Accident Insurance for Mine Workers

ABSTRACT

Objective: This study aimed to determine the reflection of the Mine Workers Compulsory Personal Accident Insurance (MWCPAI) on forensic medicine practices and to identify its possible deficiencies compared to the Regulation of Permanent Disabilities (RPD).

Method: The subject of the study was the cases for the recourse claim from the insurance company for the compensation paid by the workplace to the miners injured due to occupational accidents. The study encompassed cases submitted by judicial authorities requesting the determination of coverage percentage (CP) according to the MWCPAI.

Results: All cases (n = 18) were male, with a mean age of 36.05 ± 5.4 years. The two most common events were object fall or impact (n = 6, 33.3%) and crushing (n = 5, 27.8%), respectively. The most prevalent injury was lower extremity injury. Although 44.4% of the cases recovered without sequelae, half of these cases were deemed disabled according to RPD. When the cases were assessed based on the handicap coverage list (HCL) of the MWCPAI, it was found that eight cases (44.4%) who recovered without sequelae did not qualify for coverage. As 50% of the cases did not precisely match any categories in the HCL, they were chosen using a comparative method, and CPs were determined by discretion, considering the extent of functional limitation.

Conclusions: In response to the occupational accidents prevalent in the mining sector, it is imperative to develop MWCPAI. MWCPAI would not only furnish insurance coverage for miners but also play a pivotal role in fostering safe working environments within the mining sector. MWCPAI is attributable to its mandate for insurance companies to assess the risks inherent in mining operations.

Keywords: Mining, Occupational Accident, Insurance, Disability.

Maluliyet Değerlendirmesinde Farklı Bir Kavram: Maden Çalışanları Zorunlu Ferdi Kaza Sigortası

ÖZET

Amaç: Çalışmada Maden Çalışanları Zorunlu Ferdi Kaza Sigortası'nın (MÇZFKS) adli tıp uygulamalarına yansıması ve maluliyet yönetmeliğine göre olası eksikliklerinin saptanması amaçlanmaktadır.

Yöntem: İş kazasına bağlı yaralanma sonucu madencilere ödenen tazminatın, işyerince kazanın yaşandığı dönemi içerisine alan MÇZFKS'yi yapan şirketten rücuen istenmesine yönelik açılan dava dosyalarından MÇZFKS'ye göre teminat yüzdesi belirlenmesi istemiyle gönderilmiş olgular çalışmaya dahil edildi.

Bulgular: Olguların tamamı (n: 18) erkek olup, yaş ortalaması 36,05±5,4 idi. En sık iki olay sırasıyla cisim düşmesi veya çarpması (n: 6, %33,3) ve ezilmeydi (n: 5, %27,8). En sık alt ekstremite yaralanması olduğu belirlendi. Olguların %44,4'ünün yaralanmasının sekelsiz iyileşmesine rağmen bu olguların yarısının maluliyet yönetmeliğine göre karşılığı bulunduğundan maluliyet oranı hesaplanmıştı. Olgular MÇZFKS sakatlık teminat listesine göre değerlendirildiğinde sekelsiz iyileşen 8 olgunun (%44,4) teminat yüzdesinin olmadığı saptandı. Olguların %50,0'sinin ise sakatlık teminatı listesinde tam karşılığı bulunmadığından kıyas yöntemi ile seçilerek fonksiyonel kısıtlılığı ölçüsünde takdir uygulanıp teminat yüzdesi belirlendi.

Sonuç: Madencilik sektöründe meydana gelen iş kazalarının göz önüne alınarak madencilerin hukuki kazanımları açısından MÇZFKS geliştirilmelidir. MÇZFKS, madencilere sigorta güvencesinin sağlamasının yanında maden ocaklarının sigorta şirketlerince yaptırılacak risk incelemesini de içerdiğinden madencilik sektöründe güvenli çalışma ortamlarının sağlanmasına da katkı sağlayacaktır.

Anahtar Kelimeler: Madencilik, İş Kazası, Sigorta, Maluliyet.

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

INTRODUCTION

The International Labour Organization (ILO) reports that approximately 3 million employees lose their lives each year due to work-related occupational diseases and accidents, according to its latest global estimates. In addition to the suffering experienced by workers and their families, work-related deaths also impose an economic burden at both national and international levels. According to ILO data, disruptions in production, compensation, lost workdays, and health expenditures account for 3.94% of the global annual GDP (1,2). The country with the highest occupational death rate is Cuba, while Costa Rica emerges as the country with the highest rate of non-fatal occupational injuries. Turkey ranks high with a 6.3 occupational death rate and a 2,459 non-fatal occupational injury rate per 100,000 workers (3). In 2022, 1,517 workers in Turkey died in work accidents, while 588,823 workers experienced work-related accidents. Reports indicate that workers who died due to work accidents in Zonguldak accounted for 0.9% (n = 14) of the total work-related fatalities (4). According to the report (4,5), 8.2% of the employees in Zonguldak have had work-related accidents.

The Regulation of Permanent Disabilities (RPD), published in the Official Gazette on 11th October 2008 with the number 27021, is used to determine the disability rate (DR) for compensation cases due to tort for physical injuries and death that occurred after this date as a result of occupational accidents (6,7). Compensation for work accidents is calculated through an actuarial formula that considers the DR of the victim.

Miners working in unhealthy environments underground may face many hazards. Despite technological advances, workplace hazards persist, making occupational health and safety (OHS) a continuing concern for the public. There is a positive correlation relationship between economic development and the implementation of OHS measures and practices. Developed countries provide a broader perspective on organization and legislation in OHS. ILO provides financial support for measures aimed at preventing occupational accidents and diseases. Insurance is one of the ILO's financial instruments for OHS (8). Due to the high-risk nature of mining areas, individuals and legal entities operating in the mining sector were obliged to obtain Mine Workers Compulsory Personal Accident Insurance (MWCPAI) through the Council of Ministers Decision No. 2015/7249 published in the Official Gazette on 6th February 2015 (9). Accordingly, institutions or organizations employing personnel in the mining sector obtain insurance policies from insurance companies to cover their personnel against the consequences of work accidents that may occur during mining activities.

MWCPAI is a fixed sum insurance. Consequently, the sum insured must be paid if the risk materializes. In case a miner faces a work accident that results in their death within two years, the miner's family is provided with compensation in the form of death benefits. If the miner becomes disabled, they are entitled to receive disability benefits per the disability types and rates mentioned in the relevant terms and conditions (9).

This study aimed to determine the reflection of the MWCPAI on forensic medicine practices and to identify its possible deficiencies compared to the RPD. This issue has not been previously addressed in forensic medical literature. Despite the limited number of cases in the study, the uniqueness of the topic adds significance to our findings.

MATERIAL AND METHODS

The subject of the study was the cases for the recourse claim from the insurance company for the compensation paid by the workplace to the miners injured due to occupational accidents between 2016 and 2019. This study adhered to the ethical guidelines outlined in the Declaration of Helsinki and obtained approval by the local ethics committee (Decision number: 01). We conducted a retrospective analysis of cases forwarded to us by the judicial authorities, requesting a determination of coverage percentage (CP) according to the MWCPAI. The data set included sociodemographic information, the type of accident, the type of injury, whether the patient underwent surgery due to the accident, the cause of disability, if any, DRs, and the percentage of insurance coverage. The data from the study were analyzed using SPSS 22.0 software for descriptive statistics such as mean, median, minimum, maximum, standard deviation, and frequency.

RESULTS

All 18 patients were male, with a mean age of 36.05±5.4 (SD) years (minimum 22, maximum 45) at the time of the event, with a median age of 36.5 years. The results indicated that 66.7% (n = 12) of the cases were in the 31-40 age group, 22.2% (n = 4) were in the 41-50 age group, and 11.1% (n = 2) were in the 21-30 age group.

The subject of all cases was recourse compensation, and they were all filed in the civil court of first instance, functioning as the commercial court of first instance (CCFI). The two most prevalent types of accidents were falling or impacting by an object (n = 6, 33.3%) and crushing (n = 5, 27.8%), respectively (Table 1).

Table 1. Distribution of cases according to accident type

Accident	n	%
Falling or impacting objects	6	33.3
Crush	5	27.8
Jamming	4	22.2
Fall (from the same lavel)	2	11.1
Falling from a height	1	5.6
Total	18	100.0

Seven cases exhibited multiple work-related accidents. However, upon closer examination, the injury locations differed from those observed in other cases within the same case group. A total of 17 cases (94.4%) exhibited injuries from a single anatomical localization, while one case (5.6%) exhibited injuries from both the shoulder and ankle. Upon analysis of the distribution of traumatic lesions according to anatomical sites, it was determined that 57.8% of cases involved lower extremity injuries (n = 11), 31.6% involved upper extremity injuries (n: 6), 5.3% involved both lower and upper extremity injuries (n = 1), and 1 case involved eye injuries (5.3%). The largest group among lower extremity injuries was foot/ankle localization (n = 6, 54.5%). Table 2 illustrates the distribution of cases according to the affected body region, as per the malfunction list in Schedule A of the RPD annex. The dominant side of all cases with upper extremity injuries was right.

Table 2. Distribution of cases by injured body parts

Sequelae list*	n	%
Pelvis and lower extremities malfunctions	11	57.8
Fingers of the hand malfunctions	5	26.3
Wrist and hand malfunctions	1	5.3
Shoulder and arm malfunctions	1	5.3
Eye malfunctions	1	5.3
Total**	19	100.0

*According to the RPD. **In one case, fractures of both the talus and humerus occurred.

It was determined that 44.4% (n = 8) of the patients underwent surgery for the injury, and the others (n = 10, 55.6%) were treated conservatively. After all the treatments were applied, there was finger functional limitation or amputation in four cases, ankle functional limitation in three cases, knee functional limitation in two cases, and decreased vision in one case. The mean DR of the operated cases was 7.75, with a mean CP of 3.68. In contrast, the mean DR of the conservatively followed cases was 5.72, with a mean CP of 2.64. Comparative analysis of cases in terms of DR and CP revealed that DR and CP were the same in 4 (22.2%) cases, DR was higher in 12 (66.7%) cases, and CP was higher in 2 (11.1%) cases. Although eight cases (44.4%) had healed without functional residuals, four (50.0%) had a DR calculated according to RPD. The remaining four cases (50.0%) were found to have no disability. The average DR of the cases with a sequelae was 8.5. Upon evaluation of cases according to the handicap coverage list (HCL) of the general conditions of MWCPAI, it was determined that eight cases (44.4%) who recovered without leaving sequelae did not have a CP. Nine cases (50.0%) did not have a direct equivalent in the HCL of the general conditions of MWCPAI. Therefore, the coverage item was selected through a comparative method, and the CP was determined by applying discretion based on the extent of functional limitation. Only one case (5.6%) was to have an exact equivalent in the said list. The average CP of the cases whose CP was determined was 6.5. Table 3 presents information on the type of injury, functional limitations, DR, and CP of the cases.

DISCUSSION

Despite the advancements in technology and the implementation of rigorous safety measures, underground coal mining remains a high-risk occupation concerning accidents and fatalities. In small-scale enterprises in certain countries, the number of individuals engaged in informal mining activities may surpass those employed in the formal mining sector. Due to the non-adherence to international and national standards in small-scale enterprises, accident rates can be approximately 6-7 times higher than in larger mining enterprises, even within developed countries (10). Moreover, the prevalence of unofficial mines, such as those in Zonguldak, poses a significant and inevitable danger. The high number of work-related accidents in Zonguldak and Manisa provinces, despite their relatively minor populations, can be explained by the harsh working conditions in the mining sector (11).

Mining constitutes only 1% of the global workforce yet accounts for approximately 8% of fatal occupational accidents (10). According to data from July 2022, Turkey employs 222,067 workers in mining and quarries (5). According to recent reports, workers in the mining industry experience occupational accidents at a rate over six times higher and a fatality rate over seven times higher than workers in other sectors across the country (12). Figure 1 illustrates the occupational accidents that occurred in Turkey between 2016 and 2022, specifically highlighting those that transpired within the challenging coal mining sector.

In our study, all cases were male. According to the Working Life Statistics 2021 report, 94.83% of individuals who experienced occupational accidents in our country were male, while 5.17% were female (5). Furthermore, it was reported that nearly all miners (n = 4949) who suffered occupational accidents in hard coal mining in 2022 were male, with only a small fraction (n = 2) being female (4). Reviewing the literature, a study assessing occupational accidents treated in emergency departments found that approximately 90% of cases were male (13).

Table 3. Demographic and sequela characteristics of the cases

Case no	Age/Sex	Type of injury	Cause of disability	Disabilty rate	Comparably selected collateral item	Percentage of insurance coverage
1	40/M	Knee ligament rupture	Functional limitation	14.1	Ankylosis of the knee joint	2.9
2	39/M	Amputation of the 4th digit from the DIP joint	Amputation	7	Complete loss of the ring finger alone	2.7
3	41/M	4th digit phalangeal fracture	Functional limitation	7.2	Complete loss of the ring finger alone	4
4	43/M	Tibia fracture	Uncomplicated recovery	5.1	No comparable electable equivalent	0
5	35/M	Radius fracture	None	0	None	0
6	38/M	Knee ligament rupture	Functional limitation	14	Ankylosis of the knee joint	4
7	31/M	Femur fracture	None	0	None	0
8	22/M	4th digit phalangeal fracture	None	0	None	0
9	33/M	Tibia-fibula fracture	Uncomplicated recovery	4.3	No comparable electable equivalent	0
10	41/M	Humerus-talus fracture	Uncomplicated recovery	9.1	No comparable electable equivalent	0
11	33/M	1st toe SFT	None	0	None	0
12	30/M	Tibia fracture	Functional limitation	4.2	Ankylosis of the ankle joint	3.8
13	33/M	Ocular trauma	Functional limitation	7.1	Enucleation of one eye or loss of half of the vision in both eyes	3.6
14	45/M	Tibia fracture	Functional limitation	20.2	Ankylosis of the ankle joint	15
15	37/M	1st digit SFT	Functional limitation	4.3	Complete loss of the index finger alone	10
16	35/M	Talus fracture	Functional limitation	10.3	Ankylosis of the ankle joint	9
17	37/M	Tibia fracture	Uncomplicated recovery	4.3	No comparable electable equivalent	0
18	36/M	1st digit phalangeal fracture	Functional limitation	8	Amputation of the thumb alone	10

DIP: distal interphalangeal; SFT: soft tissue trauma

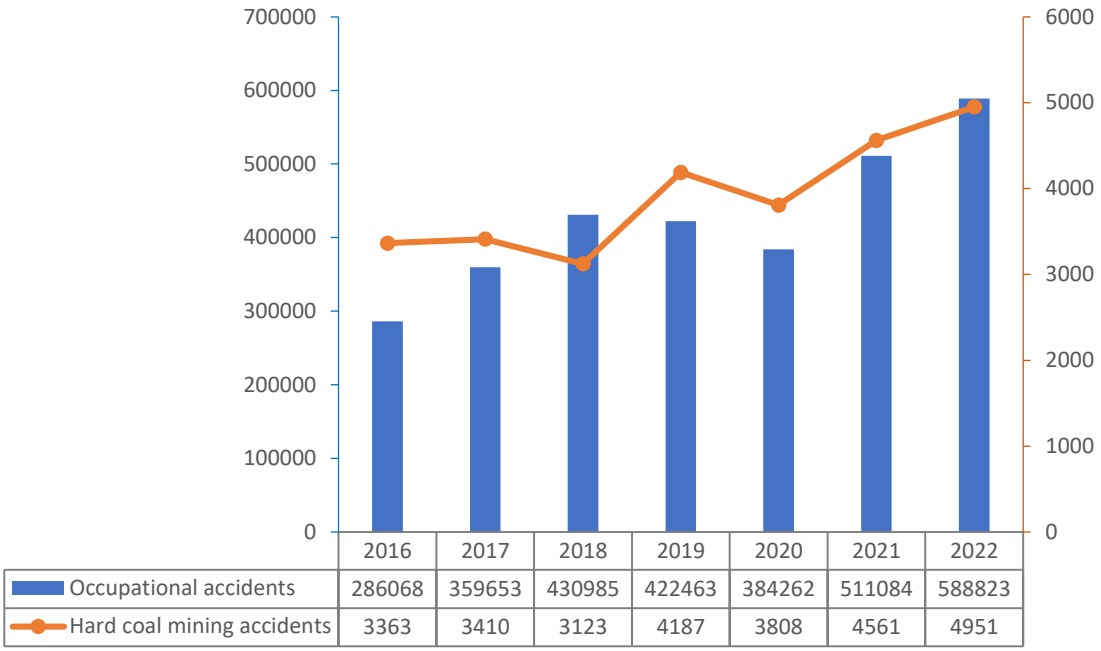

Figure 1. Occupational accidents (total) and hard coal mining accidents, Türkiye, 2016-2022 (4)

Similarly, in a survey conducted by Acara et al., 90.6% of cases were male (14), and in another study by Kadıoğlu et al., 89.3% were male (15). This male predominance in gender distribution is believed to stem from the higher involvement of males in the workforce, particularly in hazardous and physically demanding sectors like mining, as observed in our study, leading to increased susceptibility to occupational accidents.

The mean age of the study sample was 36.05 years. The highest incidence of accidents was observed during the third decade of life, which was consistent with findings from studies evaluating cases admitted to healthcare institutions due to occupational accidents: Oğuzlar et al. (13) reported the highest number of occupational accidents occurring within the 28-37 age range, while Orhan et al. (16) and Acara et al. (14) reported it to be within the 25-34 age range. In a survey study involving miners working in underground coal mines, more than half of the participants (50.8%) were between the ages of 26-40 (11). Although age group classifications vary across the literature, it can be inferred that occupational accidents primarily affect individuals aged 21-40. This pattern may be attributed to the active engagement of this age group in the workforce, coupled with the inexperience of those newly entering the workforce, propensity for risk-taking behavior among the younger demographic, and a more socially active lifestyle prone to various traumas. Additionally, in the mining sector, which comprises the sample group of our study, a significant decline in employment rates at older ages is natural due to the strenuous working conditions and heightened risk of occupational diseases or accidents. A study examining fatal fall-related occupational accidents in the United States of America reported that while young individuals experienced more accidents, older individuals generally suffered more severe injuries (17). In our study, the fact that the decrease in working capacity was higher in relatively older patients supports this finding.

They were all filed in the civil court of first instance, functioning as the CCFI. The legal authority responsible for adjudicating commercial disputes is the CCFI. Given that cases stemming from insurance law fall within the scope of commercial matters, such disputes are resolved by the CCFI. In jurisdictions lacking a dedicated CCFI, commercial cases are adjudicated by the civil court of first instance, functioning as the CCFI (18).

Looking at the distribution of the type of injury of occupational accidents that occurred in Turkey in 2022, it was reported that 44.9% were wounds and superficial injuries, 15.1% were dislocations, sprains, or strains, 4.3% were bone fractures, and 0.1% were amputations (4). A study on occupational accidents reported that most of the injuries are so mild that they can be remedied by a simple medical intervention (19). Another study analyzing occupational accidents admitted to the Social Security High Health Committee reported fractures (28.8%) and amputations (24.8%) as the most common types of injuries in the construction, furniture, and equipment manufacturing, as well as coal mining industries (20). Similarly, a study on occupational accidents in Denizli found that the most common cause of presentation was soft tissue trauma, followed by fractures (21). In our study, fractures accounted for 66.7% of the cases. While there are

sectoral variations in these studies, it is evident that severe injuries can occur in high-risk working environments such as the mining sector.

The study observed that the two most common accidents were falling or impacting objects (33.3%) and crushing (27.8%). Occupational accidents may occur in mines due to falling or slipping of materials, nail penetration, and falls. The strength, grip, and anti-slip or abrasion properties of boots are crucial for personal protection against injuries caused by falls. It has been reported that over three-quarters of occupational accidents in underground mines occur because hand, foot, and wrist protectors, which are essential personal protective equipment, do not fully meet the required standards (22).

Our study observed that all patients (94.4%) except one, who sustained an eye injury due to a stone impact, experienced injuries to the extremities in terms of anatomical regions. The most prevalent injury was lower extremity injury, with foot/ankle localization constituting the largest subgroup (n = 6, 54.5%). A thesis study assessing occupational accidents reported that around 70% of lower extremity traumas were related to the foot and ankle (21). There is a consistency in the frequency of injury sites in occupational accidents, as reported in the literature. In two separate studies analyzing occupational accidents presented at the emergency department, extremity trauma emerged as the most prevalent injury site (15,23). However, there were proportional differences when the extremities were categorized into upper and lower extremities. Studies reported upper extremity injuries as the largest group, with lower extremity injuries as the second most common (14,15,24). The higher incidence of lower extremity injuries in our study may be due to the difference in the lines of work and the work performed between our research and other studies.

Çolak et al. reported that approximately 90% of patients received medical treatment, while the remaining underwent surgery (23). Conversely, in this study, it was found that 44.4% of cases required surgery. This variance is presumed to be attributable to differences in the study population. It was observed that the mean DR and the mean CP were higher in cases that underwent surgery compared to those managed conservatively. Surgical applications come to the forefront in deplased or complicated fractures or injuries with high severity (25,26).

In approximately one-fourth of cases (22.2%), the DR and the CP were found to be identical. Remarkably, this concordance between DR and CP was observed solely in cases that had healed without sequelae, a category not encompassed by either list. The RPD has disability provisions for uncomplicated healed patella, calcaneus, talus, fibula, and tibia fractures. Half of the patients who recovered without functional limitations were assigned a DR in the present study.

It was determined that the cases that healed without functional disability did not have a provision in the disability coverage list of the general conditions of the MWCPAI. While the literature underscores the limitations of the RPD (27–29), it is evident that the disability coverage list within the MWCPAI requires further elaboration to mitigate the risk of mine workers losing their entitlements.

In compensation law, when evaluating permanent bodily damages, there are instances where the exact equivalent of a person's impairment may not be explicitly listed. This situation may lead to the selection of the closest defect to the sequelae in the person by comparison and to assess the extent of the functional limitation of the person (30). In our study, half of the cases had their CP determined by selecting from the HCL through a comparison method and applying discretion regarding the rate of functional loss. This approach may result in contradiction among report preparers in cases where sequelae do not precisely match any listed impairments. We argue that updating the HCL for the MWCPAI to encompass a broader range of clinical conditions encountered in practice would ensure standardization.

Actuarial calculation is essential in determining compensation in financial compensation cases filed due to death or bodily injury. The calculation considers active and passive period earnings the disability and fault rates, if applicable. It is critical to meticulously examine all medical records to ascertain any sequelae forming the basis of the DR, leaving no room for doubt. Determining disability in cases where an individual has previously sustained injuries for any reason poses challenges (31). When determining the DR and the CP for the same functionally affected area in different incidents, it is necessary to calculate the difference between pre- and post-trauma. In our study, although seven cases involved multiple occupational accidents, the injury localizations differed from each other on a case-by-case basis. Hence, a calculation method based on differential calculation was not required. Yet, a detailed reexamination of cases necessitating difference calculation is crucial. The availability of pre-incident medical records can aid in distinguishing sequelae caused solely by the incident. Moreover, thorough performance of re-employment and periodic examinations can ensure accurate assessments in determining the amount of compensation due to the DR in cases of occupational diseases or accidents.

Hand injuries frequently occur due to failure to comply with occupational safety principles (32). Studies examining hand injuries have reported that the dominant side is often affected, given its increased usage during work (32–34). Furthermore,

hand injuries typically necessitate prolonged treatment and commonly result in functional impairment (35). Our study shows that the dominant side of all patients with upper extremity injuries was right, and most recovered with sequelae reinforces the literature. As the RPD specifies DR for the dominant upper extremity, a 1/5 discount is applied for sequelae in the non-dominant extremity during DR calculations. Similarly, the HCL for the MWCPAI highlights rates for the dominant upper extremity. Hence, it is evident that both lists are arranged in favor of the dominant side.

Limitations: One limitation of the study is that it was limited to recourse cases, excluding others. Since it was a retrospective study, information on the factors leading to accidents and working conditions could not be obtained. Being single-centered poses a challenge in generalizing the findings to the broader population. One possible reason for the low number of cases is the existence of an unregistered labor force, which is also known to the public as the invisible tip of the iceberg.

CONCLUSION

Based on a proactive approach, OHS aims to prevent occupational accidents by conducting risk analyses that may cause the accident to occur. Despite being inherently high-risk environments, it is asserted that nearly all accidents in mines are preventable (11). Statistical data presented in OHS studies can contribute significantly to understanding the root causes of accidents and diseases and facilitate the implementation of necessary preventive measures (8). However, despite the obligation imposed on mining companies to comply with the MWCPAI, the upward trend in occupational accidents in hard coal mining has persisted since 2015. This suggests that regulatory measures alone are insufficient to mitigate the adverse effects of occupational accidents. Given the classification of the mining sector as highly hazardous according to OHS hazard classifications, it is essential to provide comprehensive training to miners tailored to the specific hazards they may encounter and create a safe working environment. Such measures are believed to be crucial in effectively reducing the severity of work accidents in the mining sector (12).

There is a need to improve OHS policies in the mining sector in light of global developments. The MWCPAI provides insurance coverage to miners and requires risk assessments in the mining sector. The primary goal should be the minimization of occupational accidents in mining operations. However, we contend that there is a need for improvement in the general conditions, disability classifications, and principles outlined in the MWCPAI to ensure legal protection for miners in the event of unavoidable accidents. Additionally, as the MWCPAI represents a relatively new form of insurance, the number of such cases is expected to increase as awareness among the mining workforce grows.

Declarations:

Funding: No funding was received for conducting this study.

Ethics: This study adhered to the ethical guidelines outlined in the Declaration of Helsinki and obtained approval by the Ethics Committee of Zonguldak Bulent Ecevit University (Decision number: 2024/01).

Conflicts of Interest: None.

Authorship Contributions: This study is entirely author's own work and no other author contribution.

REFERENCES

1. International Labour Organization. International labour standards on occupational safety and health [Internet]. [cited 2023 Sep 5]. Available from: https://www.ilo.org/global/standards/subjects-covered-by-international-labour-standards/occupational-safety-and-health/lang--en/index.htm
2. Uluslararası Çalışma Örgütü. İş sağlığı ve güvenliği profili Türkiye [Internet]. Ankara; 2016. Available from: https://www.ilo.org/wcmsp5/groups/public/---europe/---ro-geneva/---ilo-ankara/documents/publication/wcms_498818.pdf
3. International Labour Organization-ILOSTAT. Statistics on safety and health at work [Internet]. [cited 2023 Sep 6]. Available from: https://ilostat.ilo.org/topics/safety-and-health-at-work/#
4. Sosyal Güvenlik Kurumu. İş kazası ve meslek hastalığı istatistikleri [Internet]. [cited 2023 Sep 5]. Available from: https://www.sgk.gov.tr/Istatistik/Yillik/fcd5e59b-6af9-4d90-a451-ee7500eb1cb4/
5. Çalışma ve Sosyal Güvenlik Bakanlığı Çalışma Genel Müdürlüğü. Çalışma hayatı istatistikleri 2021 [Internet]. Ankara; Available from: https://www.csgb.gov.tr/media/89862/calisma_hayati_2021.pdf
6. Çalışma Gücü ve Meslekte Kazanma Gücü Kaybı Oranı Tespit İşlemleri Yönetmeliği. Resmi Gazete Tarihi: 11.10.2008 Resmi Gazete Sayısı: 27021 [Internet]. [cited 2023 Sep 6]. Available from: https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=12511&MevzuatTur=7&MevzuatTertip=5
7. Onğun AC. Trafik ve iş kazasında Yargıtay uygulamaları ile aktüeryal tazminat hesaplamaları. İstanbul Barosu Derg. 2017;91(5):100–90.
8. Alper Y. Bazı ülkelerde işçi sağlığı- işgüvenliği uygulamaları ve Türkiye'deki uygulama ile karşılaştırılması. J Soc Policy Conf. 1992;(37–38):83–101.

9. Kubilay H, Akdemir P. Maden çalışanları zorunlu ferdi kaza sigortası taraflarının yükümlülükleri. Uyuşmazlık Mahkemesi Derg. 2017;10:455–73.
10. International Labour Organization. Mining: a hazardous work [Internet]. [cited 2023 Sep 6]. Available from: https://www.ilo.org/safework/areasofwork/hazardous-work/WCMS_356567/lang--en/index.htm
11. Dizlek OA. Kömür maden ocağında iş güvenliği için alınan önlemlerin değerlendirilmesi. Tarsus Üniversitesi Lisansüstü Eğitim Enstitüsü; 2021.
12. Sekmen M, Zengin MA. Türkiye madencilik sektörü iş kazalarının analizi ve gelecek perspektifleri. Int J Adv Eng Pure Sci. 2023;35(2):246–58.
13. Oğuzlar FÇ, Armağan HH, Bedel C, Tomruk Ö, Beceren NG. Acil servise başvuran iş kazalarının değerlendirilmesi. Genel Tıp Derg. 2021;31(1):12–7.
14. Acara AÇ, Yanturalı S, Canacık Ö, Bolatkale M. Acil servise iş kazası nedeniyle başvuran hastalarda iş kazası nedenleri, iş kazalarının oluşum mekanizmaları ve çözüm önerilerinin incelenmesi. Kafkas J Med Sci. 2021;11(Ek 1):184–9.
15. Kadıoğlu E, Karaman S, Arık Ö. İş kazası nedeniyle acil servise başvuran hastaların demografik analizi. Gaziosmanpaşa Üniversitesi Tıp Fakültesi Derg. 2016;8(3):163–73.
16. Orhan Ç, Çakmak F, Akdeniz YS, İpekçi A, İkizceli İ. Cerrahpaşa Tıp Fakültesi Acil Tıp Anabilim Dalına başvuran iş kazası olgularının analizi. J Med. 2022;36(3):139–44.
17. Shishlov KS, Schoenfisch AL, Myers DJ, Lip-scomb HJ. Non-fatal construction industry fall-related injuries treated in US emergency departments, 1998-2005. Am J Ind Med. 2011;54(2):128–35.
18. Kurt Konca N. Yeni Türk Ticaret Kanunu'na göre asliye ticaret mahkemeleri. TAAD. 2013;(15):79–126.
19. Orhan Ç, Çakmak F, Akdeniz YS, İpekci A, İkizceli İ. Cerrahpaşa Tıp Fakültesi Acil Tıp Anabilim Dalına başvuran iş kazası olgularının analizi. J Med. 2022;36(3):139–44.
20. Saygun M, Tunçbilek A. SSK Yüksek Sağlık Kurulu'na başvuran iş kazası olgularının değerlendirilmesi. Ankara Üniversitesi Tıp Fakültesi Mecmuası. 2000;53(3):193–9.
21. Pehlivanoğlu B. Pamukkale Üniversitesi Hastanesi Acil Servisine 2018-2021 yılları arasında başvuran iş kazalarının retrospektif değerlendirilmesi [Internet]. Pamukkale Üniversitesi; 2022. Available from: https://gcris.pau.edu.tr/bitstream/11499/45767/1/baybars_pehlivanoglu_tez.pdf
22. Uğur S, Topkaya Ö. Kömür madenciliği sektörü ve Uluslararası Çalışma Örgütü'nün 176 sayılı sözleşmesi ile bu sektöre yönelik yürürlüğe konan iş sağlığı ve güvenliği düzenlemeleri. Res J Polit Econ Manag. 2015;3:241–56.
23. Çolak T, Yılmaz S, Kalaycıoğlu O, Çolak Z, Çelik K. İş kazası nedeniyle acil servise başvuran olguların demografik özellikleri ve maliyet analizi. ASUJMS [Internet]. 2022;3(1):1–5. Available from: https://dergipark.org.tr/en/download/article-file/2134960
24. El-Hady RA, Thabet H, Ghandour N, Mandor AM. Medico-Legal aspects of permanent infirmities as a sequel of different types of injuries (A retrospective study in Assiut Governorate, Egypt). Ain Shams J Forensic Med Clin Toxicol. 2013;20:92–8.
25. Keklikçi K, Çilli F, Pehlivan Ö, Kuşkucu M. Femur boyun kırıkları. TOTBİD. 2009;8(1–2):1–6.
26. Ateş Y, Ömeroğlu H, Uçar HD, Korkusuz Z. Tibia cisim kırıklarında farklı tedavi metodlarının karşılaştırılması. Acta Orthop Traumatol Turc. 1994;28(90–3).
27. Düzcan AM, Durak D, Fedakar R, Türkmen İnanır N. Adli Tıp Ana Bilim Dalı tarafından düzenlenen maluliyet raporlarının retrospektif incelenmesi. Uludağ Üniversitesi Tıp Fakültesi Derg [Internet]. 2023;49(1):9–16. Available from: https://dergipark.org.tr/tr/download/article-file/2742784
28. Koçak M, Garbioğlu A, Özbay M. Sekel bırakarak iyileşmiş tibia ve talus kırıklarında maluliyet değerlendirmesi: Bir olgu sunumu. J Med. 2021;35(1):34–8.
29. Hilal A. Maluliyet hesaplamalarında karşılaşılan sorunlar. Bull Leg Med. 2016;21(2).
30. Garbioğlu A. Adli tıp genel kurulunda karara bağlanan maluliyet dosyalarında tespit edilen çelişki ve yaklaşım farklılıklarının değerlendirilmesi [specilization in medicine]. [İstanbul (Turkey)]: Adli Tıp Kurumu; 2018.
31. Ünal V, Ünal EÖ, Yener Z, Çetinkaya Z, Çağdır S. Fark hesabına dayalı maluliyet oranı tespiti. Türkiye Klin J Foren Med. 2015;12(2):37–46.
32. Trybus M, Lorkowski J, Brongel L, Hladki W. Causes and consequences of hand injuries. Am J Surg. 2006;192(1):52–7.
33. Altan L, Akın S, Bingöl Ü, Özbek S, Yurtkuran M. El Yaralanması Ciddiyet Skoru'nun endüstriyel el yaralanmalarında prognozu belirlemedeki rolü. Ulus Travma Derg. 2004;10(2):97–101.
34. Aslan A, Aslan İ, Özmeriç A, Atay T, Çaloğlu A, Konya MN. Acil el yaralanmalarında deneyimlerimiz: 5 yıllık verilerin epidemiyolojik değerlendirmesi. TAF Prev Med Bull. 2013;12(5):563–70.
35. Wu Z, Guo Y, Gao J, Zhou J, Li S, Wang Z, et al. The epidemiology of acute occupational hand injuries treated in emergency departments in Foshan City, South China. Ulus Travma Acil Cerrahi Derg. 2018;24(4):303–10.

RESEARCH ARTICLE

Simge Ozbakıs Ozkaynak[1]
Ilknur Suer[2*]
Murat Kaya[3]
Sukru Ozturk[3]

[1] Istanbul University, Institute of Graduate Studies in Health Sciences, Department of Genetics, Istanbul, Türkiye
[2] Istanbul University, Istanbul Faculty of Medicine, Department of Medical Genetics, Istanbul, Türkiye
[3] Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics, Istanbul, Türkiye

Corresponding Author:
Ilknur Suer
mail: ilknursuer@istanbul.edu.tr

Received: 17.12.2024
Acceptance: 14.02.2025
DOI:10.18521/ktd.1600640

Preliminary findings of the study were presented as an oral presentation at the 9th International Medicine and Health Sciences Researches Congress by SOO (March 2022-Ankara, Presentation ID 144).

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Enhanced Expression of miR-638-5p May Suppress Acute Myeloid Leukemia *in vitro* Cell Proliferation Through *PGK1 and PIM1*

ABSTRACT

Objective: miR-638-5p is a crucial tumor suppressor miRNA in several cancer types including, Acute Myeloid Leukemia (AML). This study aimed to analyze the role of miR-638-5p and its potential target genes in HL-60 and NB4 acute promyelocytic leukemia cell lines using *in vitro* method.

Method: After the miR-638-5p mimic transfection into AML cells, the effect on cell viability was examined by the WST-8 method, and the effect on apoptosis was measured via the Caspase-3 quantification method. *In silico* tools such as miRWalk, miRDB, and miRTarBase were used to select the possible target genes of miR-638-5p. The expression levels of selected genes were investigated by qRT-PCR. The overall survival (OS) rate of AML patients was explored via the BloodSpot database, the Enrichr tool was used for enrichment analysis, and correlation analysis was performed using the Correlation AnalyzeR tool.

Results: Decreased proliferation and increased apoptosis were determined in miR-638-5p mimic transfected cells compared to the controls. *MECP2*, *PIM1*, *MEF2C*, *PGK1*, and *SPAG1* genes were selected as the potential targets of miR-638-5p for *in vitro* study. *PGK1* and *PIM1* expression levels were significantly suppressed in cells transfected with the miR-638-5p mimic. The OS investigation revealed that overexpression of *MECP2*, *MEF2C*, and *PGK1* does not affect the survival of AML patients; however, overexpression of *SPAG1* and *PIM1* has a detrimental effect on AML survival. Also, a positive correlation was detected between *PIM1* and *PGK1* genes via enrichment analysis.

Conclusions: miR-638-5p may contribute to AML pathogenesis by targeting the *PGK1* and *PIM1* genes, and this situation may indicate its potential as a biomolecule for regulating cell proliferation in AML cells.

Keywords: miR-638-5p, Acute Myeloid Leukemia, HL-60, NB4, microRNA Mimic Transfection.

miR-638-5p'nin Ekspresyon Artışı *PGK1* ve *PIM1* Aracılığıyla Akut Miyeloid Lösemide *in vitro* Hücre Proliferasyonunu Baskılayabilir

ÖZET

Amaç: miR-638-5p, Akut Miyeloid Lösemi (AML) dahil olmak üzere çeşitli kanser türlerinde önemli bir tümör baskılayıcı miRNA'dır. Bu çalışma, miR-638-5p'nin ve potansiyel hedef genlerinin HL-60 ve NB4 akut promyelositik lösemi hücre hatları üzerindeki rolünü *in vitro* ortamda incelemeyi amaçlamaktadır.

Yöntem: miR-638-5p mimik transfeksiyonundan sonra AML hücrelerinin canlılığı üzerindeki etki WST-8 yöntemi ile apoptoz üzerindeki etki ise Caspase-3 kantifikasyon yöntemi ile incelendi. miRWalk, miRDB ve miRTarBase gibi *in silico* araçlar miR-638-5p'nin olası hedef genlerini seçmek için kullanıldı. Seçilen genlerin ifade düzeyleri qRT-PCR ile araştırıldı. AML hastalarının genel sağkalım (OS) oranı BloodSpot veritabanı üzerinden araştırıldı, zenginleştirme analizi için Enrichr aracı kullanıldı ve korelasyon analizi Correlation AnalyzeR aracı kullanılarak gerçekleştirildi.

Bulgular: Kontrollerle karşılaştırıldığında miR-638-5p mimik transfekte hücrelerde azalmış proliferasyon ve artmış apoptoz belirlendi. *MECP2*, *PIM1*, *MEF2C*, *PGK1* ve *SPAG1* genleri *in vitro* çalışma için miR-638-5p potansiyel hedefleri olarak seçildi. *PGK1* ve *PIM1* ekspresyon seviyeleri miR-638-5p mimic transfekte her iki hücrede de önemli ölçüde baskılandı. OS araştırması, *MECP2*, *MEF2C ve PGK1*'in aşırı ekspresyonunun AML hastalarının sağkalımını etkilemediğini; ancak *SPAG1* ve *PIM1*'in aşırı ekspresyonunun AML sağkalımı üzerinde zararlı bir etkiye sahip olduğunu ortaya koydu. Ayrıca, zenginleştirme analizi yoluyla *PIM1* ve *PGK1* genleri arasında pozitif bir korelasyon tespit edildi.

Sonuç: miR-638-5p'nin *PGK1* ve *PIM1* genlerini hedef alarak AML patogenezine katkıda bulunabileceği ve bu durumun AML hücrelerinde hücre proliferasyonunu düzenleyen bir biyomolekül olarak potansiyeline işaret edebileceği düşünülmektedir.

Anahtar Kelimeler: miR-638-5p, Akut Miyeloid Lösemi, HL-60, NB4, mikroRNA Mimik Transfeksiyonu.

INTRODUCTION

Acute Myeloid Leukemia (AML) is a type of leukemia that occurs with the accumulation of myeloid cells that cannot mature during the blood cell formation process. It is the most common type of acute leukemia among adults. It is responsible for 80% of adult acute leukemias and is a rare type of childhood leukemia. The disease mostly occurs due to de novo mutations in healthy individuals (1, 2).

MicroRNAs (miRNAs) are small, non-coding RNAs, consisting of 17- 25 nucleotides (3). It is possible to functionally investigate the roles of miRNAs in cellular processes through target genes by providing gain or loss of miRNA function in the cell through miRNA mimic and inhibitor transfection into the cell. (4). Although there are different methods, miRNA mimic transfection is generally applied via the lipofectamine-mediated method (5). miRNAs bind to target mRNA transcripts, causing transcript degradation or negative regulation of gene expression by repressing translation (6, 7). Sequences encoding miRNAs, whose main function is to regulate protein levels by causing mRNA degradation, constitute 1-3% of the human genome (8, 9). miRNAs are involved in many processes in cancer biology, such as apoptosis, metastasis, and proliferation (10, 11). Therefore, because miRNA expression can be associated with cancer type, cancer stage, patient response to treatment, and clinical changes, studies on the use of miRNAs as biomarkers, especially for cancer diagnosis and prognosis, have increased in recent years (12).

Studies have shown that miR-638-5p may have a significant role in many malignancies, including hepatocellular carcinoma (13), gliomas (14), lung cancer (15), breast cancer (7), stomach cancer (16), and leukemia (17). In these studies, decreased miR-638-5p levels were observed, and decreased miR-638-5p was associated with metastasis, poor prognosis, and a low survival rate for these disease types. miR-638-5p, which may have different target genes for each disease, causes a decrease in the expression of target genes by targeting oncogenes and thus acts as a tumor suppressor in the cell. Because of this feature, miR-638-5p, which has a tumor suppressor effect on cells, has been determined as a suitable candidate for therapeutic studies in various types of cancer and solid tumors (18).

Only one study has investigated the relationship between AML and miR-638-5p, which showed that decreased miR-638-5p was associated with deterioration of disease status, poor prognosis, and increased metastasis for AML (19). The effect of the association between miR-638-5p and its potential target genes on AML processes requires elucidation. Therefore, the present study investigated the function of miR-638-5p and potential target genes in AML cells. Initially, potential target genes of miR-638-5p were identified using *in silico* tools. The functional effects of ectopic miR-638-5p on AML cells were assessed, and the expression levels of possible target genes of miR-638-5p in AML cells were examined utilizing *in vitro* methods. The protein-protein interactions (PPI) association between possible target genes of miR-638-5p and their impact on AML survival was examined using *in silico* databases.

MATERIAL AND METHODS

Proliferation, Passage, and Cryopreservation of Cells: The HL-60 and NB-4 cells were cultured in RPMI-1640 medium containing 10% FBS and 1% antibiotic. Cells were cultured in an incubator at 37°C temperature condition with 5% CO_2 and passaged as needed. Cells were frozen and stored at -80°C in RPMI-1640 freezing medium containing 10% DMSO for use in other experimental stages.

miR-638-5p and Non-Targeting miRNA Mimic Transfection: Cells were counted, and 4x105 cells were placed in each well of a 6-well plate. RPMI-1640 medium containing 10% FBS without antibiotics was used for transfection. 24 hours after transplantation, miR-638-5p mimic transfection, and non-targeting miRNA mimic transfection were performed according to the Lipofectamine 2000 reagent protocol (Invitrogen, Carlsbad, CA, USA).

Observation of the Effect of miR-638-5p on Cellular Processes: The CVDK-8 kit (EcoTech Biotechnology) was used to examine the proliferation changes in cells transfected with the miR-638-5p mimic and non-targeting miRNA mimic via the WST8 method. The Caspase-3 Assay was used for the evaluation of the effects of miR-638-5p on apoptosis.

Identification of Potential Target Genes of miR-638-5p: In this study, some criteria were considered while selecting potential target genes of miR-638-5p; a) Since miR-638-5p is a tumor suppressor miRNA, genes reported to be overexpressed in cancers were selected, b) Genes targeted by miR-638-5p in at least one of the *in silico* databases miRWalk, miRDB, TargetScan, and miRTarBase were selected, c) Each of the selected genes has been shown to contribute to the cancer process by being targeted by miR-638-5p in cancer types other than AML.

Determination of the Effect of Potential Target Genes of MiR-638-5p on AML Overall Survival: The BloodSpot is a useful database that includes information about gene expression from AML patients that is crucial to the formation and maturation of blood cells. This tool has facilitated the rapid acquisition of an overview of gene expression patterns in healthy and malignant hematopoiesis for many researchers (20). By the

BloodSpot database, the effects of potential target genes of miR-638-5p on the overall survival (OS) rate of AML patients were explored in this research.

Enrichment Analysis: KEGG pathways and hub genes associated with the selected potential target genes of miR-638-5p were investigated using the Enrichr (21) tool, and correlation analysis was performed using the Correlation AnalyzeR (22) tool.

RNA Isolation: Total RNA was isolated 24 h after mimic transfection using TRIzol (Invitrogen). NanoDrop ND-2000c spectrophotometer (Thermo Fisher) was used to measure the concentration and purity of the isolated RNAs. The isolated RNAs were stored at -80 °C to be used in the necessary stages of the study.

cDNA Synthesis and qRT-PCR Analysis: The expression level of miR-638-5p was analyzed to determine the transfection level in HL-60 and NB4 cell lines. For this purpose, the synthesized cDNA samples were used in qRT-PCR analysis. qRT-PCR experiment was performed using TaqMan Universal Master Mix (Thermo Fisher), TaqMan miRNA probes (Thermo Fisher) and TaqMan control probes (RNU43) on the LightCycler480 (Roche). cDNA synthesis was performed with the SCRIPT Reverse Transcriptase kit and the β-actin gene was used in normalization as a housekeeping gene. The 5x HOT FIREPol EvaGreen qPCR Supermix (Solis Bio Dyne) was used in accordance with the manufacturer's protocol. Expression analysis of selected genes as candidate targets of miR-638-5p was performed.

Statistical Analysis: SPSS 28 software was used for the statistical analysis of the study data. Student's t-test was used to evaluate statistical significance, and according to the test results, data with a p-value less than 0.05 were considered significant. The $2^{-\Delta\Delta Ct}$ method was used for relative quantitation analysis of the qRT-PCR results. GraphPad Prism 9.3 software was used to create figures.

RESULTS

Ectopic expression of miR-638-5p inhibits AML Cell Proliferation: qRT-PCR analysis revealed that miR-638-5p levels were substantially elevated in transfected cells in comparison to control groups, indicating that transfection had occurred as expected (Figure 1). Cell viability was considerably reduced in HL-60 and NB4 cells transfected with mimic miR-638-5p, as assessed by the WST-8 method at 48 and 72 hours ($p<0,05$) (Figure 2A).

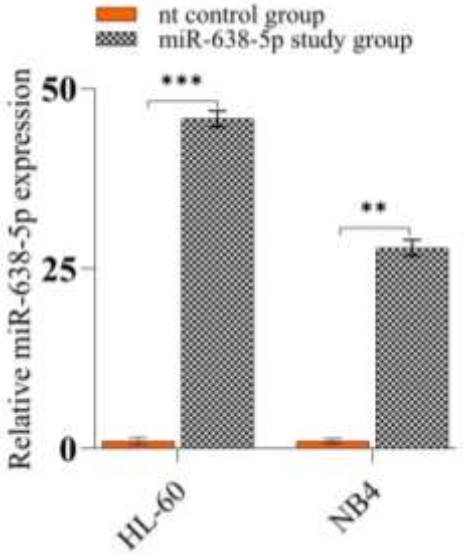

Figure 1. Validation of miR-638-5p mimic transfection efficiency (**p <0.01, ***p <0.001).

Overexpression of miR-638-5p Induces Apoptosis in AML Cells: After miR-638-5p transfection into NB4 cells, the amount of Caspase 3 showed a statistically significant increase compared to the control group (Figure 2B). The significant elevation of Caspase 3, recognized as an apoptosis marker, in miR-638-5p transfected cells relative to the control group suggests that miR-638-5p may induce apoptosis in AML cells.

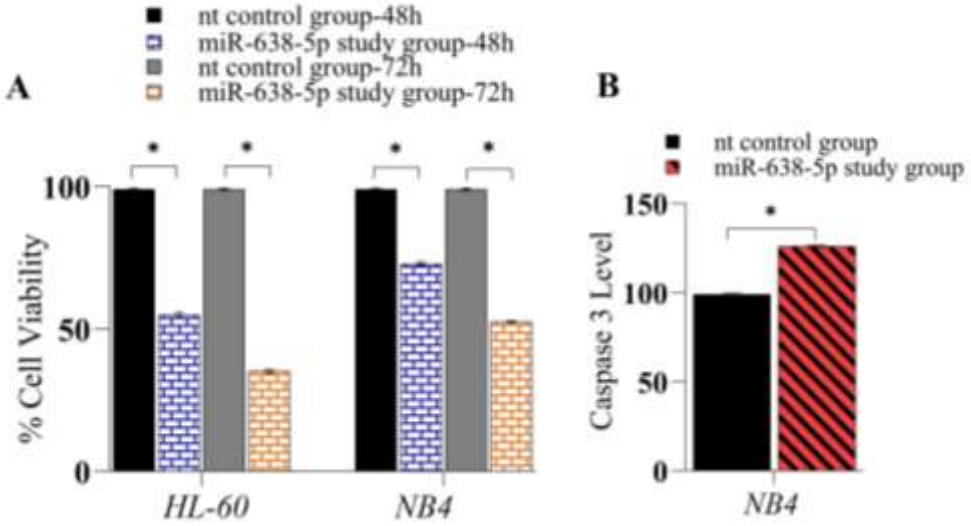

Figure 2. miR-638-5p mimic transfection A) inhibited proliferation and B) elevated apoptosis of the acute myeloid leukemia cells (**p <0.01, ***p <0.001).

miR-638-5p may Affect AML Prognosis via Potential Target Genes: According to the determined criteria; the *MECP2, PIM1, MEF2C, PGK1,* and *SPAG1* genes were selected as the targets of miR-638-5p for *in vitro* study. *In silico* investigations revealed that *PIM1* expression was elevated in complex cytogenetic anomalies, a poor prognostic indicator in AML (Figure 3). Furthermore, the results of the overall survival (OS) investigation showed that overexpression of *MECP2, MEF2C,* and *PGK1* does not affect the survival of AML patients; however, overexpression of *SPAG1* and *PIM1* has a detrimental effect on AML survival (Figure 4). Enrichment analysis results showed a positive correlation between *PIM1* and *PGK1* genes (Figure 5).

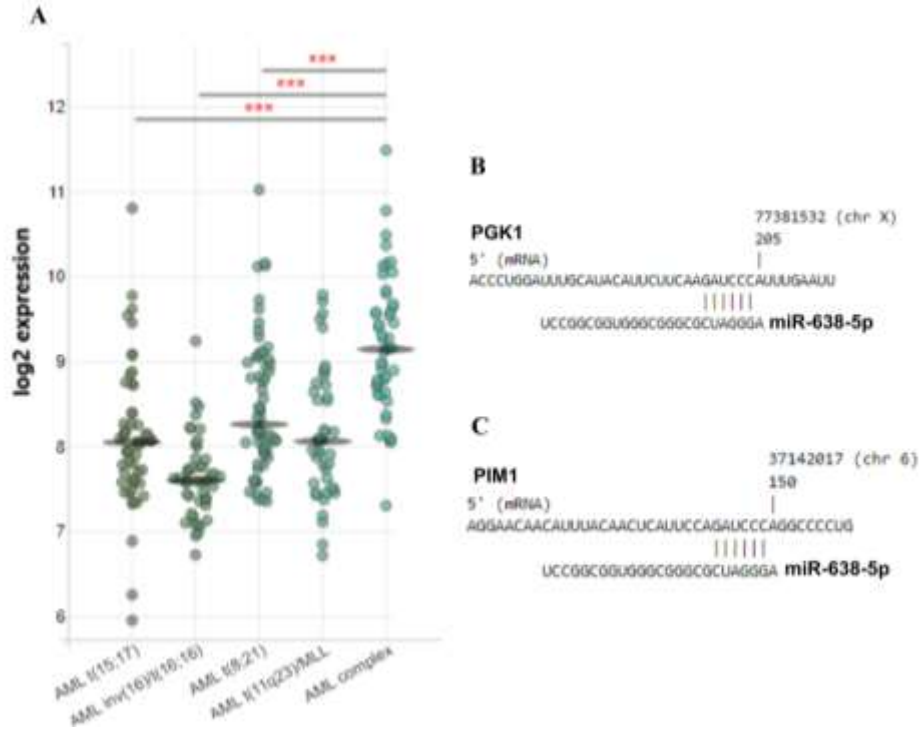

Figure 3. Analysis of the relationship between miR-638-5p, a selected target gene, and AML *in silico* A) Comparison of *PIM1* gene expression in cases of prevalent cytogenetic abnormalities associated with AML cases, B) Base pairing between miR-638-5p and *PGK1* and *PIM1*.

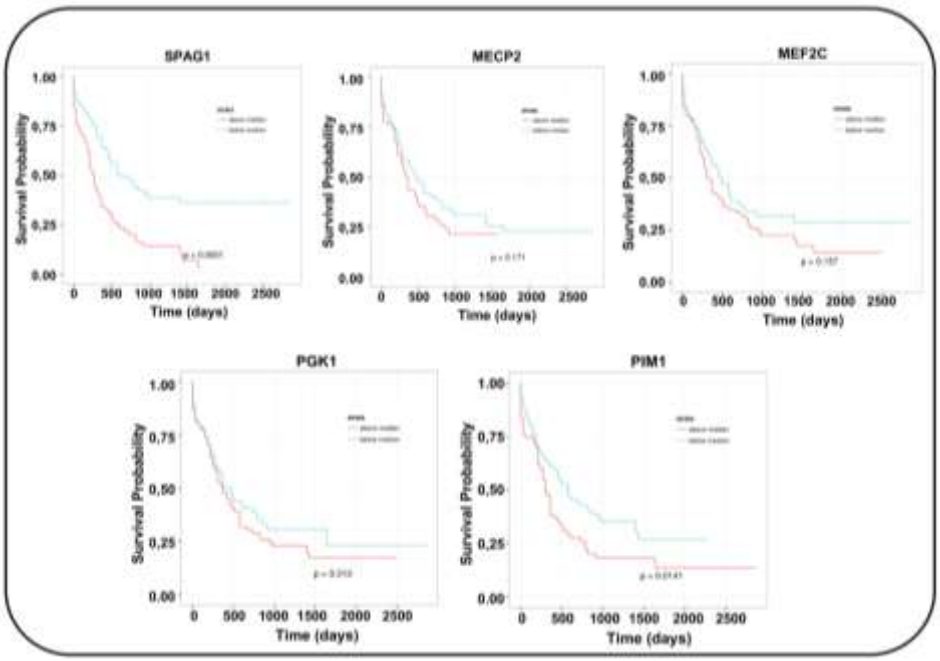

Figure 4. Determination of the impact of the potential target genes of miR-638-5p on the survival of AML patients. The overexpression of *MECP2, MEF2C,* and *PGK1* does not influence the survival of AML patients; conversely, the overexpression of *SPAG1* and *PIM1* adversely impacts AML survival.

Evaluation of the Expression Levels of Target Genes: The selected genes' (*MECP2*, *PIM1*, *MEF2C*, *PGK1*, and *SPAG1*) primer sequences were added to Table 1. *PIM1* and *PGK1* gene expressions were detected to be decreased in the miR-638-5p transfected group compared to the control group in both HL-60 (p=0.019 and p=0.003) and NB4 (p=0.010 and p=0.016) cell lines. On the other hand, it was determined that the expressions of *MECP2*, *MEF2C*, and *SPAG1* genes did not show a statistically significant change (Figure 5, Figure 6).

Table 1. Primer sequences used in qRT-PCR

	Primer Sequence	Reference
PIM1-F	5′- CCGTCTACACGGACTTCGAT-3′	(50)
PIM1-R	5′- CTGGCCCCTGATGATCTCTT-3′	
PGK1-F	5'-TCACTCGGGCTAAGCAGATT-3	(51)
PGK1-R	5'-CAGTGCTCACATGGCTGACT-3'	
MECP2-F	5'-ACTTCTGGCCCTGGTTAGGT-3'	(52)
MECP2-R	5'-CCGTGACCGAGAGAGTTAGC-3'	(52)
MEF2C-F	5'-GCACCAACAAGCTGTTCCAG-3'	(53)
MEF2C-R	5'-TGTCTGAGTTTGTCCGGCTC-3'	
SPAG1-F	5'-CCGCAGTGGTATAGCAACAG-3'	(54)
SPAG1-R	5'-GGCTTTCACGTTCCCATCAG-3'	
β-actin-F	5'-GCCTCGCCTTTGCCGATC-3'	(55)
β-actin-R	5'-CCCACGATGGAGGGGAAG-3'	

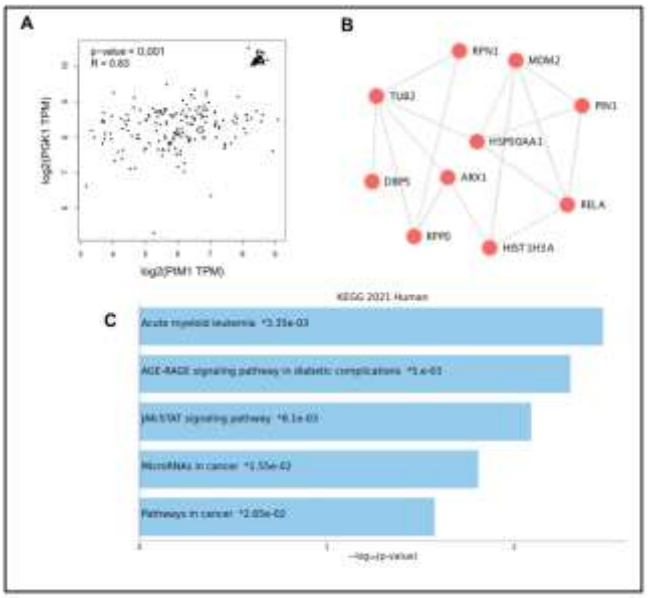

Figure 5. Enrichment analysis results of *PIM1*, a potential target gene of miR-638-5p. A) Positive correlation between *PIM1* and *PGK1* genes, potential target genes of miR-638-5p, B) 10 hub genes of *PIM1*, C) Pathways associated with *PIM1* according to KEGG 2021.

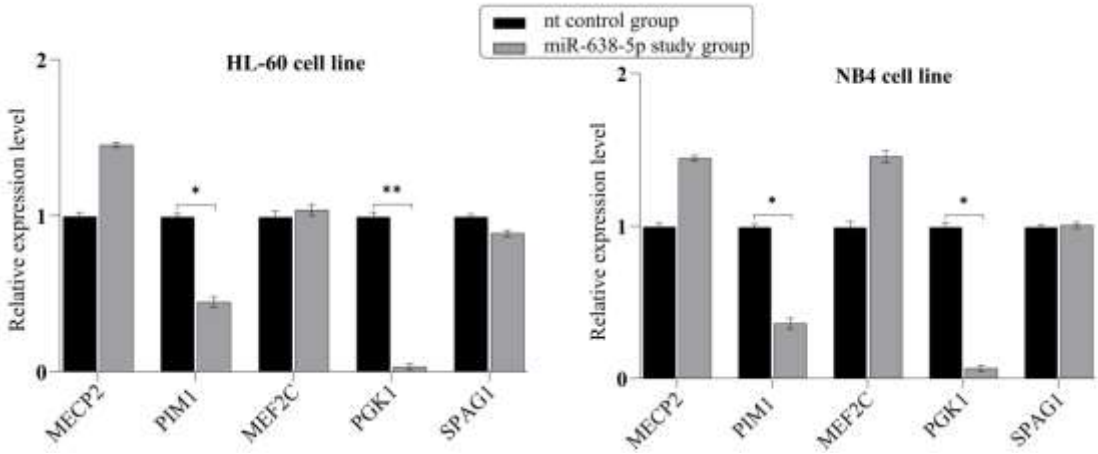

Figure 6. The relative expression level of potential target genes of miR-638-5p selected via *in silico* approaches A) HL-60 cells, B) NB4 cells (*p <0.05, **p <0.01).

DISCUSSION

Acute Myeloid Leukemia (AML) is a highly aggressive cancer, especially prevalent in the elderly population, characterized by poor therapeutic response, therefore presenting a substantial health challenge (23). Recent findings indicate that dysregulated miRNAs significantly influence AML pathogenesis by controlling cell differentiation, proliferation, and apoptosis. For instance, overexpression of miR-155 is frequently seen in AML and is associated with poor prognosis (24). The miR-181 family members are often downregulated in AML. Their inhibition results in increased expression of oncogenes and facilitates leukemic transformation (25). MiRNAs may serve as biomarkers for diagnosing AML and differentiating it from ALL. Garzon et al. showed that overexpression of the miR-20a, miR-25, miR-191, miR-199a, and miR-199b in AML were associated with clinical outcomes of AML patients such as poor prognosis and low survival rate (26).

miR-638-5p is one of the miRNAs that is associated with many diverse types of cancer and has been reported to play a role in the cancer formation process. miR-638-5p, which has been found to support tumor formation in cancers such as lung cancer (27), breast cancer (28), and melanoma (29), has shown tumor suppressor properties in types such as stomach cancer (16), colorectal cancer (30), and leukemia (31). In breast cancer and osteosarcoma, it can act both as a promoter of tumor formation and a suppressor of tumor formation by targeting different genes (32). Few studies have examined the relationship between miR-638-5p and AML (17-19). In one of these studies, miR-638-5p expression was found to be repressed in primary myeloid cells (19).

In this study, miR-638-5p transfection reduced the proliferation of both NB4 and HL-60 cells. This result was confirmed by data obtained from the measurement of apoptosis in NB4 cells. The data obtained were compatible with literature data, and miR-638-5p was observed to reduce cell viability and increase apoptosis in AML cells, and it was confirmed that it had a tumor suppressor effect on AML (14, 33, 34).

According to our gene expression results, a statistically significant decrease in the expression of *PIM1* and *PGK1* was observed in both NB4 and HL-60 cell lines in the miR-638-5p transfected group compared to the control group. On the other hand, no significant change was observed in the expression of *MECP2, MEF2C,* and *SPAG1* among the target genes in either cell line.

In our study *in silico* analyses showed that *PIM1* overexpression may be associated with poor survival in AML patients. Correlation analysis results revealed a significant positive correlation between *PIM1* and *PGK1*. Based on the findings of enrichment analysis, it seems that the *PIM1* gene may significantly contribute to the development of several malignancies, including AML. As a result of hub gene analysis, many of the 10 genes identified to be associated with *PIM1* were determined to be among the genes that play an important role in the AML process. For instance, *MDM2* serves as a negative regulator of the tumor suppressor *p53* and is often overexpressed in acute myeloid leukemia (AML) and many solid malignancies (35). *RPN1*, one of the *PIM1* hub genes, is crucial in the progression of many malignancies (36). *EVI1* is a low-expressed gene in normal hematopoietic cells; nevertheless, AML-characterized t(3;3), inv(3), or ins(3;3) chromosomal abnormalities lead to the juxtaposition of the promoter for the housekeeping gene *RPN1*, situated upstream of the *EVI1*, causing significant upregulation of *EVI1* (37). These examples as well as additional studies in the literature suggest that *PIM1* may be essential to AML processes via several AML-associated genes.

Studies show that miR-638-5p may inhibit cancer cell proliferation via *PIM1* in some cancers. For example, in a study by Wang et al., *PIM1* expression was increased in osteosarcoma cells, and it was concluded that it was associated with a low survival rate for this type of cancer, and increased miR-638-5p expression was reported to play a tumor suppressor role by inhibiting *PIM1* gene expression (38). On the other hand, the relationship between miR-638-5p and *PIM1* has not been investigated in AML as of yet. *PGK1* is a proto-oncogene associated with many types of cancer because it participates in DNA repair and angiogenesis. Increased *PGK1* expression in solid tumors, such as stomach cancer (39), ovarian cancer (40), brain tumors (41, 42), breast cancer (43), hepatocellular carcinoma (44), and prostate cancer (45), has been associated with tumor development and metastasis (46). miR-638-5p has been shown to contribute to the disease process via *PGK1* in human aortic endothelial cells (47). However, the miR-638-5p/*PGK1* axis has not been investigated in AML.

The current study investigated the role of ectopic miR-638-5p expression in AML cells, revealing that miR-638-5p may suppress AML cells via the *PIM1* and *PGK1* genes. We conclude that PIM1 and PGK1 genes may have the potential to play a key role in the pathogenesis of AML. The results of our study will contribute to the literature by demonstrating miR-638-5p/*PGK1* and miR-638-5p/*PIM1* relationships in AML cells.

Studies using mimic and inhibitor transfection approaches to investigate the functions and mechanisms of miRNAs have made it possible to investigate relevant miRNA-disease relationships *in vitro*. However, miRNA-mediated approaches are disadvantaged by the *in vivo* stability and availability of these molecules, which unfortunately limits the clinical applicability of miRNAs. Current studies have focused on alternative techniques such

as nanoparticle-mediated methods for the enhancement of miRNA stability and targeted delivery. Thus, miRNAs can be much more efficient in clinical applications in the future by overcoming these issues (48, 49).

Our study results indicate that miR-638-5p may play a role as a tumor suppressor in AML cells via the *PGK1* and *PIM1* genes and may be a candidate therapeutic miRNA for future studies. Even though these data at the mRNA level require confirmation with further studies. As a further study, the protein levels of *PIM1* and *PGK1* after miR-638-5p transfection can be investigated by western blot. Confirming whether miR-638-5p targets the 3' UTR of these target genes using the luciferase reporter assay method is another possibility for further investigation. It is particularly important to confirm the findings obtained in cell lines in the peripheral blood and bone marrow samples of patients with AML. Overall, these data will be valuable for the design of future *in vivo* animal experiments.

CONCLUSION

miR-638-5p transfection reduced proliferation, increased apoptosis, and targeted *PIM1* and *PGK1* oncogenes, reducing their expression in two AML cell lines, HL-60 and NB4. The results obtained in this study are especially important as preliminary data for future studies that will investigate the potential of miR-638-5p as a therapeutic biomarker in AML by targeting the *PIM1* and *PGK1* genes.

Financial Disclosure: This study was supported by the Research Fund of Istanbul University (Project No: 37151).

Conflict of Interest: There is no conflict of interest.

Ethics Committee Approval and Informed Consent: Ethics committee approval is not required, patient samples were not incorporated, and only cell lines were included in this study.

Authorship Contributions: SOO (conception and design, or analysis and interpretation of the data; drafting the article or revising it critically for important intellectual content and approval of the final version), IS (conception and design, or analysis and interpretation of the data; drafting the article or revising it critically for important intellectual content and approval of the final version), MK (drafting the article or revising it critically for important intellectual content and approval of the final version), SO (conception and design, or analysis and interpretation of the data and approval of the final version).

REFERENCES

1. De Kouchkovsky I, Abdul-Hay M. 'Acute myeloid leukemia: a comprehensive review and 2016 update'. Blood Cancer J. 2016;6(7):e441.
2. Lyengar V SA. Leukemia: StatPearls Publishing, Treasure Island (FL) 2020
3. Kaya M, Abuaisha A, Süer İ, Alptekin MS, Abanoz F, Emiroğlu S, et al. Overexpression of CDC25A, AURKB, and TOP2A Genes Could Be an Important Clue for Luminal A Breast Cancer. Eur J Breast Health. 2024;20(4):284-91.
4. Wang S, Xu J, Guo Y, Cai Y, Zhu W, Meng L, et al. Successful Transfection of MicroRNA Mimics or Inhibitors in a Regular Cell Line and in Primary Cells Derived from Patients with Rheumatoid Arthritis. Bio Protoc. 2023;13(18):e4823.
5. Kaya M, Abuaisha A, Suer I, Emiroglu S, Önder S, Onay Ucar E, et al. Let-7b-5p sensitizes breast cancer cells to doxorubicin through Aurora Kinase B. PLoS One. 2025;20(1):e0307420.
6. Suer I, Kaya M. Is the AURKB Gene Involved in Aml Cell Proliferation Since It is Targeted by miR-34a-5p and let-7b-5p? Konuralp Medical Journal. 2023;15(1):16-23.
7. Kaya M, Abuaisha A, Suer I, Emiroglu S, Abanoz F, Palanduz S, et al. Turmeric Inhibits MDA-MB-231 Cancer Cell Proliferation, Altering miR-638-5p and Its Potential Targets. Eur J Breast Health. 2024;20(2):102-9.
8. Wang J, Chen J, Sen S. MicroRNA as Biomarkers and Diagnostics. J Cell Physiol. 2016;231(1):25-30.
9. Condrat CE, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, et al. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. Cells. 2020;9(2).
10. Erdogan C, Suer I, Kaya M, Ozturk S, Aydin N, Kurt Z. Bioinformatics analysis of the potentially functional circRNA-miRNA-mRNA network in breast cancer. PLoS One. 2024;19(4):e0301995.
11. Kaya M. Bioinformatics evaluation of the circRNA–miRNA–mRNA axis in cervical squamous cell carcinoma. Exploration of Medicine. 2024;5:553-65.
12. Lee YS, Dutta A. MicroRNAs in cancer. Annu Rev Pathol. 2009;4:199-227.
13. Shi M, Jiang Y, Yang L, Yan S, Wang YG, Lu XJ. Decreased levels of serum exosomal miR-638 predict poor prognosis in hepatocellular carcinoma. J Cell Biochem. 2018;119(6):4711-6.
14. Zheng DH, Wang X, Lu LN, Chen DL, Chen JM, Lin FM, et al. MiR-638 serves as a tumor suppressor by targeting HOXA9 in glioma. Eur Rev Med Pharmacol Sci. 2018;22(22):7798-806.
15. Wang F, Lou J-f, Cao Y, Shi X-h, Wang P, Xu J, et al. miR-638 is a new biomarker for outcome prediction of non-small cell lung cancer patients receiving chemotherapy. Experimental & Molecular Medicine. 2015;47(5):e162-e.

16. Yao Y, Suo AL, Li ZF, Liu LY, Tian T, Ni L, et al. MicroRNA profiling of human gastric cancer. Mol Med Rep. 2009;2(6):963-70.
17. Bai Y, Chen C, Guo X, Ding T, Yang X, Yu J, et al. miR-638 in circulating leukaemia cells as a non-invasive biomarker in diagnosis, treatment response and MRD surveillance of acute promyelocytic leukaemia. Cancer Biomark. 2020;29(1):125-37.
18. Zhou J, Guo H, Yang Y, Zhang Y, Liu H. A meta-analysis on the prognosis of exosomal miRNAs in all solid tumor patients. Medicine (Baltimore). 2019;98(16):e15335.
19. Lin Y, Li D, Liang Q, Liu S, Zuo X, Li L, et al. miR-638 regulates differentiation and proliferation in leukemic cells by targeting cyclin-dependent kinase 2. J Biol Chem. 2015;290(3):1818-28.
20. Gíslason MH, Demircan GS, Prachar M, Furtwängler B, Schwaller J, Schoof EM, et al. BloodSpot 3.0: a database of gene and protein expression data in normal and malignant haematopoiesis. Nucleic Acids Res. 2024;52(D1):D1138-d42.
21. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016;44(W1):W90-7.
22. Miller HE, Bishop AJR. Correlation AnalyzeR: functional predictions from gene co-expression correlations. BMC Bioinformatics. 2021;22(1):206.
23. Almeida AM, Ramos F. Acute myeloid leukemia in the older adults. Leuk Res Rep. 2016;6:1-7.
24. Narayan N, Bracken CP, Ekert PG. MicroRNA-155 expression and function in AML: An evolving paradigm. Exp Hematol. 2018;62:1-6.
25. Weng H, Lal K, Yang FF, Chen J. The pathological role and prognostic impact of miR-181 in acute myeloid leukemia. Cancer Genet. 2015;208(5):225-9.
26. Garzon R, Volinia S, Liu CG, Fernandez-Cymering C, Palumbo T, Pichiorri F, et al. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood. 2008;111(6):3183-9.
27. Wang F, Lou JF, Cao Y, Shi XH, Wang P, Xu J, et al. miR-638 is a new biomarker for outcome prediction of non-small cell lung cancer patients receiving chemotherapy. Exp Mol Med. 2015;47:e162.
28. Lin QY, Wang JQ, Wu LL, Zheng WE, Chen PR. miR-638 represses the stem cell characteristics of breast cancer cells by targeting E2F2. Breast Cancer. 2020;27(1):147-58.
29. Bhattacharya A, Schmitz U, Raatz Y, Schonherr M, Kottek T, Schauer M, et al. miR-638 promotes melanoma metastasis and protects melanoma cells from apoptosis and autophagy. Oncotarget. 2015;6(5):2966-80.
30. Zhang J, Fei B, Wang Q, Song M, Yin Y, Zhang B, et al. MicroRNA-638 inhibits cell proliferation, invasion and regulates cell cycle by targeting tetraspanin 1 in human colorectal carcinoma. Oncotarget. 2014;5(23):12083-96.
31. Zhu DX, Zhu W, Fang C, Fan L, Zou ZJ, Wang YH, et al. miR-181a/b significantly enhances drug sensitivity in chronic lymphocytic leukemia cells via targeting multiple anti-apoptosis genes. Carcinogenesis. 2012;33(7):1294-301.
32. Chong ZX, Yeap SK, Ho WY. Dysregulation of miR-638 in the progression of cancers. Pathology - Research and Practice. 2021;220:153351.
33. Wang H, Yao H, Yi B, Kazama K, Liu Y, Deshpande D, et al. MicroRNA-638 inhibits human airway smooth muscle cell proliferation and migration through targeting cyclin D1 and NOR1. J Cell Physiol. 2018;234(1):369-81.
34. Zhao LY, Yao Y, Han J, Yang J, Wang XF, Tong DD, et al. miR-638 suppresses cell proliferation in gastric cancer by targeting Sp2. Dig Dis Sci. 2014;59(8):1743-53.
35. Brummer T, Zeiser R. The role of the MDM2/p53 axis in antitumor immune responses. Blood. 2024;143(26):2701-9.
36. Wang X, Zhu HQ, Lin SM, Xia BY, Xu B. RPN1: a pan-cancer biomarker and disulfidptosis regulator. Transl Cancer Res. 2024;13(5):2518-34.
37. Shearer BM, Sukov WR, Flynn HC, Knudson RA, Ketterling RP. Development of a dual-color, double fusion FISH assay to detect RPN1/EVI1 gene fusion associated with inv(3), t(3;3), and ins(3;3) in patients with myelodysplasia and acute myeloid leukemia. Am J Hematol. 2010;85(8):569-74.
38. Wang XX, Liu J, Tang YM, Hong L, Zeng Z, Tan GH. MicroRNA-638 inhibits cell proliferation by targeting suppress PIM1 expression in human osteosarcoma. Tumour Biol. 2017.
39. Huang B, Cai W, Wang Q, Liu F, Xu M, Zhang Y. Gankyrin Drives Malignant Transformation of Gastric Cancer and Alleviates Oxidative Stress via mTORC1 Activation. Oxid Med Cell Longev. 2018;2018:9480316.
40. Wu Y, Deng Y, Zhu J, Duan Y, Weng W, Wu X. Pim1 promotes cell proliferation and regulates glycolysis via interaction with MYC in ovarian cancer. Onco Targets Ther. 2018;11:6647-56.
41. Irshad K, Mohapatra SK, Srivastava C, Garg H, Mishra S, Dikshit B, et al. A combined gene signature of hypoxia and notch pathway in human glioblastoma and its prognostic relevance. PLoS One. 2015;10(3):e0118201.

42. Ameis HM, Drenckhan A, von Loga K, Escherich G, Wenke K, Izbicki JR, et al. PGK1 as predictor of CXCR4 expression, bone marrow metastases and survival in neuroblastoma. PLoS One. 2013;8(12):e83701.
43. Xu D, Aka JA, Wang R, Lin SX. 17beta-hydroxysteroid dehydrogenase type 5 is negatively correlated to apoptosis inhibitor GRP78 and tumor-secreted protein PGK1, and modulates breast cancer cell viability and proliferation. J Steroid Biochem Mol Biol. 2017;171:270-80.
44. Ai J, Huang H, Lv X, Tang Z, Chen M, Chen T, et al. FLNA and PGK1 are two potential markers for progression in hepatocellular carcinoma. Cell Physiol Biochem. 2011;27(3-4):207-16.
45. Chen J, Cao S, Situ B, Zhong J, Hu Y, Li S, et al. Metabolic reprogramming-based characterization of circulating tumor cells in prostate cancer. J Exp Clin Cancer Res. 2018;37(1):127.
46. He Y, Luo Y, Zhang D, Wang X, Zhang P, Li H, et al. PGK1-mediated cancer progression and drug resistance. Am J Cancer Res. 2019;9(11):2280-302.
47. Zhang X, Guan MX, Jiang QH, Li S, Zhang HY, Wu ZG, et al. NEAT1 knockdown suppresses endothelial cell proliferation and induces apoptosis by regulating miR638/AKT/mTOR signaling in atherosclerosis. Oncol Rep. 2020;44(1):115-25.
48. Jin HY, Gonzalez-Martin A, Miletic AV, Lai M, Knight S, Sabouri-Ghomi M, et al. Transfection of microRNA Mimics Should Be Used with Caution. Front Genet. 2015;6:340.
49. Manikkath J, Jishnu PV, Wich PR, Manikkath A, Radhakrishnan R. Nanoparticulate strategies for the delivery of miRNA mimics and inhibitors in anticancer therapy and its potential utility in oral submucous fibrosis. Nanomedicine (Lond). 2022;17(3):181-95.
50. Karatas OF, Wang J, Shao L, Ozen M, Zhang Y, Creighton CJ, et al. miR-33a is a tumor suppressor microRNA that is decreased in prostate cancer. Oncotarget. 2017;8(36):60243-56.
51. Song Y, Selak MA, Watson CT, Coutts C, Scherer PC, Panzer JA, et al. Mechanisms underlying metabolic and neural defects in zebrafish and human multiple acyl-CoA dehydrogenase deficiency (MADD). PLoS One. 2009;4(12):e8329.
52. Lee W, Kim J, Yun JM, Ohn T, Gong Q. MeCP2 regulates gene expression through recognition of H3K27me3. Nat Commun. 2020;11(1):3140.
53. Huang H, Zhao Y, Shang X, Ren H, Zhao Y, Liu X. CAIII expression in skeletal muscle is regulated by Ca(2+)-CaMKII-MEF2C signaling. Exp Cell Res. 2019;385(1):111672.
54. Boaretto F, Snijders D, Salvoro C, Spalletta A, Mostacciuolo ML, Collura M, et al. Diagnosis of Primary Ciliary Dyskinesia by a Targeted Next-Generation Sequencing Panel: Molecular and Clinical Findings in Italian Patients. J Mol Diagn. 2016;18(6):912-22.
55. Suer I, Karatas OF, Yuceturk B, Yilmaz M, Guven G, Buge O, et al. Characterization of stem-like cells directly isolated from freshly resected laryngeal squamous cell carcinoma specimens. Curr Stem Cell Res Ther. 2014;9(4):347-53.

RESEARCH ARTICLE

Abdullah Belada[1]
Ahmet Koder[2]
Mehmet Ali Sungur[3]

[1] Duzce University Medical Faculty, Department of ENT, Duzce, Türkiye
[2] Trakya University Medical Faculty, Department of ENT, Edirne, Türkiye
[3] Duzce University Medical Faculty, Department of Biostatistic, Duzce, Türkiye

Corresponding Author:
Abdullah Belada
mail: abdullahbelada@yahoo.com

Received: 31.10.2024
Acceptance: 14.02.2025
DOI:10.18521/ktd.1576850

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Did Increase of Rates of Sudden Hearing Loss During COVID-19?

ABSTRACT

Objective: This study aims to assess the impact of the COVID-19 pandemic on cases of sudden hearing loss (SHL).

Method: We examined two patient groups diagnosed with SHL at Düzce University Medical Faculty Hospital and Düzce Atatürk State Hospital in Turkey. The first group, diagnosed between March 2019 and March 2020, represents pre-Covid-19. The second group includes patients diagnosed from March 2020 to March 2021, corresponding to the initial year of the Covid-19 pandemic in this region. We evaluated patient numbers, age, and gender across these groups to identify any increase in SHL cases potentially associated with the pandemic.

Results: The ratio of SHL cases per population in Group 1 was 51 out of 450,000, or approximately 0.011333. In Group 2, this ratio was 32 out of 450,000, or roughly 0.007111. Statistical analysis (p=0.037) revealed a notably higher SHL rate in the pre-Covid-19 period than during the pandemic.

Conclusions: The suggested etiological explanations for SHL remain hypothetical. Although COVID-19 has been widely considered a possible factor, our findings do not support increased SHL cases due to the virus.

Keywords: COVID-19 Pandemic, Sudden Hearing Loss, Infection.

Covid-19 Sürecinde Ani İşitme Kaybi Oranlari Artiş Gösterdi Mi?

ÖZET

Amaç: Bu çalışmanın amacı, Covid-19 salgının ani işitme kaybı üzerindeki etkisini araştırmaktır.

Yöntem: Çalışmamızı oluşturan 1. grup, Covid-19 öncesi Türkiye-Düzce Mart 2019 den Mart 2020 tarihine kadar Düzce Üniversitesi Tıp Fakültesi Hastanesi ile Düzce Atatürk Devlet Hastanesi' nde Ani İşitme Kaybı tanısı almış hastaları içerir. İkinci grup ise yine aynı popülasyonda Covid-19 enfeksiyonun görülmeye başladığı Mart 2020 ile Mart 2021 ile tarihleri arasında AİK tanısı almış hastaları içermektedir. Bu hastaların sayısı, cinsiyeti ve yaşı incelenip, iki grubun sayısı arasında AİK tanısı alan hastalarında Covid-19 salgını nedenli bir artış olup olmadığının değerlendirilmesi amaçlanmaktadır.

Bulgular: Grup 1 deki hastaların nüfusa oranı 51/450000 = 0,011333 dir. Grup 2 deki hastaların nüfusa oranı 32/450000 = 0,007111 dir. Bu iki oran 51/450000 = 0,011333 ile 32/450000 = 0,007111 karşılaştırıldığında p=0,037 olup istatistiksel olarak Covid-19 öncesinde AİK oranı, Covid-19 esnasındaki AİK oranından anlamlı olarak yüksek bulunmuştur.

Sonuç: Etyoloji de ortaya konan fikirler hipotez düzeyindedir. Tüm dünyayı etkisi altına alan Covid-19 da AİK nın olağan şüphelileri arasındadır. Bizim bulgularımız Covid-19 un AİK nı artırmadığı yönündedir.

Anahtar Kelimeler: Covid-19 Salgını, Ani İşitme Kaybı, Enfeksiyon.

INTRODUCTION

Sudden hearing loss (SHL) is considered an otorhinolaryngology emergency, characterized by a rapid sensorineural hearing decrease of 30 dB or more across three sequential frequencies within 72 hours. Each year in the United States, about 5 to 27 out of every 100,000 individuals are diagnosed with SHL (1). Given the condition's relatively rare occurrence, its causes and treatments are not fully understood (2). Studies indicate that 32-65% of SHL cases may resolve on their own (3,4). Possible causes include viral infections (5), vascular blockages (6), cochlear stress responses (7), and immune-related processes (8). Viral agents such as herpes simplex (HSV), HIV, hepatitis, measles, rubella, mumps, lassa virus, and enterovirus have been associated with SHL (2,9,10).

Covid-19, identified in December 2019 as a zoonotic virus from a seafood market (11), typically shows symptoms within 2 to 14 days of exposure. Common symptoms are fever, cough, sore throat, headache, muscle aches, and changes in taste or smell, though severe cases can lead to respiratory distress, multi-organ failure, or death, especially in immunocompromised individuals (12,13).

While various viruses are known to trigger SHL, it is uncertain whether Covid-19 is one of them. This study investigates whether there was an increase in SHL cases within one year following the onset of the Covid-19 pandemic.

MATERIAL AND METHODS

A total of 83 patients with SHL were included in this study. To meet the study's objective, participants were divided into two groups, each spanning the same one-year timeframe: the pre-pandemic group and the pandemic-period group. The pre-pandemic group consists of patients diagnosed with SHL at Duzce University Medical Faculty Hospital and Duzce Atatürk State Hospital between March 2019 and March 2020 in Duzce, Turkey, before Covid-19 emerged. The pandemic group includes those diagnosed with SHL between March 2020 and March 2021, marking the period when Covid-19 began affecting this area. The study was conducted retrospectively based on sudden hearing loss diagnoses entered into the system. By comparing the number of SHL cases, along with patient age and gender, this study aims to determine if there was an increase in SHL cases during the pandemic. The Clinical Research Ethics Committee of Duzce University approved the study protocol (2020/160)

Statistical Analysis: Statistical analyses were performed using IBM SPSS version 22 (IBM Corp., 2013, Armonk, NY). The Shapiro-Wilk test was applied to check data distribution, and the Levene test was used to assess homogeneity of variance. For comparisons between groups, an independent samples t-test was conducted, while categorical data were evaluated using a chi-square test. Results were presented as mean, standard deviation, frequency, and percentage, with a statistical significance threshold set at 0.05.

RESULTS

In this study, 83 patients in total were assessed. The pre-pandemic group included 51 patients, with 26 males (50.98%) and 25 females (49.02%), and an average age of 44.71±16.27 years. In the pandemic group, there were 32 patients, comprising 22 males (68.75%) and 10 females (31.25%), with a mean age of 44.03±14.98 years (Table 1). No statistically significant differences in age (p=0.850) or gender distribution (p=0.111) were observed between the pre-pandemic and pandemic groups. SHL incidence rates per 100,000 people were calculated for each one-year period, with the population of Düzce considered as 450,000. The pre-pandemic rate was 11.33 per 100,000, while the pandemic rate was 7.11 per 100,000. A significant difference was found between these rates (p=0.037), with the SHL rate being notably higher before Covid-19 compared to during the pandemic. (Figure 1).

Table 1. Comparison of before and pandemic-period groups

	Before Pandemic (n=51)	**Pandemic Period (n=32)**	**p**
Sex, n (%)			
Male	26 (50.98)	22 (68.75)	0.111
Female	25 (49.02)	10 (31.25)	
Age (year)	44.71±16.27	44.03±14.98	0.850

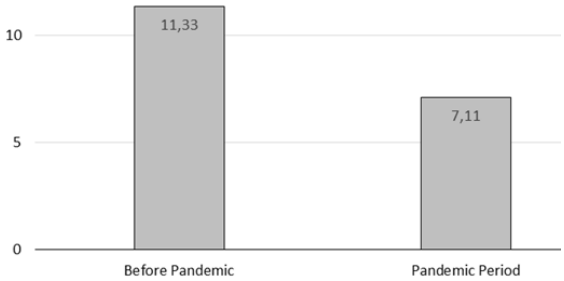

Figure 1. The ratio of sudden hearing loss in the before and pandemic period

DISCUSSION

Coronaviruses (CoVs) have led to three significant outbreaks in the past 25 years: Severe Acute Respiratory Syndrome (SARS), Middle Eastern Respiratory Syndrome (MERS), and currently COVID-19. The COVID-19 outbreak began in Wuhan, Hubei province, China, and was identified as a zoonotic disease associated with a novel betacoronavirus, SARS-CoV (14). Key symptoms of COVID-19 include fever, cough, sore

throat, muscle pain, respiratory failure, and disorders of smell and taste (15).

Viral infections can lead to peripheral facial paralysis and sensory disorders related to cranial nerve involvement (16,17). Various viruses, such as herpes simplex, HIV, hepatitis, measles, rubella, mumps, Lassa, and enterovirus, have been implicated in sudden hearing loss (SHL) (17,18). Several theories suggest that viral infections might cause SHL through mechanisms such as direct invasion of the cochlear nerve, reactivation of latent viruses in the inner ear, or systemic cross-reactions between antigens and antibodies triggered by the virus (2,17,18).

Numerous studies have explored the relationship between COVID-19 and SHL, primarily in the form of case reports. In one study by Osman Kılıç et al (19), out of five SHL patients tested for COVID-19, one tested positive, exhibiting SHL as the sole symptom. The authors cautioned that SHL could be a unique manifestation of COVID-19. Lamaunier et al (20) documented a case of SHL in the right ear of a 67-year-old female patient following COVID-19. Similarly, Lang et al (21) described a nurse diagnosed with COVID-19 who later experienced sudden hearing loss in her right ear despite treatment.

Drouet et al (22) found elevated plasma serotonin levels in SHL patients in a study involving 133 participants. Building on this, Harenberg et al (23) proposed that increased serotonin may lead to coagulation disorders and microthrombosis, suggesting a potential link between COVID-19 and SHL via ACE2 receptors. Fidan and colleagues (24) evaluated the incidence of sudden hearing loss during the COVID-19 pandemic, revealing an increase in SHL diagnoses compared to pre-COVID-19 rates. In a cohort study conducted by Kim et al. (25), it was found that Covid-19 infection increased sudden hearing loss.

In our research conducted from March 2020 to March 2021, during the onset of COVID-19 in Turkey, 39 patients were admitted to hospitals in Düzce province with SHL, compared to 54 in the pre-COVID-19 period. Interestingly, this comparison indicates a decline in SHL cases during the pandemic. Although viral infections are known to cause SHL, specific virus types have been associated with this condition. A review of the literature shows no documented increase in SHL related to SARS or MERS, indicating that the connection between COVID-19 and SHL remains largely anecdotal.

During this unprecedented period, factors that cannot be analyzed with current data may have contributed to the decrease in SHL cases. These could include reduced exposure to acoustic trauma due to restrictions on gatherings such as hunting, sports events, and nightlife. The widespread use of masks may have led to a decline in other upper respiratory tract infections, potentially reducing SHL cases from non-COVID-19 viruses. Additionally, some patients may have avoided seeking treatment for SHL in hospitals due to concerns about disease transmission. These factors warrant further investigation. The study includes patients from a specific region, and a much larger sample size and longer follow-up period would be needed to assess the effects of COVID-19 more accurately

CONCLUSION

The low incidence of SHL and the inability to perform pathological examinations leave its etiology unclear. Current theories are largely hypothetical, with COVID-19 being considered a potential contributor to SHL. Our findings suggest that COVID-19 does not increase the risk of SHL, and further studies are needed to clarify this issue.

REFERENCES

1. Chandrasekhar SS, Tsai Do BS, Schwartz SR, Bontempo LJ, Faucett EA, Finestone SA, et al. Clinical Practice Guideline: Sudden Hearing Loss (Update). Otolaryngol Head Neck Surg. 2019;161 (8):1-45.
2. Chen X, Fu YY, Zhang TY. Role of viral infection in sudden hearing loss. J Int Med Res. 2019;47(7):2865-72.
3. Dishoeck H and Bierman T. Sudden perceptive deafness and viral infection (report of the first one hundred patients). Ann Otol Rhinol Laryngol. 1957; 66(4): 963–80
4. Rasmussen H. Sudden deafness. Acta Otolaryngol. 1949; 37: 65–70.
5. Ovet G, Alatas N and Kocacan FN. Sudden sensorineural hearing loss: is antiviral treatment really necessary? Am J Otolaryngol. 2015; 36(4):542–6.
6. Veldman JE. Cochlear and retrocochlear immune-mediated inner ear disorders. Pathogenetic mechanisms and diagnostic tools. Ann Otol Rhinol Laryngol. 1986; 95(1):535-40.
7. Mattox DE, Simmons FB. Natural history of sudden sensorineural hearing loss. Ann Otol Rhinol Laryngol. 1977;86(8):463-80.
8. Stachler RJ, Chandrasekhar SS, Archer SM, Bontempo LJ, Faucett EA, Finestone SA, et al. Clinical practice guideline: sudden hearing loss. Otolaryngol Head Neck Surg. 2012;146(3):1-35
9. Mateer EJ, Huang C, Shehu NY, Paessler S. Lassa fever-induced sensorineural hearing loss: a neglected public health and social burden. PLoS Negl Trop Dis. 2018; 12(2):1-11
10. Chen HC, Chung CH and Wang CH. Increased risk of sudden sensorineural hearing loss in patients with hepatitis virus infection. PLoS One. 2017; 12(4):1-11

11. Mackenzie J, Smith D. COVID-19: a novel zoonotic disease caused by a coronavirus from China: what we know and what we don't. Microbiol Aust. 2020;17(3):41:45
12. Baj J, Karakuła-Juchnowicz H, Teresiński G, Buszewicz G, Ciesielka M, Sitarz E et al. COVID-19: specific and non-specific clinical manifestations and symptoms: the current state of knowledge. J Clin Med. 2020; 9(6):1753
13. Abdel Rhman S, Abdel Wahid A. COVID-19 and sudden sensorineural hearing loss, a case report. Otolaryngology Case Reports. 2020;16:1-3
14. Harrison AG, Lin T, Wang P. Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. Trends Immunol. 2020;41(12):1100-15.
15. Vaira LA, Salzano G, Deiana G, De Riu G. Anosmia and Ageusia: Common Findings in COVID-19 Patients. Laryngoscope. 2020;130(7):1787.
16. Kennedy PG. Herpes simplex virus type 1 and Bell's palsy-a current assessment of the controversy. J Neurovirol. 2010;16(1):1-5.
17. Mateer EJ, Huang C, Shehu NY, Paessler S. Lassa fever-induced sensorineural hearing loss: A neglected public health and social burden. PLoS Negl Trop Dis. 2018; 12(2):1-11
18. Cohen BE, Durstenfeld A, Roehm PC. Viral causes of hearing loss: a review for hearing health professionals. Trends Hear. 2014;29(18)
19. Kilic O, Kalcioglu MT, Cag Y, Tuysuz O, Pektas E, Caskurlu H, Cetın F. Could sudden sensorineural hearing loss be the sole manifestation of COVID-19? An investigation into SARS-COV-2 in the etiology of sudden sensorineural hearing loss. Int J Infect Dis. 2020;97(8):208-11.
20. Lamounier P, Franco Gonçalves V, Ramos HVL, Gobbo DA, Teixeira RP, Dos Reis PC, Bahmad F Jr, Cândido Costa C. A 67-Year-Old Woman with Sudden Hearing Loss Associated with SARS-CoV-2 Infection. Am J Case Rep. 2020; 3(21):1-6
21. Lang B, Hintze J, Conlon B. Coronavirus disease 2019 and sudden sensorineural hearing loss. J Laryngol Otol. 2020; 1(10):1-3.
22. Drouet L, Hautefort C, Vitaux H, Kania R, Callebert J, Stepanian A, Siguret V, Eliezer M, Vodovar N, Launay JM. Plasma Serotonin is Elevated in Adult Patients with Sudden Sensorineural Hearing Loss. Thromb Haemost. 2020;120(9):1291-9.
23. Harenberg J, Marchetti M, Falanga A. Acquired Autoimmune Hemophilia Following SARS-CoV-2 Vaccines: Dual-Drug Effects on Blood Coagulation and the Scylla and Charybdis Phenomenon. Thromb Haemost. 2021;121(12):1555-7.
24. Fidan V, Akin O, Koyuncu H. Rised sudden sensorineural hearing loss during COVID-19 widespread. Am J Otolaryngol. 2021;42(5):102996.
25. Kim HJ, Jeong S, Kim K, Lee JD, Oh YH, Suh MJ. Incidence of hearing loss following COVID-19 among young adults in South Korea: a nationwide cohort study. EClinicalMedicine. 2024;29 (6);75:102759.

RESEARCH ARTICLE

Merve Balci[1]
Kamile Marakoglu[2]
Duygu Ilke Yildirim[2]
Muslu Kazim Korez[3]

[1] Aksaray Central Community Health Center, Aksaray, Türkiye
[2] Department of Family Medicine, Selcuk University Medical Faculty, Konya, Türkiye
[3] Biostatistics, Selcuk University Faculty of Medicine, Konya, Türkiye

Corresponding Author:
Merve Balci
mail: mervegulbahar05@gmail.com

Received: 21.05.2024
Acceptance: 14.01.2025
DOI:10.18521/ktd.1479646

Presented as an e-oral presentation at the 21st International Eastern Mediterranean Family Medicine Congress held in Adana/Turkey.

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Evaluation of COVID-19 Vaccination Status and Influencing Factors among Research Assistant Physicians and Sixth-Year Medical Students at a Faculty of Medicine

ABSTRACT

Objective: This study aimed to evaluate the COVID-19 vaccination status of research assistant physicians and sixth-year medical students at a faculty of medicine, as well as the factors influencing their vaccination decisions.

Method: The study was conducted as a descriptive and cross-sectional investigation between May 2021 and June 2021 among research assistant physicians and sixth-year medical students at a faculty of medicine. Data were collected using a 47-item questionnaire administered either face-to-face or online. The questionnaire included items on participants' sociodemographic characteristics, COVID-19 history, and vaccination history regarding COVID-19 and other vaccines. Data were analyzed using SPSS version 21, with statistical significance set at $p<0.05$.

Results: A total of 501 participants were included in the study, comprising 352 research assistant physicians and 149 sixth-year medical students. Among the participants, 88.2% (n=442) had received two doses of the COVID-19 vaccine. The most frequently cited reason for vaccination, reported by 54.4% of vaccinated participants, was "acquiring immunity against the disease." In contrast, the most common reason for vaccine hesitancy, reported by 42.6% of unvaccinated participants, was "lack of sufficient data concerning the efficacy and safety of vaccines." COVID-19 vaccine acceptance was significantly higher among men compared to women (p=0.023), single individuals compared to married individuals (p=0.006), and those who feared transmitting COVID-19 to their family members compared to those without such concerns (p=0.007).

Conclusions: The majority of participants in this study received the COVID-19 vaccine. The high level of vaccine acceptance among healthcare professionals, who serve as important role models for society, is crucial in combating the COVID-19 pandemic. It is recommended that vaccination campaigns consider the factors influencing vaccine acceptance and hesitancy to enhance their effectiveness.

Keywords: COVID-19, Vaccine, Research Assistant Physicians, Medical Students, Healthcare Workers.

Bir Tıp Fakültesindeki Araştırma Görevlisi Doktorların ve Dönem VI Tıp Fakültesi Öğrencilerinin COVID-19 Aşısı Olma Durumları ve Etkileyen Faktörlerin Değerlendirilmesi

ÖZET

Amaç: Bu çalışmada araştırma görevlisi doktorların ve dönem VI tıp fakültesi öğrencilerinin COVID-19 aşısı olma durumları ve bunu etkileyen faktörlerin değerlendirilmesi amaçlandı.

Yöntem: Bu çalışma Mayıs 2021 - Haziran 2021 tarihlerinde bir tıp fakültesindeki araştırma görevlisi doktorlar ve dönem VI tıp fakültesi öğrencilerinde gerçekleştirilen tanımlayıcı ve kesitsel tipte bir araştırmadır. Araştırma verileri katılımcıların sosyo-demografik özellikleri, COVID-19 anamnez bilgileri, COVID-19 aşısı ve diğer aşılarla ilgili anamnez bilgilerine yönelik sorulardan oluşan 47 soruluk yüz yüze veya çevrim içi doldurulan anket formu aracılığıyla toplandı. Veriler SPSS versiyon 21 kullanılarak analiz edildi. İstatistiksel anlamlılık $p<0,05$ düzeyinde değerlendirildi.

Bulgular: Bu çalışmaya katılan 501 katılımcının 352'si araştırma görevlisi doktor ve 149'u dönem VI tıp fakültesi öğrencisi idi. Çift doz COVID-19 aşısı yaptıranlar katılımcıların %88,2'sini (n=442) oluşturmaktadır. Katılımcılardan COVID-19 aşısı yaptıranların belirttiği en sık aşı yaptırma nedeni %54,4 oran ile "hastalığa karşı bağışıklık kazanmak" iken COVID-19 aşısı yaptırmayanların en sık aşı yaptırmama nedeni %42,6 oran ile 'aşının güvenliği ve etkinliği açısından verilerin yeterli olmaması' idi. Erkeklerde kadınlara göre (p=0,023), bekarlarda evlilere göre (p=0,006), COVID-19'u ailesine bulaştırmaktan korkanlarda korkmayanlara göre (p=0,007) COVID-19 aşı kabulü anlamlı derecede yüksek bulundu.

Sonuç: Bu çalışmada katılımcıların önemli bir çoğunluğu COVID-19 aşısını yaptırdı. Toplum için iyi bir rol model olan sağlık çalışanlarının aşı kabulünün yüksek olması COVID-19 pandemisi ile mücadelede önem arz etmektedir. Aşı kampanyaları oluşturulurken aşı reddi ve kabulüne etki eden bu faktörlerin göz önünde bulundurulmasının faydalı olacağı düşünülmelidir.

Anahtar Kelimeler: COVID-19, Aşı, Araştırma Görevlisi Doktorlar, Tıp Öğrencileri, Sağlık Çalışanları.

INTRODUCTION

Several pneumonia cases of unknown cause were reported in Wuhan in December 2019 (1). In January 2020, a new type of coronavirus, called 2019 novel coronavirus (2019-nCoV), was identified in throat swab samples taken from patients (2). Later, this virus was named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) due to its similarity to SARS-CoV, and the disease caused by the virus was termed COVID-19.

While COVID-19 is asymptomatic or manifests with mild symptoms in some patients, it may cause clinical conditions progressing to severe respiratory failure and even multiple organ failure in other cases (3). Since there is no specific method proven in terms of efficacy and safety in the treatment of COVID-19, the treatment approach is more focused on symptoms (3,4). Therefore, the most crucial method for fighting against the disease is immunization through vaccination (5).

Vaccines that received emergency use approval began to be administered in late 2020. As of December 31, 2023, approximately 13,6 billion doses of vaccine had been administered across the world (5,6). Four vaccines received approval for emergency use in Turkey: the Pfizer/BioNTech (BNT162b2) mRNA vaccine, the Sinovac (CoronaVac) inactive vaccine, the Gamaleya (Sputnik V) non-replicative viral vector vaccine, and the Turcovac (Erucov-Vac) inactive vaccine (5,7). In Turkey, approval for emergency use was first granted for the Sinovac (CoronaVac) vaccine by the Turkish Medicines and Medical Devices Agency on January 13, 2021 (8). The strategy for implementing COVID-19 vaccination was announced by the Republic of Turkey Ministry of Health, and the priority groups to be vaccinated were determined by evaluating the risks of exposure to the disease, severe disease, and transmission, as well as the negative impact of the disease on the functioning of social life. Healthcare workers were considered to have the highest priority in this evaluation (9).

As of December 10, 2024, the rate of individuals aged 18 and over in Turkey who received the first dose of either vaccine is 93.4%, and the rate of those who received the second dose of either vaccine was 85.7%. The total number of vaccines administered to date is 152 million 737 thousand 320, of which 57 million 962 thousand 188 belong to the first dose, 53 million 195 thousand 230 to the second dose, and 28 million 237 thousand 406 to the third dose (10).

The high vaccine acceptance among healthcare professionals, who serve as critical role models for society, is of significant importance in the fight against the COVID-19 pandemic (11, 12). This study aimed to evaluate the COVID-19 vaccination status of research assistant physicians and sixth-year medical students at a faculty of medicine, as well as the factors influencing their vaccination decisions.

MATERIAL AND METHODS

Sample and Data Collection: The study population consisted of 210 sixth-year medical students enrolled at Selçuk University Faculty of Medicine during the 2020–2021 academic year and 420 research assistant physicians working at Selçuk University Faculty of Medicine Hospital. The study aimed to include the entire population, excluding those who declined participation. Among the sixth-year medical students, all were approached (210/210), but 61 declined participation, resulting in a response rate of 70.95% (149/210). Similarly, all research assistant physicians were approached (420/420), with 68 declining participation, yielding a response rate of 83.8% (352/420). Considering the combined data from both groups, a total of 610 individuals were reached, and 501 were included in the sample, resulting in a final participation rate of 79.52%. The data for the study was collected from May 6, 2021, through June 30, 2021, using a 47-question survey completed face-to-face or online. The survey form consisted of questions regarding the participants' sociodemographic characteristics and anamnesis information about COVID-19 disease, COVID-19 vaccination, and other vaccinations.

Data Collection Forms

Sociodemographic Data Form: This form was prepared by the researchers and included questions to obtain information about the participants' age, gender, marital status, history of receiving other vaccines, and other sociodemographic characteristics related to the COVID-19 pandemic.

COVID-19 Vaccine Data Form: Using this form, the participants were asked whether they were concerned about the side effects of COVID-19 vaccines, whether they had been vaccinated against COVID-19, and whether they would recommend COVID-19 vaccination to their families, friends, and patients. Individuals who reported having received the COVID-19 vaccination were presented with an open-ended question to determine their reasons for vaccination. The responses of the participants to this question were grouped into the following categories: "acquiring immunity against the disease", "protecting family and close circle against the disease", "contributing to social immunity", "reducing the risk of severe disease", "reducing transmission", and "possibility of restrictions being imposed on unvaccinated individuals". Individuals who reported not having received the COVID-19 vaccination were asked whether they would be willing to be vaccinated in the future. These participants were also presented with an open-ended question to determine their reasons for not being vaccinated, and their

responses were classified into the following groups: "not enough time having elapsed since contracting the disease to receive vaccination", "lack of sufficient data concerning the efficacy and safety of vaccines", "concerns about the short- and long-term side effects of vaccines", and "lack of studies on vaccination during pregnancy and breastfeeding".

This study was approved by the ethics committee of the Selcuk University Faculty of Medicine (decision number: 2021/240, date: May 5, 2021). Prior the study, informed consent was obtained from each participant in compliance with the Declaration of Helsinki of the World Medical Association.

Statistical Analysis: All data was evaluated using IBM SPSS v. 21.0 statistical package program. Before the analyses, the suitability of the variables to the normal distribution was examined using Q-Q plots and the Shapiro-Wilk test. Descriptive statistics for the data were defined as mean ± standard deviation or median (minimum–maximum) for numerical variables, and as frequencies (n) and percentages (%) for categorical variables. Relationships between categorical variables were investigated with the Pearson chi-square, Yates continuity corrected chi-square, Fisher's exact chi-square, and Fisher-Freeman-Halton tests. Logistic regression models were constructed to determine factors affecting COVID-19 vaccination status. The results were evaluated at the 95% confidence interval, and the significance level was taken as $p < 0.05$.

RESULTS

A total of 501 volunteer research assistant doctors working at a medical school and term VI medical faculty students participated in the study. Of the participants, 53.9% (n = 270) were women, and 46.1% (n = 231) were men. The mean age of the participants was 27.5 ± 3.09 (23-40) years. Table 1 presents the participants' sociodemographic characteristics and anamnesis information related to other vaccines.

Table 1. Participants' sociodemographic characteristics and anamnesis information about other vaccines (n = 501)

Parameters	n Median (min-max)	%
Age	27 (23-40)	
Age group		
23-29 years	382	76.2
30-40 years	119	23.8
Gender		
Female	270	53.9
Male	231	46.1
Marital status		
Married	186	37.1
Single	315	62.9
Title		
Term VI medical faculty student	149	29.7
Research assistant doctor	352	70.3
Department employed		
Basic sciences	28	5.6
Internal sciences	208	41.5
Surgical sciences	116	23.2
Term VI medical faculty student	149	29.7
Years of profession		
<1 year	158	31.5
1-5 years	304	60.7
>5 years	39	7.8
Flu vaccination status for the 2020-2021 season		
Unvaccinated	383	76.4
Vaccinated	118	23.6
History of adverse reactions to previous vaccines		
Absent	478	95.4
Present	23	4.6
Total	**501**	**100.0**

min: minimum, max: maximum

Of the participants, 88.2% of the participants reported that they had received the COVID-19 vaccination, and 11.8% reported that they had not received the COVID-19 vaccination. The most common reason cited by vaccinated participants for receiving the COVID-19 vaccine was "acquiring immunity against the disease", while unvaccinated participants most frequently reported "lack of sufficient data concerning the efficacy and safety of vaccines" as their reason for refusal (Table 2).

Table 2. Participants' reasons for accepting or rejecting covid-19 vaccination

	n	%
Reasons for being vaccinated against COVID-19 (n = 494)*		
Acquiring immunity against the disease	268	54.4
Protecting family and close circle against the disease	48	9.7
Contributing to social immunity	58	11.7
Reducing the risk of severe disease	85	17.2
Reducing transmission	17	3.4
Possibility of restrictions being imposed on unvaccinated individuals	18	3.6
Reasons for not being vaccinated against COVID-19 (n = 68)*		
Not enough time having elapsed since contracting the disease to receive vaccination	15	22.2
Lack of sufficient data concerning the efficacy and safety of vaccines	29	42.6
Concerns about the short- and long-term side effects of vaccines	12	17.6
Lack of studies on vaccination during pregnancy and breastfeeding	12	17.6

*More than one response was allowed.

Table 3 presents the evaluation of the participants' COVID-19 vaccination status according to their sociodemographic characteristics and anamnesis related to other vaccines.

Table 4 presents the evaluation of the participants' COVID-19 vaccination status according to their working status in the pandemic unit and anamnesis information related to COVID-19 infection and vaccine.

Logistic regression analysis was applied to parameters that were found to be statistically significant in influencing participants' COVID-19 vaccination status (Table 5).

Table 3. Evaluation of participants' covid-19 vaccination status according to their sociodemographic characteristics and anamnesis related to other vaccines

	COVID-19 vaccination status				Total		X^2	*p*
	Vaccinated		Unvaccinated					
	n	%	n	%	n	%		
Gender								
Female	230	85.2	40	14.8	270	100.0		
Male	212	91.8	19	8.2	231	100.0	5,203[1]	**0.023**
Age group								
23-29 years	338	88.5	44	11.5	382	100.0		
30-40 years	104	87.4	15	12.6	119	100.0	0.025[2]	0.874
Marital status								
Married	154	82.8	32	17.2	186	100.0		
Single	288	91.4	27	8.6	315	100.0	7.578[2]	**0.006**
Title								
Term VI student	132	88.6	17	11.4	149	100.0		
Research assistant	310	88.1	42	11.9	352	100.0	<0.001[2]	0.989
Department employed								
Basic sciences	25	89.3	3	10.7	28	100.0		
Internal sciences	181	87.0	27	13.0	208	100.0		
Surgical sciences	104	89.7	12	10.3	116	100.0		
Term VI student	132	88.6	17	11.4	149	100.0	0.569[1]	0.904
Years of profession								
<1 year	139	88.0	19	12.0	158	100.0		
1-5 years	270	88.8	34	11.2	304	100.0		
>5 years	33	84.6	6	15.4	39	100.0	0.601[1]	0.741
Flu vaccination status for the 2020-2021 season								
Unvaccinated	328	85.6	55	14.4	383	100.0		
Vaccinated	114	96.6	4	3.4	118	100.0	9.420[1]	**0.002**
History of adverse reactions to previous vaccines								
Absent	425	88.9	53	11.1	478	100.0		
Present	17	73.9	6	26.1	23	100.0	0.001[3]	**0.042**
Total	**442**	**88.2**	**59**	**11.8**	**501**	**100.0**		

[1]Pearson chi-square test, [2]Yates chi-square test, [3]Fisher chi-square test

Table 4. Evaluation of participants' COVID-19 vaccination status according to their employment in the pandemic unit and anamnesis information regarding COVID-19 infection and vaccine

	COVID-19 vaccination status				Total		X^2	*p*
	Vaccinated		Unvaccinated					
	n	%	n	%	n	%		
Active employment in the pandemic unit								
No	174	88.3	23	11.7	197	100.0		
Yes	268	88.2	36	11.8	304	100.0	0.001[2]	1.000
Use of personal protective equipment								
Partial	124	89.2	15	10.8	139	100.0		
Full	318	87.8	44	12.2	362	100.0	0.072[2]	0.788
Fear of contracting COVID-19								
Absent	172	86.9	26	13.1	198	100.0		
Present	270	89.1	33	10.9	303	100.0	0.383[2]	0.536
Fear of transmitting COVID-19 infection to family								
Absent	14	66.7	7	33.3	21	100.0		
Present	428	89.2	52	10.8	480	100.0	0.001[3]	**0.007**
History of COVID-19 infection (n = 501)								
Absent	311	91.2	30	8.8	341	100.0		
Present	131	81.9	29	18.1	160	100.0	8.243[2]	**0.004**
Concerns about COVID-19 vaccine side effects								
Absent	280	94.3	17	5.7	297	100.0		
Present	162	79.4	42	20.6	204	100.0	0.001[3]	**<0.001**
Recommending COVID-19 vaccination to family and friends								
Yes	426	91.2	41	8.8	467	100.0		
Not sure	16	47.1	18	52.9	34	100.0	0.001[3]	**<0.001**
Recommending COVID-19 to patients								
Yes	429	90.1	47	9.9	476	100.0		
Not sure	13	52.0	12	48.0	25	100.0	0.001[3]	**<0.001**

[1]Pearson chi-square test, [2]Yates chi-square test, [3]Fisher's chi-square test, [4]Fisher-Freeman-Halton test.

Table 5. Logistic regression analysis of factors affecting covid-19 vaccination status

Variable	Odds ratio (95% CI)	*p*
Gender		
Female	1.000	
Male	1.941 (1.090-3.456)	**0.024**
Marital status		
Married	1.000	
Single	2.216 (1.281-3.835)	**0.004**
History of adverse reactions to previous vaccines		
Present	1.000	
Absent	2.830 (1.069-7.492)	**0.036**
Flu vaccination status		
Unvaccinated	1.000	
Vaccinated	4.779 (1.694-13.482)	**0.003**
Fear of transmitting COVID-19 infection to family		
Absent	1.000	
Present	4.115 (1.589-10.661)	**0.004**
History of COVID-19 infection		
Present	1.000	
Absent	2.295 (1.324-3.977)	**0.003**
Recommending COVID-19 vaccination to family and friends		
Not sure	1.000	
Yes	11.689 (5.545-24.642)	**<0.001**
Recommending COVID-19 vaccination to patients		
Not sure	1.000	
Yes	8.426 (3.636-19.525)	**<0.001**

CI: confidence interval

DISCUSSION

In this study, the majority of participants were found to have received the COVID-19 vaccine. Korkmaz et al., in their study conducted with 768 healthcare workers in Turkey in February 2021, reported a vaccination rate of 80.6% among healthcare workers (13). Similarly, Yılmaz et al. determined that the COVID-19 vaccination rate was 85.0% among 4,201 healthcare workers in Istanbul in April 2021 (14). In another study undertaken with 793 healthcare workers in Nigeria in May 2021, Abubakar et al. reported a vaccination rate of 90% (15). In their April 2021 study from China, Xu et al. found that 86.2% of 1,051 healthcare workers had been vaccinated (16). Schrading et al., who conducted a study with 1,398 healthcare workers in the USA in January 2021, showed that 86.0% of healthcare workers were vaccinated against COVID-19 (17). George et al., evaluating 7,763 healthcare workers in South Africa in 2022, similarly reported that 89% were vaccinated (18). The high vaccination rate observed in the current study, consistent with the literature, can be attributed to healthcare workers' awareness of the importance of vaccination in combating the pandemic.

In a study conducted by Shaw et al. with 4,537 healthcare workers in the USA in March 2021, it was observed that the frequency of vaccination was significantly higher among men than among women (19). Štěpánek et al., evaluating 3,550 healthcare workers in the Czech Republic in 2021, also reported a significantly higher frequency of vaccination among men compared to women (20). In their study with 2,761 healthcare workers in Canada in December 2020, Dzieciolowska et al. found that men were significantly more likely to be vaccinated than women (21). The higher acceptance of vaccination among men in the current study, consistent with the literature, may be due to women's higher levels of concern regarding vaccine safety and side effects.

Pacella-LaBarbara et al., in their study conducted with 475 healthcare workers in the USA in February 2021, found no significant relationship between marital status and vaccination frequency (22). In contrast, in a study conducted with 1,574 healthcare workers in Turkey in December 2020, Kaplan et al. observed that married healthcare workers had a higher vaccine acceptance rate than single healthcare workers (23). The higher vaccination rate among single participants in the current study can be attributed to fewer concerns about pregnancy and breastfeeding.

Kozak et al. carried out research with 3,401 healthcare workers in Germany in April 2021 and determined that the acceptance of the COVID-19 vaccine was significantly higher among healthcare workers who received the flu vaccine compared to those who did not receive the flu vaccine (24). In another study, Štěpánek et al. reported that the frequency of COVID-19 vaccination was significantly higher among healthcare workers who had received the flu vaccine at any time in the past and during the 2020-2021 flu season, compared to those without a history of flu vaccination (20). In a study conducted with 529 healthcare workers in Lebanon in February 2021, Nasr et al. observed that those who had received the influenza vaccine during the 2020–2021 season were significantly more likely to receive the COVID-19 vaccine than those who had not (25). The positive correlation between influenza vaccination and COVID-19 vaccination observed in the current study, consistent with the literature, may reflect healthcare workers' awareness of the importance of vaccination in combating infectious diseases.

In a study conducted by Holzmann-Littig et al. with 4,500 healthcare workers in Germany in February 2021, it was observed that the frequency of COVID-19 vaccination was statistically significantly lower among those who had previously experienced any post-vaccine side effects compared to those without this history (26). In the current study, participants with a history of adverse reactions to vaccines were less likely to be vaccinated against COVID-19, possibly due to concerns about experiencing similar side effects.

In a study undertaken by Pacella-LaBarbara et al., the vaccine acceptance of healthcare workers with a history of COVID-19 infection was reported to be significantly lower than that of healthcare workers who had never had this infection (22). Similarly, in an international study carried out by Qunaibi et al. with 5,708 healthcare workers in January 2021, the vaccine acceptance of healthcare workers with a history of COVID-19 infection was observed to be significantly lower than that of healthcare workers without a history of COVID-19 infection (27). In the current study, the lower vaccination rate among participants who had previously contracted COVID-19 may be explained by their belief that they had acquired immunity or the necessity of waiting a certain period after infection before vaccination. It is expected that unvaccinated participants with a history of COVID-19 infection may choose to be vaccinated at a later stage.

In a study by Nasr et al., the main reasons healthcare workers cited for receiving the COVID-19 vaccine were "protecting family members against infection", "protecting themselves from infection", and "contributing to herd immunity and ending the pandemic" (25). Similarly, Sirikalyanpaiboon et al., in their study with 705 physicians in Thailand in April 2021, reported that healthcare workers were motivated to vaccinate to "reduce the risk of severe illness" and "acquire immunity against the disease" (28). Nzaji et al. found that healthcare workers were motivated by the desire to "protect themselves from infection" and "protect their friends, family, and loved ones"

(29). In a study undertaken by Kozak et al., the reasons for healthcare workers to receive COVID-19 vaccination were related to the protection of their patients, family members, and themselves from infection, reducing transmission, and easing of restrictions (24). In the current study, the primary motivations for vaccination among participants were acquiring immunity against the disease, reducing the risk of severe illness, contributing to herd immunity, protecting family and community, potential restrictions for the unvaccinated, and reducing transmissibility. These findings highlight the global consistency of motivations for vaccination.

Nzaji et al. also observed that the primary reasons for vaccine hesitancy among healthcare workers were concerns about safety, side effects, and efficacy (29). In a study conducted by Sirikalyanpaiboon et al. reported that healthcare workers declined COVID-19 vaccination due to concerns about its short- and long-term side effects, efficacy, and safety, as well as insufficient data on vaccination during pregnancy (28). Kozak et al. observed that healthcare workers did not receive the COVID-19 vaccination due to their concerns about the short- and long-term side effects of vaccines, as well as those about the efficacy and safety of vaccines (24). In the current study, the reasons cited by unvaccinated participants for refusing the vaccine included a lack of sufficient data on the efficacy and safety of vaccines, insufficient time elapsed since recovery from COVID-19, concerns about the short- and long-term side effects of vaccines, and a lack of studies on vaccination during pregnancy and breastfeeding. The consistency of these findings with those reported in the literature underscores the need for further efforts to address concerns and knowledge gaps regarding vaccination.

In a study conducted by Kaplan et al., it was reported that vaccine acceptance was higher among individuals who recommended vaccination to others than among those who expressed hesitancy in recommending it (23). In another study, Sirikalyanpaiboon et al. observed that individuals who advocated for COVID-19 vaccination to their family, friends, and patients had a statistically significantly higher frequency of vaccination compared to those who did not recommend it or expressed hesitancy (28). In light of these findings, healthcare workers' recommendation of vaccination to others seems to be an indicator of their own vaccine acceptance.

CONCLUSION

The COVID-19 pandemic has placed a huge burden on the healthcare system due to high rates of morbidity and mortality. Healthcare professionals serve as important role models for society. COVID-19 vaccine acceptance by healthcare workers plays an important role in the fight against the pandemic by ensuring the uninterrupted operation of healthcare services and boosting public trust in vaccination (11,12). Vaccine acceptance is affected by various factors, including gender, marital status, history of flu vaccination, history of adverse reactions to other vaccines, history of COVID-19 infection, fear of transmitting COVID-19 to family, and willingness to recommend COVID-19 vaccination to family, friends, and patients. It would be useful to consider these factors affecting vaccine acceptance or rejection when developing vaccination campaigns.

REFERENCES

1. Phelan AL, Katz R, Gostin LOJJ. The novel coronavirus originating in Wuhan, China: challenges for global health governance. 2020;323(8):709-10.
2. Ahmed SSJJoaim, research m. The Coronavirus disease 2019 (COVID-19): a review. 2020;32(4):1-9.
3. Cascella M. Features, evaluation, and treatment of coronavirus (COVID-19). StatPearls Publishing; 2020.
4. T.C. Sağlık Bakanlığı Halk Sağlığı Genel Müdürlüğü. COVID-19 (SARS-CoV-2 Enfeksiyonu) Erişkin Hasta Tedavisi . 7 Mayıs 2021 2021 [Available from: https://covid19.saglik.gov.tr/TR-66926/eriskin-hasta-tedavisi.html.
5. Dayan SJDTD. COVID-19 ve Aşı. 2021;48:98-113.
6. World Health Organization. COVID-19 vaccination, World data 2024 [Available from: https://data.who.int/dashboards/covid19/vaccines.
7. Pavel STI, Yetiskin H, Uygut MA, Aslan AF, Aydın G, İnan Ö, et al. Development of an inactivated vaccine against SARS CoV-2. 2021;9(11):1266.
8. T.C. Sağlık Bakanlığı Türkiye İlaç ve Tıbbi Cihaz Kurumu. Duyuru (Acil Kullanım Onayı) 2021 [Available from: https://www.titck.gov.tr/haber/kamuoyunun-dikkatine-13012021185623.
9. T.C. Sağlik Bakanliği COVID-19 Aşisi Bİlgİlendİrme Platformu. COVID-19 Aşısı Ulusal Uygulama Stratejisi 2024 [Available from: https://covid19asi.saglik.gov.tr/TR-77706/covid-19-asisi-ulusal-uygulama-stratejisi.html.
10. T.C. Sağlik Bakanliği COVID-19 Aşisi Bİlgİlendİrme Platformu. Günlük Aşi Verİlerİ 2024 [Available from: https://covid19asi.saglik.gov.tr.
11. Aci OS, Kackin O, Karaaslan S, Ciydem EJIjonk. Qualitative examination of the attitudes of healthcare workers in Turkey regarding COVID-19 vaccines. 2022;33(2):136-46.
12. Aldakhlan HA, Khan AS, Alabdulbaqi DJC. Hesitancy Over the COVID-19 Vaccine Among Various Healthcare Workers: An International Narrative Review. 2024;16(1).

13. Korkmaz N, Kul GJJoCM. Has the Approach of Healthcare Workers to Influenza Vaccine Changed due to the Pandemic? What are their Perspectives on COVID-19 Vaccine? 2021;11(6):870-4.
14. Yilmaz S, Çolak FÜ, Yilmaz E, Ak R, Hökenek NM, Altıntaş MMJDm, et al. Vaccine hesitancy of health-care workers: another challenge in the fight against COVID-19 in Istanbul. 2022;16(3):1134-40.
15. Abubakar AT, Suleiman K, Ahmad SI, Yahaya SS, Bello UI, Suleiman BA, et al. Acceptance of COVID-19 vaccine and associated factors among healthcare workers in Katsina state: a cross-sectional study in Northwest Nigeria. 2023;13(12):e063281.
16. Xu B, Gao X, Zhang X, Hu Y, Yang H, Zhou Y-HJV. Real-world acceptance of COVID-19 vaccines among healthcare workers in perinatal medicine in China. 2021;9(7):704.
17. Schrading WA, Trent SA, Paxton JH, Rodriguez RM, Swanson MB, Mohr NM, et al. Vaccination rates and acceptance of SARS-CoV-2 vaccination among US emergency department health care personnel. 2021;28(4):455.
18. George G, Nota PB, Strauss M, Lansdell E, Peters R, Brysiewicz P, et al. Understanding COVID-19 vaccine hesitancy among healthcare workers in South Africa. 2023;11(2):414.
19. Shaw J, Hanley S, Stewart T, Salmon DA, Ortiz C, Trief PM, et al. Healthcare personnel (HCP) attitudes about coronavirus disease 2019 (COVID-19) vaccination after emergency use authorization. 2022;75(1):e814-e21.
20. Štěpánek L, Janošíková M, Nakládalová M, Štěpánek L, Boriková A, Vildová HJV. Motivation to COVID-19 vaccination and reasons for hesitancy in employees of a Czech tertiary care hospital: A cross-sectional survey. 2021;9(8):863.
21. Dzieciolowska S, Hamel D, Gadio S, Dionne M, Gagnon D, Robitaille L, et al. Covid-19 vaccine acceptance, hesitancy, and refusal among Canadian healthcare workers: A multicenter survey. 2021;49(9):1152-7.
22. Pacella-LaBarbara ML, Park YL, Patterson PD, Doshi A, Guyette MK, Wong AH, et al. COVID-19 vaccine uptake and intent among emergency healthcare workers: a cross-sectional survey. 2021;63(10):852-6.
23. Kaplan AK, Sahin MK, Parildar H, Adadan Guvenc IJIJoCP. The willingness to accept the COVID-19 vaccine and affecting factors among healthcare professionals: A cross-sectional study in Turkey. 2021;75(7):e14226.
24. Kozak A, Nienhaus AJIjoer, health p. COVID-19 vaccination: Status and willingness to be vaccinated among employees in health and welfare care in germany. 2021;18(13):6688.
25. Nasr L, Saleh N, Hleyhel M, El-Outa A, Noujeim ZJBOH. Acceptance of COVID-19 vaccination and its determinants among Lebanese dentists: A cross-sectional study. 2021;21:1-10.
26. Holzmann-Littig C, Braunisch MC, Kranke P, Popp M, Seeber C, Fichtner F, et al. COVID-19 vaccination acceptance and hesitancy among healthcare workers in Germany. 2021;9(7):777.
27. Qunaibi E, Basheti I, Soudy M, Sultan IJV. Hesitancy of Arab healthcare workers towards COVID-19 vaccination: a large-scale multinational study. 2021;9(5):446.
28. Sirikalyanpaiboon M, Ousirimaneechai K, Phannajit J, Pitisuttithum P, Jantarabenjakul W, Chaiteerakij R, et al. COVID-19 vaccine acceptance, hesitancy, and determinants among physicians in a university-based teaching hospital in Thailand. 2021;21:1-12.
29. Nzaji MK, Kamenga JdD, Lungayo CL, Bene ACM, Meyou SF, Kapit AM, et al. Factors associated with COVID-19 vaccine uptake and hesitancy among healthcare workers in the Democratic Republic of the Congo. 2024;4(2):e0002772.

RESEARCH ARTICLE

Irfan Kucuk[1]
Suleyman Bas[2]

[1] University of Health Sciences, Kartal Dr. Lütfi Kırdar City Hospital, Department of Gastroenterology, İstanbul, Türkiye
[2] University of Health Sciences, Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital, Department of Internal Medicine, Istanbul, Türkiye

Corresponding Author:
Irfan Kucuk
mail: drirfn@gmail.com

Received: 21.10.2024
Acceptance: 23.02.2025
DOI:10.18521/ktd.1570077

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Serum Procalcitonin Values Relate to Disease Activity in Patients with Inflammatory Bowel Diseases

ABSTRACT

Objective: In inflammatory bowel disease (IBD) patients, the correlation between disease activity and serum procalcitonin (SPCT) values remains elusive. By using a number of clinical and laboratory phenotypes of disease activity in conjunction with the degree of mucosal inflammation in patients with ulcerative colitis (UC), we sought to determine whether the blood SPCT levels of IBD patients could be useful as a biomarker.

Method: This retrospective case-control study was conducted with 132 UC patients, 83 Crohn's disease (CD) patients, and 72 healthy controls (HCs). In UC, endoscopic and clinical activity were identified using the Mayo Clinical Scoring System (MCS), and the histological activity index (HAI) was calculated using the Truelove and Richards technique. The Crohn's disease activity index (CDAI) of CD patients was calculated. The Montreal classification was preferred for determining disease localization in IBD patients.

Results: The median SPCT levels were higher in the UC and CD patients compared to the HC (0.07 vs 0.26 vs 0.03 ng/ml, respectively, $p<0.001$). The MCS of UC and the CDAI of CD patients having active disease showed higher median SPCT levels than the patients in remission ($p<0.001$, $p=0.033$, respectively). The CD patients with a fistula and/or an abscess had higher SPCT concentrations than CD patients without a fistula and/or abscess ($p<0.001$). In the UC group, SPCT levels were positively correlated to the MCS and HAI values ($p<0.001$ for both values).

Conclusions: For the disease activity of IBDs, SPCT values may be a cost-effective and practical biomarker.

Keywords: Disease Activity, Inflammatory Bowel Disease, Procalcitonin.

İnflamatuvar Bağırsak Hastalarında Serum Prokalsitonin Değerleri Hastalık Aktivitesi ile İlişkilidir

ÖZET

Amaç: İnflamatuar barsak hastalıkları (İBH) olan vakalarda hastalık aktivitesi ile serum prokalsitonin (SPCT) değerleri arasındaki korelasyonlar ile ilgili veriler çelişkili olup tam olarak belirlenememiştir. Çalışmamızda ülseratif kolitli (ÜK) hastalarda mukozal inflamasyonun derecesine ilave olarak, İBH hastalık aktivitesinin değişik klinik ve laboratuvar fenotipleri ile SPCT düzeyleri değerlendirilmiş ve SPCT'nin yararlı bir biyobelirteç olup olamayacağı araştırılmıştır.

Yöntem: Bu retrospektif vaka-kontrol çalışmasına 132 ÜK hastası, 83 Crohn hastalığı (CH) vakası ve 72 sağlıklı kontrol (SK) dahil edilmiştir. ÜK klinik aktivitesi için Mayo klinik skorlama sistemi (MKS), histolojik aktivite indeksi (HAİ) için Truelove ve Richards yöntemleri kullanıldı. CH'da klinik aktivite için Crohn hastalığı aktivite indeksi (CHAİ) kullanıldı. İBH'nın endoskopik lokalizasyonu Montreal sınıflandırmasına göre yapılmıştır.

Bulgular: Median SPCT düzeyleri ÜK ve CH hastalarında SK''e göre daha yüksekti (0.07 vs 0.26 vs 0.03 ng/ml, sırasıyla, $p<0.001$). ÜK'de MKS ve CH'da CHAİ'e göre aktif hastalığı olanlarda remisyonda olanlara göre median SPCT değerleri daha yüksekti ($p<0.001$, $p=0.033$, sırasıyla). Fistülü ve/veya apsesi olan CH vakalarında olmayanlara göre daha yüksek median SPCT seviyeleri tespit edildi ($p<0,001$). ÜK hastalarında SPCT düzeyleri ile MKS ve HAİ değerleri arasında pozitif korelasyon saptandı ($p<0.001$ her ikisi için).

Sonuç: Hastalık aktivitesinin belirlenmesinde İBH'da SPCT değerleri uygun maliyetli ve pratik bir biyobelirteç olarak kullanılabilir.

Anahtar Kelimeler: Hastalık Aktivitesi, İnflamatuar Bağırsak Hastalığı, Prokalsitonin

INTRODUCTION

Inflammatory bowel disorders (IBDs) have been becoming more widespread worldwide. Two main subtypes of IBDs, ulcerative colitis (UC) and Crohn's disease (CD) have relapsing and remitting courses. Assessment of the disease activity plays a pivotal role, especially for the choice of treatment modality in the follow-up sessions of IBD patients (1-3). Until now, several clinical and endoscopic modalities were developed for the assessment of the disease severity and biomarkers that correlate to the clinical, endoscopic, and laboratory features of IBDs are gaining interest (4-8). The resolution of mural inflammation is the ideal therapeutic goal in IBDs and a useful biomarker should display the inflammatory activity in the gut wall (9,10).

The thyroid gland's parafollicular C-cells secrete procalcitonin (PCT), a 116 amino acid precursor of calcitonin that plays a role in calcium control. Normally, serum procalcitonin (SPCT) is detected in low ranges or is undetectable (11). Gram-negative bacteremia has a strong potential to stimulate the secretion of pro-inflammatory cytokines and tumor necrosis factor-alpha (TNF-α), which further induces calcitonin-related polypeptide gene 1 expression. Thus, extra-thyroidal synthesis of PCT occurs in severe bacterial infections, including sepsis, in which supranormal circulating SPCT can be encountered (12,13).

In previous reports, as an easily obtained and economic biomarker, SPCT has gained interest for clinical use in IBDs (14-18). However, studies about SPCT have revealed conflicting results (12,19-22). As a practical marker, the diagnostic and prognostic utility of SPCT in IBDs needs to be further elucidated. In this regard, we aimed to evaluate whether SPCT can be linked to the various clinical and laboratory traits of IBDs along with the severity of mucosal inflammation in patients with UC.

MATERIAL AND METHODS

Data Collection: This research was a single-center retrospective, cross-sectional study. Patients with IBDs who were admitted to the gastroenterology department at the institute and who underwent a colonoscopy procedure between October January 2012 and July 2023 were retrospectively evaluated. In this group, both newly and previously diagnosed patients were included. A second cohort of healthy subjects served as the control group. The local ethics committee approved the study (2023/203).

Subjects with clinical conditions that can alter their SPCT values, such as infections whether acute or chronic, sepsis, inflammatory and autoimmune diseases, severe organ failure, any malignancy, and any physical trauma, as well as patients who underwent a bowel resection and/or had a history of surgery within the previous six months, were excluded from the study. The patients who underwent colonoscopy for non-IBD indications and whose colonoscopy results were normal constituted the healthy control group.

The medical records of all patient and healthy control files were reviewed and the records of 215 eligible patients with IBDs and 72 healthy controls were evaluated. SPCT, C-reactive protein (CRP), erythrocyte sedimentation (ESR), and total blood count values were noted from the subject files prior to undergoing colonoscopy.

Evaluation of Patients' Clinical and Endoscopic Activities: For the clinical activity of patients with UC, the scores ranged from 0 to 12 using the Mayo Clinical Score (MCS). A score of ≤2 was categorized as clinical remission, while a score of >2 referred to an activation (5). Proctitis, left-sided colitis, and widespread or pancolitis were the three categories used to classify the illness severity of UC patients. While pancolitis and extensive colitis were identified as extensive diseases, proctitis and left-sided colitis were identified to be limited disorders. The endoscopic activity of ulcerative colitis was classified using the Mayo Endoscopic Sub-scoring (MES) index; values ranging from 0 to 3 denote remission and mild, moderate, and severe colitis, respectively (5). A score of 0 and 1 was recorded as an endoscopic inactive disease, while a score of 2 and 3 was recorded as an endoscopic active disease.

For the clinical activity of CD patients, the Crohn's disease activity index (CDAI) was identified, and a score below 150 was defined as clinical remission while a score of ≥150 referred to activation (6). Ileal, colonic, and ileocolonic diseases were identified as locations of CD.

An Analysis of Ulcerative Colitis using Histopathology: A skilled pathologist who was blinded examined the formalin-fixed, paraffin-embedded, and H&E-stained colonic biopsies and graded them using a scale created by Truelove and Richards (23). The scale consisted of three components: active inflammation (0-3), chronic inflammation (0-2), and crypt distortion (0-3). The histologic activity index (HAI) was calculated by summing the scores of these components. The histological remission was defined as scores less than 5, whereas activation was defined as values more than 5 (16).

Statistical Analysis: The statistical analysis was performed in SPSS 23.0 (SPSS Inc., Chicago, IL). The normality tests were conducted using the Kolmogorov Smirnov tests and when both tests resulted in p values of > 0.05, the distribution was assumed to be normal. Median (interquartile range) was used for the data with non-normal distribution and frequencies with percentages were presented for the categorical data. The Mann-Whitney U and Kruskal-Wallis tests were used to compare non-parametric continuous variables. Additionally, equity with categorical variables was tested using

the chi-square test. Diagnostic accuracy was studied by the receiver operating characteristics (ROC) curved analysis. A *p*-value of <0.05 was accepted as statistically significant.

RESULTS

Totally, age and sex matched for the 132 UC patients (86 males and 46 females), for the 83 CD patients (54 males and 29 females), and for the 72 healthy controls (HCs) (47 males and 25 females) that were included in the study. Table 1 shows the demographic, clinical, and laboratory characteristics of the subjects. The disease duration was similar between the patient groups. The ESR, CRP, and platelet values of IBD patients were higher compared to those of the HCs (*p<0.001*). In comparison to the HCs, the leucocyte and neutrophil counts were higher in the CD patients (*p=0.003* and *p<0.001*, respectively).

Table 1. The sample's demographic, clinical, and laboratory characteristics.

	UC Patients (n=132)	**CH Patients (n=83)**	**Control Group (n=72)**	***p***
Gender, n (%)				
Female	46 (34.8)	29 (34.9)	25 (34.7)	0.989 [a]
Male	86 (65.2)	54 (65.1)	47 (65.3)	
Age (years), median (IQR)	35 (25-50)	36 (25-46)	38 (29.25-48.75)	0.706 [b]
Disease duration (years), median (IQR)	2.25 (0.50-6)	3 (0.50-5)		0.785 [c]
CRP (mg/L), median (IQR)	16.90 (3.64-76.65)	15.91 (4.58-42.65)	2.36 (0.94-4.09)	**<0.001** [b,1,*]
ESR (mm/h), median (IQR)	36.50 (14-66.75)	30 (14-50)	9.50 (3-17)	**<0.001** [b,1,*]
Leukocytes (x10^3/μL), median (IQR)	7.71 (6.46-9.53)	8.59 (6.93-10.80)	7.27 (6.07-8.85)	**0.012** [b,2,*]
Neutrophils (x10^3/μL), median (IQR)	5.04 (3.79-6.45)	5.99 (4.57-8.36)	4.58 (3.68-5.63)	**<0.001** [b,3,*]
Platelets (x10^3/μL), median (IQR)	319.50 (262.50-403)	313 (274-409)	244.50 (211-270.75)	**<0.001** [b,1, *]
Procalcitonin (ng/ml)	0.07 (0.03-1.05)	0.26 (0.04-1.04)	0.03 (0.03-0.05)	**<0.001** [b,1, *]
UC Localization, n (%)				
Limited disease	82 (62.1)			
Extensive disease	50 (37.9)			
CH Localization, n (%)				
Ileal		49 (59)		
Colonic		12 (14.5)		
Ileocolonic		22 (26.5)		
Mayo Endoscopic Score of UC, n (%)				
İnactive disease	2 (1.5)			
Mild disease	51 (38.6)			
Moderate disease	47 (35.6)			
Severe disease	32 (24.2)			
IBDs in first degree relatives, n (%)	18 (13.6)	15 (18.1)		0.380 [a]
Mayo Clinical Score of UC, median (IQR)	6 (3-9)			
Remission (score ≤ 2), n (%)	28 (21.2)			
Activation (score>2), n (%)	104 (78.8)			
Crohn's Disease Activity Index, median (IQR)		182 (108-272)		
Remission (score < 150), n (%)		35 (42.2)		
Activation (score ≥150), n (%)		48 (57.8)		
Histological Activity Index in UC, median (IQR)	6 (5-7)			
Fistula and/or abscess		18 (21.7)		
Extra-intestinal manifestations n (%)	13 (9.8)	26 (31.3)		**<0.001** [a,*]

Abbreviations: CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; IQR: Interquartile range; UC: Ulcerative colitis; CD: Crohn's disease; IBDs: Inflammatory bowel diseases.

Footnotes: 1: Significant difference in the comparison of UC vs controls, CD vs controls (*p*<0.001, *p*<0.001); **2:** Significant difference in the comparison of CD vs HCs (*p*=0.003); **3:** Significant difference in the comparison of CD vs HCs (*p*<0.001). a: Chi-Square Test, b: Kruskal-Wallis Test, c: Mann-Whitney U Test. *: Statistically significant (*p*<0.05).

Most of the UC patients had limited disease detected in the colonoscopy procedure, whereas terminal ileum was the most common location in the CD patients. With respect to the clinical activity scores, most of the patients with IBDs had an active disease. The ratio of the patients who were under treatment and the patients with IBDs in first degree relatives were similar between the UC and CD groups. Extra-intestinal manifestations were more prevalent in the CD patients than in the UC patients (*p<0.001*) (Table 1).

The median SPCT levels of the UC and CD patients were higher than those of the HCs (0.07 [0.03-1.05] vs. 0.26 [0.04-1.04] vs 0.03 [0.03-0.05] ng/ml, respectively, *p<0.001*). CD patients had the highest median SPCT values (Table 1). Regarding the treatment status and presence of IBDs in first-degree relatives, the median SPCT values were not statistically significantly different in both the UC and CD groups (Table 2). According to the MCS values of the UC patients and the CDAI values of the CD patients, the patients with active disease had higher median SPCT levels than the patients in remission (*p<0.001* and *p=0.033*, respectively). In the CD group, the patients with a fistula and/or abscess had higher SPCT levels than the patients without a fistula and/or abscess (*p<0.001*) (Table 2).

Table 2. Serum procalcitonin values according to the disease phenotypes in the patients with IBDs.

				Procalcitonin		
Ulcerative Colitis		**n**	**%**	**Median**	**IQR**	***p***
Mayo clinical scoring	Remission (score ≤ 2)	28	21.2	0.03	0.02-0.04	**<0.001** [a,*]
	Activation (score >2)	104	78.8	0.89	0.04-1.07	
Localization of UC	Limited disease	82	62.1	0.04	0.02-0.06	**<0.001** [a,*]
	Extensive disease	50	37.9	1.07	1.03-1.76	
IBDs in first degree relatives	Positive	18	13.6	0.50	0.03-2.05	0.383 [a]
	Negative	114	86.4	0.07	0.03-1.04	
Extra-intestinal manifestations	Positive	13	9.8	0.04	0.03-1.05	0.789 [a]
	Negative	119	91.2	0.07	0.03-1.05	
Crohn's disease						
Treatment status	No Treatment	34	41	0.22	0.05-1.03	0.550 [a]
	Under Treatment	49	59	0.82	0.03-1.07	
CDAI Score	Remission (score< 150)	35	42.2	0.06	0.03-1.02	**0.033** [a,*]
	Activation (score ≥150)	48	57.8	0.84	0.09-1.06	
Localization of CH	Ileal	49	59	0.08	0.03-1.02	**0.010** [b,1,2,*]
	Colonic	12	14.5	1.05	0.38-1.82	
	Ileocolonic	22	26.5	0.94	0.18-1.04	
IBDs in first degree relatives	Positive	15	18.1	0.93	0.14-1.07	0.187 [a]
	Negative	68	81.9	0.25	0.03-1.04	
Extra-intestinal manifestations	Positive	26	31.3	1.02	0.45-1.42	**0.001** [a,*]
	Negative	57	68.7	0.14	0.03-1.02	
Fistula and/or abscess	Positive	18	21.7	1.07	0.91-2.02	**<0.001** [a,*]
	Negative	65	78.3	0.14	0.03-1.02	

Abbreviations: CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; IQR: Interquartile range; UC: Ulcerative colitis; CD: Crohn's disease; CDAİ: Crohn's Disease Activity Index

Footnotes: 1: Significant difference between ileal localization and colonic localization in patients with CD (p=0.014); **2:** Significant difference between ileal localization and ileocolonic localization in patients with CD (p=0.026).

a: Mann-Whitney U Test, b: Kruskal-Wallis Test. *: Statistically significant (*p*<0.05).

No statistically significant correlation was determined between the disease duration and the SPCT levels in patients with IBDs. In the UC group, the SPCT levels were positively correlated to the MCS, HAI, CRP, ESR, leukocytes, neutrophil, and platelet values (*p<0.001* for all). In the patients with CD, there were positive correlations between the SPCT values and the CRP, ESR, and platelet counts (*p<0.001, p=0.004, p=0.039*, respectively) (Table 3).

Table 3. Correlation analysis of the serum procalcitonin values with clinical and laboratory parameters in the patients with IBDs

	Procalcitonin	
Ulcerative Colitis	rho	*p*
Disease duration	-0.058	0.506
Mayo Clinical Score	0.624	**<0.001 ***
Histological Activity Index	0.757	**<0.001 ***
C-Reactive Protein	0.861	**<0.001 ***
Erythrocyte sedimentation rate	0.524	**<0.001 ***
Leukocytes	0.313	**<0.001 ***
Neutrophils	0.323	**<0.001 ***
Platelets	0.442	**<0.001 ***
Crohn's disease	r	*p*
Disease duration	0.141	0.204
Crohn's Disease Activity Index	0.194	0.078
C-Reactive Protein	0.676	**<0.001 ***
Erythrocyte sedimentation rate	0.313	**0.004 ***
Leukocytes	0.016	0.883
Neutrophils	0.009	0.935
Platelets	0.227	**0.039 ***

Footnotes: 1: Significant difference in the comparison of UC vs HCs, CD vs HCs (p<0.001, p<0.001); **2:** Significant difference in the comparison of CD vs HCs (p=0.003); **3:** Significant difference in the comparison of CD vs HCs (p<0.001).
rho: Sperman Correlation Coefficient. *: Statistically significant (p<0.05)

The area under the curve (AUC) for the SPCT concentrations showed a diagnostic accuracy of 0.796 (95% CI: 0.709-0.882, p<0.001) for the clinical activity of UC (MCS), according to the receiver operating characteristic curve (ROC) analysis. For the cut-off value of ≥0.05 ng/ml, sensitivity was 67.3% and specificity was 89.3% (Figure 1).

The AUC for the SPCT concentrations had a diagnostic accuracy of 0.637 (95% CI: 0.511-0.764, p=0.033) for the clinical activity of CH (CDAI), according to the ROC analysis. For the cut-off value of≥0.18 ng/ml, the sensitivity and specificity were 70.8% and 62.9%, respectively (Figure 2).

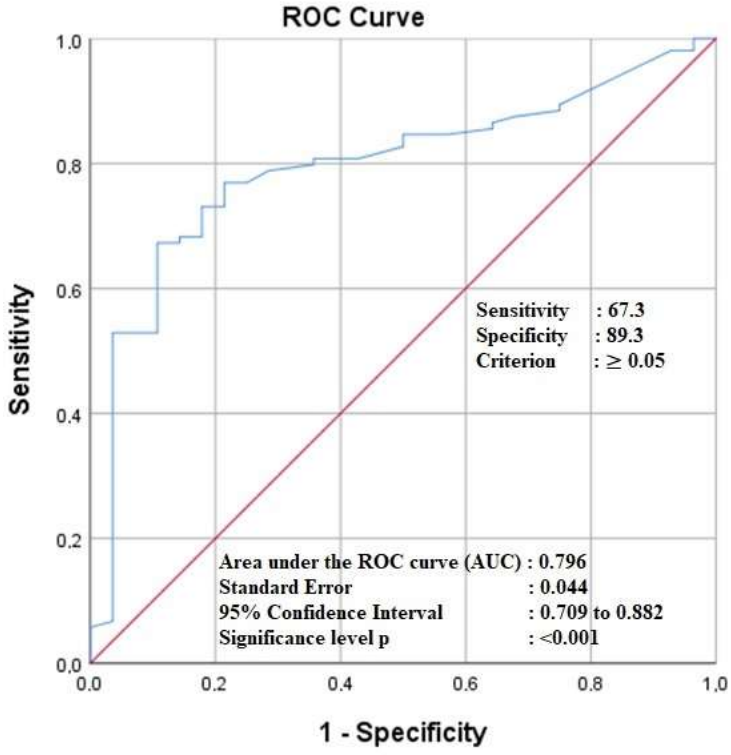

Figure 1. The predicted serum procalcitonin values for the Mayo Clinical Scoring in ulcerative colitis were analyzed using ROC curves.

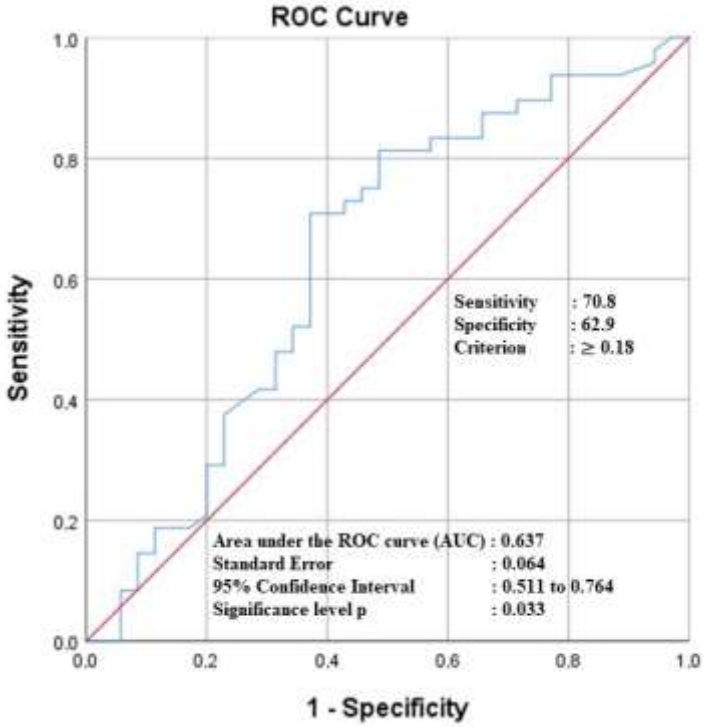

Figure 2. ROC curve analysis of the serum procalcitonin predicted concentrations for Crohn's disease CDAI scoring.

DISCUSSION

In daily clinical practice, CRP, as a traditional marker of inflammation, is widely used to assess the disease activity of IBDs patients. However, CRP has some drawbacks for the monitoring of disease activity (24). Serum CRP values depend on age, gender, weight, and smoking status, and sometimes, despite the overt inflammation, CRP may be normal or slightly elevated in 15-20% patients because of genetic polymorphisms and inter-individual variability (24). In addition, CRP may not exhibit mucosal healing (MH) in IBDs patients with lower inflammatory activity (25). SPCT levels have a tendency of early elevation and they can also rapidly normalize compared to serum CRP concentrations in bacterial infections (11). Thus, as a marker of inflammation, PCT may be more specific than CRP in infectious conditions (12,16).

Monitoring of fecal calprotectin (FCP) is a good choice in the follow up sessions of IBDs (8). However, the threshold values of FCP have not been accurately determined and it needs to be validated (8). In addition, FCP is an expensive test and obtaining the samples may cause discomfort for FCP. As a limitation, the FCP values of the IBD patients could not be noted for the comparison of FCP and SPCT levels.

Herrlinger et al. first evaluated the SPCT values in adult IBDs patients (26). In that study, regarding the discriminative efficacy of SPCT between self-limited infectious enterocolitis and IBDs, a PCT level greater than 0.4 ng/ml was noted as diagnostic for self-limited infectious enterocolitis. The SPCT concentrations were within the normal ranges in all the patients with IBDs. However, according to Truelove and Witt's severity index (TWI) and the CDAI in the patients with UC and CD respectively, approximately 40% of the patients presenting with a clinically active disease tended to have higher SPCT values than the patients with an inactive disease (26). Additionally, Oruç et al. found that patients with CD had greater SPCT values than HCs, but there was no significant difference between the SPCT values of the UC patients and the SPCT values of the HCs (14).

In a previous report, the SPCT values were higher among the UC patients (n=18) compared to the HCs (n=11), and according to the TWI and Mayo endoscopic sub-scoring, severe UC patients had higher SPCT values compared to mild and moderate patients and the HCs, but no significant difference existed between the SPCT values of mild and moderate UC patients and the SPCT values of the HCs (15). Nishio et al. noted that SPCT values correlated to the CDAI values in patients with CD, whilst no correlation existed between the MCS and SPCT values in the UC patients (20). In another report, no correlation was found between the disease location, MCS values, partial MCS values, pelvic involvement, and the SPCT concentrations (21). In the study of Chung et al., there was no correlation between the clinical activity parameters and the SPCT levels (22).

In light of the current literature, SPCT values have a tendency to increase in IBDs patients with an active disease with respect to clinical and laboratory parameters. Although the

methodological differences in the clinical trials may partly be responsible for the variable results, the diagnostic and prognostic value is still controversial. However, most probably, SPCT can be a valuable marker for the infectious complications in IBDs (12,17).

SPCT values lower than 0.05 ng/ml are normal, whereas patients with limited but not systemic infection could present with SPCT values of 0.05-0.5 ng/ml (11). In our results, the median SPCT values of the UC and CD patients were within the ranges of limited bacterial infection that can be ascribed to localized intestine bacterial inflammation, which also have a pathogenic role in IBDs (1,11,12,28). In our data, the median SPCT values were the highest in the CD patients, but there was no significant difference between the SPCT values of the UC and CD patients. In the CD patients presenting with fever episodes, elevated SPCT values were also declared to be a marker that can discriminate an intra-abdominal abscess from disease flares (17). The highest SPCT concentrations in the CD patients might be due to the formation of an intra-abdominal abscess or fistula, which is directly related to bacterial infections, and 21.7 % of the CD patients in our cohort had a fistula and/or an abscess.

With respect to SPCT concentrations, we firstly evaluated extra-intestinal manifestations in the patients with IBDs. Extra-intestinal manifestations can relate to the severity of inflammation in the intestine and the treatment of IBDs can alleviate these symptoms (1,27). In our study, the ratio of patients with extra-intestinal manifestations was higher among the CD patients compared to the UC group and it can be another explanation for the elevated SPCT values in the CD group. Our results revealed higher median SPCT levels according to the respective MCS and CDAI scoring in patients with UC and CD. Despite the different scoring systems, which were settled in different clinical trials, the SPCT values displayed the clinically active disease (14-16).

Inflammation in the gut wall relates to disease recurrence and poorer clinical outcomes, including malignity in IBDs (10). MH is defined as both endoscopic and histopathological remission (25). Current treatment modalities aim for complete MH; however, they cannot achieve complete MH (9). We found positive correlations between the SPCT values and the histological activity scores in the UC patients along with the other biochemical tests for inflammation. SPCT might a good marker for MH and disease activity in UC. A limitation is that we were unable to ascertain the CD patients' histology activity. The CDAI scores were also positively correlated to the SPCT values in CD patients. In both diseases, the patients who had a clinically active disease had higher SPCT concentrations compared to the patients in the remission phase.

According to our results, patients with extensive location of UC and patients with colonic CD had higher median SPCT values. The disruption of mucosal integrity and intestinal permeability lead to high numbers of bacteria that cause uncontrolled inflammation within mucus layer (28). Colonic involvement represents the increased bacterial content leading to induced inflammatory activity that results in elevated SPCT values. The role of intestinal microbiota in the pathogenesis of CD and UC is a well-known entity and both diseases usually present in parts of the intestine with a high bacterial content (1). In addition, the use of antibiotics has a modest benefit over placebos for the induction of remission in patients with CD (28).

Although conflicting results exist in the literature, our results were consistent with some previous reports and highlight the clinical utility of SPCT in IBDs (12,26). We also reported a diagnostic accuracy for the clinical activity of IBD patients. Different results of the SPCT values for the disease phenotypes in IBDs may also be due to variations in demographic, clinical, and laboratory characteristics of populations among the studies.

The most important limitation of the current study is the retrospective examination of a single center. We were not able to evaluate the clinical and laboratory conditions that mimic IBDs. As noted earlier, determination of the FCP values could be more valuable in the study population. The prospective evaluation of the disease traits of patients with IBDs can provide more information for the diagnostic and prognostic utility of SPCT in these patients. Moreover, the evaluation of the histopathological severity of inflammation in CD patients may be more informative.

CONCLUSION

Easily obtained and cost-effective tests have been attractive for clinicians of inflammatory diseases. Monitoring of SPCT values in the follow up sessions of IBD patients might be a practical and easy method for the determination of disease activity in these patients. SPCT concentrations might be a valuable biomarker for the severity of mucosal inflammation in UC patients.

REFERENCES

1. Osterman MT, Lichtenstein GR. Ulcerative Colitis. In: Feldman M, Friedman SL. Brandt JL editors (Eds). Sleisenger and Fordtran's Gastrointestinal and Liver Disease. Philadelphia (USA): Elselvier Saunders; 2016. p. 2023-61.
2. Kıraç Y, Yalçın B, Ustaoğlu M. The Relationship between Smoking Status, Carbon Monoxide Levels and Quality of Life, Disease Characteristics in Inflammatory Bowel Diseases. Konuralp Medical Journal. 2023;15(1):69-77.

3. Con D, Andrew B, Nicolaides S, van Langenberg DR, Vasudevan A. Biomarker dynamics during infliximab salvage for acute severe ulcerative colitis: C-reactive protein (CRP)-lymphocyte ratio and CRP-albumin ratio are useful in predicting colectomy. Intest Res. 2022;20(1):101-13.
4. Truelove SC, Wıtts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. Br Med J. 1955;2(4947):1041-8.
5. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med. 1987;317(26):1625-9.
6. Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology. 1976;70(3):439-44.
7. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. BMJ. 1989;298(6666):82-6.
8. Chen F, Hu Y, Fan YH, Lv B. Clinical Value of Fecal Calprotectin in Predicting Mucosal Healing in Patients With Ulcerative Colitis. Front Med (Lausanne). 2021;8:679264.
9. Ardizzone S, Cassinotti A, Duca P, Mazzali C, Penati C, Manes G, et al. Mucosal healing predicts late outcomes after the first course of corticosteroids for newly diagnosed ulcerative colitis. Clin Gastroenterol Hepatol. 2011;9(6):483-9.
10. Bryant RV, Winer S, Travis SP, Riddell RH. Systematic review: histological remission in inflammatory bowel disease. Is 'complete' remission the new treatment paradigm? An IOIBD initiative. J Crohns Colitis. 2014;8(12):1582-97.
11. Samsudin I, Vasikaran SD. Clinical Utility and Measurement of Procalcitonin. Clin Biochem Rev. 2017;38(2):59-68.
12. Lippi G, Sanchis-Gomar F. Procalcitonin in inflammatory bowel disease: Drawbacks and opportunities. World J Gastroenterol. 2017;23(47):8283-90.
13. Demir İ, Yılmaz İ. The Effect of Polypharmacy on Procalcitonin Levels in The Intensive Care Admission of Geriatric Patients with Sepsis. Konuralp Medical Journal. 2020;12(2):216-22.
14. Oruç N, Ozütemiz O, Osmanoğlu N, Ilter T. Diagnostic value of serum procalcitonin in determining the activity of inflammatory bowel disease. Turk J Gastroenterol. 2009;20(1):9-12.
15. Koido S, Ohkusa T, Takakura K, Odahara S, Tsukinaga S, Yukawa T, et al. Clinical significance of serum procalcitonin in patients with ulcerative colitis. World J Gastroenterol. 2013;19(45):8335-41.
16. Oussalah A, Laurent V, Bruot O, Guéant JL, Régent D, Bigard MA, et al. Additional benefit of procalcitonin to C-reactive protein to assess disease activity and severity in Crohn's disease. Aliment Pharmacol Ther. 2010;32(9):1135-44.
17. Ge X, Hu D, Cao Y, Liu Z, Ding C, Tian H, et al. Procalcitonin in Crohn's disease with fever episodes, a variable to differentiate intra-abdominal abscess from disease flares. Int J Surg. 2016;36(Pt A):34-9.
18. Wu HM, Wei J, Li J, Wang K, Ye L, Qi Y, et al. Serum Procalcitonin as a Potential Early Predictor of Short-Term Outcomes in Acute Severe Ulcerative Colitis. Dig Dis Sci. 2019;64(11):3263-73.
19. Chen JM, Liu T, Gao S, Tong XD, Deng FH, Nie B. Efficacy of noninvasive evaluations in monitoring inflammatory bowel disease activity: A prospective study in China. World J Gastroenterol. 2017;23(46):8235-47.
20. Nishio E, Saruta M, Arihiro S, Matsuoka M, Mitsunaga M, Ide D, et al. The clinical benefit of procalcitonin to assess disease activity and severity in inflammatory bowel disease. Gastroenterology. 2016;150(4, Supplement 1):995.
21. Hosomi S, Yamagami H, Itani S, Yukawa T, Otani K, Nagami Y, et al. Sepsis Markers Soluble IL-2 Receptor and Soluble CD14 Subtype as Potential Biomarkers for Complete Mucosal Healing in Patients With Inflammatory Bowel Disease. J Crohns Colitis. 2018;12(1):87-95.
22. Chung SH, Lee HW, Kim SW, Park SJ, Hong SP, Kim TI, et al. Usefulness of Measuring Serum Procalcitonin Levels in Patients with Inflammatory Bowel Disease. Gut Liver. 2016;10(4):574-80.
23. Truelove SC, Richards WCD. Biopsy studies in ulcerative colitis. Br Med J. 1956;1(4979):1315-8.
24. Brull DJ, Serrano N, Zito F, Jones L, Montgomery HE, Rumley A, et al. Human CRP gene polymorphism influences CRP levels: implications for the prediction and pathogenesis of coronary heart disease. Arterioscler Thromb Vasc Biol. 2003;23(11):2063-9.
25. Sakurai T, Saruta M. Positioning and Usefulness of Biomarkers in Inflammatory Bowel Disease. Digestion. 2023;104(1):30-41.
26. Herrlinger KR, Dittmann R, Weitz G, Wehkamp J, Ludwig D, Schwab M, et al. Serum procalcitonin differentiates inflammatory bowel disease and self-limited colitis. Inflamm Bowel Dis. 2004;10(3):229-33.
27. De Vos M. Joint involvement associated with inflammatory bowel disease. Dig Dis. 2009;27(4):511-5.
28. Khan KJ, Ullman TA, Ford AC, Abreu MT, Abadir A, Marshall JK, et al. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol. 2011;106(4):661-73.

RESEARCH ARTICLE

Samet Dinc[1]
Adem Kurtulus[2]
Necmettin Coban[3]
Cengiz Tuncer[4]
Ayhan Tekiner[5]

[1] Neurosurgery Clinic, Ankara Etlik City Hospital, Ankara, Türkiye
[2] Department of Neurosurgery, Eskisehir City Hospital, Eskisehir, Türkiye
[3] Neurosurgery Clinic, Afyonkarahisar State Hospital, Afyonkarahisar, Türkiye
[4] Department of Neurosurgery, Düzce Üniversity, Düzce, Türkiye
[5] Department of Neurosurgery, Ankara Training and Research Hospital, Ankara, Türkiye

Corresponding Author:
Samet Dinc
mail:dincsamet89@hotmail.com

Received: 26.05.2024
Acceptance: 06.01.2025
DOI:10.18521/ktd.1490160

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Retrospective Examination of the PECARN Algorithm in Pediatric Patients with Minor Head Trauma

ABSTRACT

Objective: We aimed to retrospectively evaluate and examine pediatric head trauma cases according to PECARN in the light of their neurological and clinical conditions. We wanted to contribute to the detection of TBI with minimum CT imaging rates in order to avoid radiation-related carcinogenesis.

Method: 108 pediatric patients who were admitted to the emergency department due to minor head trauma and were admitted to the neurosurgery clinic were evaluated retrospectively.

Results: During follow-up, 9 patients required intensive care and 5 patients underwent neurosurgical surgery. The most common trauma etiology was falling from one's own level with 53.7%. The most common tomography pathology was non-displaced fracture. According to the evaluations made in accordance with the PECARN algorithm, CT was recommended in 18 cases (16.7%); CT was not recommended for 32 cases.

Conclusions: The clinician's goal is to quickly and accurately diagnose clinically significant TBI while avoiding unnecessary CT imaging to protect against the adverse effects of radiation. We recommend using the PECARN algorithm for this purpose.

Keywords: Head Trauma, Carcinogenesis, Tomography.

Minör Kafa Travmalı Pediatrik Hastalarda PECARN Algoritmasının Retrospektif Olarak İncelenmesi

ÖZET

Amaç: Pediatrik kafa travması olgularını nörolojik ve klinik durumları ışığında PECARN'a göre retrospektif olarak değerlendirmeyi ve incelemeyi amaçladık. Radyasyona bağlı karsinogenezi önlemek için minimum BT görüntüleme oranlarıyla TBI tespitine katkıda bulunmak istedik.

Yöntem: Minör kafa travması nedeniyle acil servise başvuran ve beyin cerrahi kliniğine başvuran 108 çocuk hasta retrospektif olarak değerlendirildi.

Bulgular: Takip sırasında 9 hastanın yoğun bakıma ihtiyacı oldu ve 5 hastaya beyin cerrahisi ameliyatı uygulandı. En sık görülen travma etiyolojisi %53,7 ile kendi seviyesinden düşmeydi. En sık görülen tomografi patolojisi deplase olmayan kırıktı. PECARN algoritmasına göre yapılan değerlendirmelere göre 18 olguya (%16,7) BT önerildi; 32 olguya BT önerilmedi.

Sonuç: Klinisyenin amacı, radyasyonun olumsuz etkilerinden korunmak için gereksiz BT görüntülemeden kaçınarak, klinik açıdan anlamlı TBI'yı hızlı ve doğru bir şekilde teşhis etmektir. Bu amaçla PECARN algoritmasını kullanmanızı öneririz.

Anahtar Kelimeler: Kafa Travması, Karsinogenez, Tomografi

INTRODUCTION

Traumatic brain injury (TBI) is the leading cause of trauma-related morbidity and mortality in the paediatric age group (1). Falls are the most common cause of head trauma in all age groups. Birth trauma is the main etiology of almost all neonatal head traumas. The mechanism of trauma in the first 2 years is non-accidental injuries. In young children and adolescents, the most common mechanisms of TBI are falls and motor vehicle crashes (2).

Mild TBI accounts for 70-90% of head traumas in children. Patients with a Glasgow Coma Scale (GCS) score of 13-15 after blunt head trauma are defined as mild TBI (3,4). Mild TBI may also be used interchangeably with the terms minor head injury or concussion (4,5).

Although the prevalence of minor head trauma is very high, it has been documented that 3-5% have TBI and less than 1% require emergency neurosurgical procedures (6,7).

In paediatric patients presenting with head trauma, radiological assessment is performed according to the severity of the trauma as well as clinical evaluation. Since the 1970s, computed tomography (CT) has been a tool that enables rapid and accurate decision making in the evaluation of closed head traumas, detection of intracranial pathologies and determination of treatment options (8,9). There is consensus on the indication for brain CT in moderate and severe head trauma in many paediatric trauma guidelines. However, brain CT indications in mild head traumas are not clearly demarcated (10,11). Many studies have emphasised the need to establish algorithms to reduce unnecessary CT imaging in paediatric minor head injury patients (11). By performing CT imaging in the presence of certain symptoms such as vomiting, headache, unconsciousness, and otherwise observing without CT imaging, it is suggested that unnecessary CT imaging and potential harm of radiation in paediatric patients with minor head trauma can be prevented (9,12). The life expectancy after radiation exposure is longer in the paediatric age group and they are more sensitive to radiation than adults. For these reasons, the primary goal in paediatric minor head trauma is to predict TBI with minimum CT scan rates to avoid radiation-related carcinogenesis (13,14).

Algorithms for minor head injuries in children have been designed after three major clinical trials. These are CHALICE (Children's Head Injury Algorithm for the Prediction of Important Clinical Events), CATCH (Canadian Assessment of Tomography for Childhood Head Injury) and PECARN (Paediatric Emergency Care Applied Research Network) (10,15,16). PECARN is a prospective cohort study of blunt head trauma patients under 18 years of age who presented within the first 24 hours of trauma, and unlike others, patients under two years of age were analysed separately (13,15,17). Although sensitivity and negative predictive values were high in all three studies, PECARN missed fewer patients (7,18). In this study, we aimed to retrospectively evaluate pediatric head trauma cases hospitalised and treated in our centre according to the PECARN algorithm in the light of their neurological and clinical status at the time of admission.

MATERIAL AND METHODS

Study Design: In this study, 108 paediatric patients admitted to the emergency department of Afyonkarahisar State Hospital between January 2019 and June 2021 for minor head trauma and treated in the neurosurgery clinic were retrospectively assessed. Ethics committee approval was obtained from Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (date: 15.12.2023 number: 2023/12).

Patient Selection: Patients under the age of 18 who presented to the emergency department of Afyonkarahisar State Hospital in the first 24 hours of trauma due to minor head trauma and were hospitalised and treated by the neurosurgery clinic were included in the study.

Patients over 18 years of age, with a history of previous cranial surgery, known haematological disease, chronic disease/malignancy involving the central nervous system and patients with gunshot wounds/penetrating head trauma were not included in the study.

Data Collection: Patient data were obtained retrospectively from electronic health records and patient charts. Demographic (age, gender), trauma etiology, clinical (neurological status, need for operation and intensive care, etc.) and radiological data (pathology type and location) were collected.

Clinical and Radiological Evaluation: Neurological status, GCS, open wound and scalp swelling and symptoms were investigated. Symptoms included severe headache, unconsciousness and vomiting. All patients underwent detailed neurological examination and evaluation by a neurosurgeon. In addition, neurosurgical operation and intensive care needs of the patients were also mentioned.

Brain CT scans of all patients were performed using Siemens Healthineers SOMATOM Emotion CT 78560 device. The pathologies of the patients were classified as epidural haematoma, subdural haematoma, non-deplaced fracture, deplaced fracture, traumatic subarachnoid haemorrhage, contusio cerebri and pneumocephalus. Pathological locations were also specified. Cases requiring hospitalisation even though no traumatic pathology was detected on CT images were also included. The PECARN algorithm was applied retrospectively for each patient by two neurosurgeons in a consensus manner, taking into account the clinical status of the patients at the time of presentation.

Statistical Analysis: Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corpotation, Armonk, NY). Normality of distrubitions was assessed by Kolmogorov-Smirnov test. Frequency (%) analysis was used for demographic analyses. Continuous variables were expressed as mean ± standard deviation and/or median (Inter Quartile Range) and categorical data were expressed as number and percentage. Chi-square tests were used to analyse the crosstabulations created for categorical data. $p<0.05$ was considered statistically significant.

RESULTS

Baseline Demographic and Clinical Characteristics: Demographic and clinical characteristics of the patients are summarised in Table 1. The mean age in months was 47.8 ± 4.6 (mean ± SEM). The median was 24 and the range was 1-204 months. There was a male gender predominance in this cohort (71 males, 37 females).

Table 1. Baseline demographic and clinical characteristics

Variable	n (%)
Demographic	
Sex, female	40 (37)
Age (month)	
mean ± SEM	47.8 ± 4.6
median (IQR)	24 (1-204)
Trauma etiology	
Fall	100 (92.6)
from standing	58 (53.7)
from height	34 (31.5)
bicycle (unhelmeted)	3 (2.8)
swing	5 (4.6)
MVC	5 (4.6)
passenger	1 (0.9)
pedestrian	4 (3.7)
Impact with a hard object	3 (2.8)
GCS	
15	96 (88.9)
<15	12 (11.1)
Symptoms	
Headache	10 (9.3)
Vomiting	11 (10.2)
Unconsciousness	12 (11.1)
Scalp swelling	68 (63)
Wound	23 (21.3)
Additional traumatic pathology	4 (3.7)
Need for ICU	9 (8.3)
Need for surgery	5 (4.6)
Length of stay hospital (day)	
mean ± SD	3 ± 1.8
median (IQR)	2 (1-9)

SEM: *Standard Error of Mean* ***SD:*** *Standard Deviation* ***GCS:*** *Glasgow Coma Scale* ***MVC:*** *Motor Vehicle Crash* ***ICU:*** *Intensive Care Unit* ***IQR:*** *Inter Quartile Range*

GCS was <15 in 11.1% of the patients and 30.6% had symptoms. In addition, 63% had scalp swelling and 21.3% had open wounds. While 9 patients required intensive care, 5 patients underwent neurosurgical surgery (Table 1). The mean hospital stay was 3 ± 1.8 days (mean ± SD).

Trauma Etiology: Trauma etiologies of the patients are summarised in Table 1. The etiologies were subdivided into falls, motor vehicle crashes and hard object impacts. The most common pathology was fall from standing level with 53.7%. The least common trauma etiology was motor vehicle crash which was seen in 1 patient.

Radiological Characteristics: The radiological characteristics of the patients are summarised in Table 2. The most common pathology was non-deplaced fracture (80.6%). The most common pathological location was parietal region (31.5%). In 10 patients, no trauma-related intracranial radiological pathology was observed on CT imaging at the time of presentation.

PECARN Evaluation: The PECARN algorithm was utilised for each case considering the clinical and neurological status at the time of admission. In accordance with the algorithm, it was taken into consideration that the patients were under and over 2 years of age. According to the judgements made in accordance with the PECARN algorithm, CT was indicated in 18 cases (16.7%), while 32 cases (29.6%) were not indicated. Chi-square analysis of all relevant variables according to the PECARN algorithm is shown in Table 3.

Table 2. Radiological characteristics

Variables	**n (%)**
Presence of pathology	98 (90.7)
Type of pathology	
Epidural haemorrhage	10 (9.3)
Subdural haemorrhage	5 (4.6)
Traumatic subarachnoid haemorrhage	4 (3.7)
Cerebral contusion	12 (11.1)
Non-deplaced fracture	87 (80.6)
Deplaced fracture	4 (3.7)
Pneumocephalus	7 (6.5)
Location of pathology	
Frontal	30 (27.8)
Temporal	7 (6.5)
Parietal	34 (31.5)
Occipital	16 (14.8)
Frontoparietal	4 (3.7)
Frontotemporal	4 (3.7)
Frontotemporoparietal	1 (0.9)
Temporoparietal	2 (1.9)

Table 3. PECARN evaluation chi-square analysis

		PECARN			**p**
		CT required n (%)	**Observation vs. CT using shared decision-making n (%)**	**CT not indicated n (%)**	
Presence of radiological pathology	+	13 (13.3)	53 (54.1)	32 (32.7)	**0.005**
	-	5 (50)	5 (50)	0 (0)	
Non-deplaced fracture	+	8 (9.2)	50 (57.5)	29 (33.3)	**<0.001**
	-	10 (47.6)	8 (38.1)	3 (14.3)	
Deplaced fracture	+	4(100)	0 (0)	0 (0)	**0.001**
	-	14 (13.5)	58 (55.8)	32 (30.8)	
EDH	+	2 (20)	4 (40)	4 (40)	0.658
	-	16 (16.3)	54 (55.1)	28 (28.6)	
SDH	+	2 (40)	1 (20)	2 (40)	0.187
	-	16 (15.5)	57 (55.3)	30 (29.1)	
Traumatic SAH	+	0 (0)	4 (100)	0 (0)	0,236

	-	18 (17.3)	54 (51.9)	32 (30.8)	
Cerebral contusion	+	4 (33.3)	4 (33.3)	4 (33.3)	0.163
	-	14 (14.6)	54 (56.3)	28 (29.2)	
Pneumocephalus	+	1 (14.3)	3 (42.9)	3 (42.9)	0.864
	-	17 (16.8)	55 (54.5)	29 (28.7)	
Presence of symptom	+	12 (50)	12 (50)	0 (0)	**<0.001**
	-	6 (7.1)	46 (54.8)	32 (38.1)	
Headache	+	3 (30)	7 (70)	0 (0)	0.085
	-	15 (15.3)	51 (52)	32 (32.7)	
Vomiting	+	5 (45.5)	6 (55.5)	0 (0)	**0.009**
	-	13 (13.4)	52 (53.6)	32 (33)	
Unconsciousness	+	12 (100)	0 (0)	0 (0)	**<0.001**
	-	6 (6.3)	58 (60.4)	32 (33.3)	
Wound	+	6 (26.1)	6 (26.1)	11 (47.8)	**0.011**
	-	12 (14.1)	52 (61.2)	21 (24.7)	
Scalp swelling	+	7 (10.3)	49 (72.1)	12 (17.6)	**<0.001**
	-	11 (27.5)	9 (22.5)	20 (50)	
Additional traumatic pathology	+	3 (75)	1 (25)	0 (0)	**0.016**
	-	15 (14.4)	57 (54.8)	32 (30.8)	
Need for ICU	+	6 (66.7)	3 (33.3)	0 (0)	**<0.001**
	-	12 (12.1)	55 (55.6)	32 (32.3)	
Need for surgery	+	4 (80)	1 (20)	0 (0)	**0.003**
	-	14 (13.6)	57 (55.3)	32 (31.1)	
Trauma etiology	Fall from standing	4 (6.9)	29 (50)	25 (43.1)	**0.013**
	Fall from height	10 (29.4)	17 (50)	7 (20.6)	
	Fall (bicycle-unhelmeted)	1 (33.3)	2 (66.7)	0 (0)	
	Fall (swing)	1 (20)	4 (80)	0 (0)	
	MVC (passenger)	0 (0)	1 (100)	0 (0)	
	MVC (pedestrian)	0 (0)	4 (100)	0 (0)	
	Impact with a hard object	2 (66.7)	1 (33.3)	0 (0)	

PECARN: *Pediatric Emergency Care Applied Research Network* ***CT:*** *Computed Tomography* ***MVC:*** *Motor Vehicle Crash* ***ICU:*** *Intensive Care Unit* ***SAH:*** *Subarachnoid Haemorrhage* ***EDH:*** *Epidural Haemorrhage* ***SDH:*** *Subdural Haemorrhage*

DISCUSSION

The most common factor causing mortality in children is trauma, and among traumas, head traumas are both the most frequently seen and the most important cause of mortality(8,19). Falls, traffic accidents and sports injuries are the most prominent causes of head trauma and are more common in boys (8,12). In our study, it was observed that the majority of the cases were due to falls (92.6%). In addition, the majority of cases were boys (63%) in parallel with the literature. There is not yet a consensus on the indications for imaging especially in paediatric patients with minor head trauma. In the study by Easter et al. it was emphasised that clinician assessment and the PECARN algorithm define all clinically significant traumatic brain injury and PECARN is slightly more specific (20). In our trial, CT was not considered unnecessary in any patient requiring neurosurgical procedure and/or intensive care unit. In this respect, we think that the PECARN algorithm is a useful guideline for predicting traumatic brain injury and making the necessary imaging decision, regardless of the clinician's experience. Furtado et al. retrospectively examined paediatric minor head trauma patients for cost-effectiveness. For this evaluation, the PECARN algorithm was applied retrospectively and it was ascertained that CT was not required in 77.6% of the cases in which CT imaging was performed. According to this result, PECARN reduces the cost and this reduction is statistically significant (21). In our study, CT was not necessary in 32 cases (29.6%). However, some points should be remarked in this regard. Firstly, according to the PECARN algorithm, CT is not unnecessary in any patient undergoing neurosurgical procedures and/or requiring intensive care unit. Another point is that unlike other studies, our study was conducted on hospitalised patients requiring neurosurgical follow-up and treatment. These results emphasise that the PECARN algorithm is important in terms of cost reduction as well as predicting traumatic brain injury. Apart from the algorithms in the literature, there are recommendations for CT imaging indication and prediction of traumatic brain injury. Michiwaki et al. found that traumatic imaging findings were significantly more frequent in cases <1 year of age, GCS 14 and falls from height (22). Similarly, Andrade et al. showed that traumatic abnormal CT findings were statistically significantly higher in patients who fell from a distance of more than 1 meter (23). Fundaro et al. retrospectively investigated paediatric patients with mild head trauma and reported that scalp swelling and impaired consciousness were important findings in predicting traumatic brain injury requiring CT imaging (24). According to the PECARN algorithm, CT was not unnecessary in any patient with consciousness retardation in our study. In addition, CT was not necessary in 12 (17.6%) patients with scalp swelling in our study. In summary, CT indications in paediatric patients with minor head trauma should be carefully determined according to age, clinical status and mechanism of trauma, as well as the experience of the clinician. Algorithms in the literature have been tried to be established within this framework. When the sensitivity of the paediatric age group to radiation exposure with CT is considered, the aim of all these predictors is to maximally predict traumatic brain injury while minimising radiation exposure. In some studies in the literature, non-ionised imaging methods have also been researched in order to mitigate the adverse effects of radiation. Cicogan et al. evaluated point of care ultrasound (POCUS), near-infrared spectroscopy (NIRS) and rapid magnetic resonance imaging (MRI) methods. According to the PECARN algorithm, POCUS and NIRS can improve the decision-making process of the clinician in addition to PECARN in cases with moderate and severe risk for traumatic brain injury. Rapid MRI was seen as a suitable alternative to CT (25). In addition, the sensitivity of CT should be taken into consideration in the detailed diagnosis of bone pathologies.

CONCLUSION

Minor head traumas are frequently encountered especially in the paediatric age group. They are mostly not associated with traumatic brain injury and long-term sequelae. Children exposed to minor head trauma should be carefully evaluated by the clinician considering the history and findings suggestive of clinically significant TBI. The purpose of the clinician is to rapidly and accurately diagnose clinically significant TBI while avoiding unnecessary CT imaging to protect against the adverse effects of radiation. For this reason, various clinical decision-making algorithms have been proposed in the literature to assist the clinician. These algorithms are expected to have high sensitivity especially for the identification of cases requiring neurosurgical intervention and follow-up. We recommend the adoption of the PECARN algorithm for this purpose. On the other hand, similar studies should be performed in larger groups of patients requiring hospitalisation and neurosurgical follow-up and treatment as in our study.

Source of Funding: The authors declare the study received no funding.

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

Author Contributions: The authors confirm contribution to the paper as follows: study conception and design: SD, AK; data collection: SD, AK, NÇ; analysis and interpretation of results: SD, NÇ; draft manuscript preparation: AK, NÇ. All authors reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Nigrovic LE, Kuppermann N. Children With Minor Blunt Head Trauma Presenting to the Emergency Department. Pediatrics. 2019;144(6).
2. Pinto PS, Meoded A, Poretti A, Tekes A, Huisman TA. The unique features of traumatic brain injury in children. review of the characteristics of the pediatric skull and brain, mechanisms of trauma, patterns of injury, complications, and their imaging findings--part 2. J Neuroimaging. 2012;22(2):e18-41.
3. Singh N, Singhal A. Challenges in minor TBI and indications for head CT in pediatric TBI-an update. Childs Nerv Syst. 2017;33(10):1677-81.
4. Lumba-Brown A, Yeates KO, Sarmiento K, Breiding MJ, Haegerich TM, Giogia GA, et al. Centers for Disease Control and Prevention Guideline on the Diagnosis and Management of Mild Traumatic Brain Injury Among Children. JAMA Pediatr. 2018;172(11):e182853.
5. Mayer AR, Quinn DK, Master CL. The spectrum of mild traumatic brain injury: A review. Neurology. 2017;89(6):623-32.
6. Özsaraç M, Karcıoğlu Ö, Topaçoğlu H, Ayrık C, Kıyan S, Sener S, et al. Clinical indicators of traumatic brain injury and skull fracture in pediatric head trauma patients. Turkish Journal of Emergency Medicine. 2009;9(4):153-8.
7. Babl FE, Borland ML, Phillips N, Kochar A, Dalton S, McCaskill M, et al. Accuracy of PECARN, CATCH, and CHALICE head injury decision rules in children: a prospective cohort study. Lancet. 2017;389(10087):2393-402.
8. Atabaki SM. Pediatric head injury. Pediatr Rev. 2007;28(6):215-24.
9. Karaoğlu Gündoğdu D, Şahinoğlu M, Köktekir E, Karabağlı H. Pediatric Head Injuries. Türk Nöroşir Derg. 2020;30(2):287-92.
10. Dunning J, Daly JP, Lomas JP, Lecky F, Batchelor J, Mackway-Jones K. Derivation of the children's head injury algorithm for the prediction of important clinical events decision rule for head injury in children. Arch Dis Child. 2006;91(11):885-91.
11. Klemetti S, Uhari M, Pokka T, Rantala H. Evaluation of decision rules for identifying serious consequences of traumatic head injuries in pediatric patients. Pediatr Emerg Care. 2009;25(12):811-5.
12. Er A, Akman C, Alataş İ, Ünlü HB, Ceylan I, Gün C, et al. Should Children with Minor Head Injury Routinely Have CT Scan?. JOPP Derg. 2013;5(3):131-135.
13. Lyttle MD, Crowe L, Oakley E, Dunning J, Babl FE. Comparing CATCH, CHALICE and PECARN clinical decision rules for paediatric head injuries. Emerg Med J. 2012;29(10):785-94.
14. Tomar Güneysu S, Derinöz Güleryüz O. Çocuklarda Minör Kafa Travmalarına Yaklaşım. 2022.
15. Osmond MH, Klassen TP, Wells GA, Correll R, Jarvis A, Joubert G, et al. CATCH: a clinical decision rule for the use of computed tomography in children with minor head injury. CMAJ. 2010;182(4):341-8.
16. Bressan S, Eapen N, Phillips N, Gilhotra Y, Kochar A, Dalton S, et al. PECARN algorithms for minor head trauma: Risk stratification estimates from a prospective PREDICT cohort study. Acad Emerg Med. 2021;28(10):1124-33.
17. Kuppermann N, Holmes JF, Dayan PS, Hoyle JD, Atabaki SM, Holubkov R, et al. Identification of children at very low risk of clinically-important brain injuries after head trauma: a prospective cohort study. Lancet. 2009;374(9696):1160-70.
18. McGraw M, Way T. Comparison of PECARN, CATCH, and CHALICE clinical decision rules for pediatric head injury in the emergency department. CJEM. 2019;21(1):120-4.
19. Schutzman SA, Greenes DS. Pediatric minor head trauma. Ann Emerg Med. 2001;37(1):65-74.
20. Easter JS, Bakes K, Dhaliwal J, Miller M, Caruso E, Haukoos JS. Comparison of PECARN, CATCH, and CHALICE rules for children with minor head injury: a prospective cohort study. Ann Emerg Med. 2014;64(2):145-52, 52 e1-5.
21. Furtado LMF, da Costa Val Filho JA, Dos Santos AR, Furfuro R, Sandes BL, Hon Y, et al. Pediatric minor head trauma in Brazil and external validation of PECARN rules with a cost-effectiveness analysis. Brain Inj. 2020;34(11):1467-71.
22. Michiwaki Y, Maehara N, Ren N, Kawano Y, Nagaoka S, Maeda K, et al. Indications for computed tomography use and frequency of traumatic abnormalities based on real-world data of 2405 pediatric patients with minor head trauma. Surg Neurol Int. 2021;12:321.
23. Andrade FP, Montoro RN, Oliveira R, Loures G, Flessak L, Gross R, et al. Pediatric minor head trauma: do cranial CT scans change the therapeutic approach? Clinics (Sao Paulo). 2016;71(10):606-10.
24. Fundaro C, Caldarelli M, Monaco S, Cota F, Giorgio V, Filoni S, et al. Brain CT scan for pediatric minor accidental head injury. An Italian experience and review of literature. Childs Nerv Syst. 2012;28(7):1063-8.
25. Cicogna A, Minca G, Posocco F, Corno F, Basile C, Dalt LD, et al. Non-ionizing Imaging for the Emergency Department Assessment of Pediatric Minor Head Trauma. Front Pediatr. 2022;10:881461.

RESEARCH ARTICLE

Sevil Aydogan Gedik[1]
Didem Arslantas[2]
Alaettin Unsal[2]
Melih Velipasaoglu[3]

[1] Eskişehir Provincial Health Directorate, Eskişehir, Türkiye
[2] Public Health Department, Eskişehir Osmangazi University Faculty of Medicine, Eskişehir, Türkiye
[3] Gynecology and Obstetrics Department, Eskişehir Osmangazi University Faculty of Medicine, Eskişehir, Türkiye

Corresponding Author:
Sevil Aydogan Gedik
mail: aydogan.sevil@gmail.com

Received: 24.12.2024
Acceptance: 17.03.2025
DOI:10.18521/ktd.1606373

Scientific meetings where the study is presented as a paper:
1) 4th International 22nd National Public Health Congress, 13-19 December 2020.
2) 5th International and 23rd National Public Health Congress, 13-18 December 2021.

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Development of the Aydoğan-Depression Screening Scale for Pregnant and Determination of Depression Risks of Pregnant Women

ABSTRACT

Objective: The aim of the study is to develop the "Aydoğan-Depression Screening Scale for Pregnant" (A-DSP) for depression screening during pregnancy and to test its validity and reliability.

Method: This methodological study was conducted with 369 pregnant. A-DSP was designed as a 4-point Likert-type self-report scale consisting of positive and negative propositions. Content, construct and criterion validity were evaluated. Internal consistency analyses, item analysis and test-retest were performed to evaluate reliability. The cut-off score was determined by ROC analysis.

Results: The results obtained from all validity and reliability analyses of A-DSP were at a sufficient level. It was found that A-DSP consisted of 4 sub-dimensions and 21 items, total explained variance was 56.3%, and Cronbach's Alpha was 0.919. An increase in scores indicates an increase in the suspicion of depression. In addition, it is accepted that there is a suspicion of depression at a score≥41. It was determined that 29.5% of the pregnant women had a suspicion of depression.

Conclusions: It was concluded that A-DSP is a valid and reliable scale that can be used to screen for depression in pregnant women. It is thought that it would be beneficial to screen for depression using A-DSP, to monitor the mental status of pregnant women by A-DSP, to perceive it as an early warning for depression when there is a change, and to refer to a psychiatrist for further examination.

Keywords: Depression, Pregnant, Pregnancy, Validity, Reliability, Screening.

Aydoğan-Gebelere Yönelik Depresyon Tarama Ölçeği'nin Geliştirilmesi ve Gebelerin Depresyon Düzeyinin Belirlenmesi

ÖZET

Amaç: Çalışmanın amacı gebelik döneminde görülen depresyon taraması için "Aydoğan - Gebelere Yönelik Depresyon Tarama Ölçeği"nin (A-GDÖ) geliştirilmesi, geçerlik ve güvenirliğinin test edilmesidir.

Yöntem: Metodolojik tipteki çalışma 369 gebe üzerinde gerçekleştirildi. A-GDÖ 4'lü Likert tipinde olumlu ve olumsuz önermelerden oluşan bir öz bildirim ölçeği olarak tasarlandı. Kapsam geçerliği, yapı geçerliği ve ölçüt geçerliği değerlendirildi. Güvenirliği değerlendirmek için iç tutarlık analizleri, madde analizi ve test - tekrar test uygulaması yapıldı. Kestirim puanı ROC analizi ile belirlendi.

Bulgular: A-GDÖ'nün tüm geçerlik ve güvenirlik analizlerinden elde edilen sonuçların yeterli düzeyde olduğu görüldü. A-GDÖ'nün 4 alt boyut ve 21 maddeden oluştuğu, toplam açıklanan varyansın % 56.3, Cronbach Alfa güvenirlik katsayısının 0.919 olduğu bulundu. Ölçekten alınan toplam puanın artışı depresyon şüphesinin arttığını göstermektedir. Ayrıca 41 puan ve üzeri depresyon şüphesinin var olduğunu göstermektedir. Çalışmada gebelerin % 29.5'inde depresyon şüphesi olduğu tespit edildi.

Sonuç: A-GDÖ'nün gebelerde depresyonun taranması amacıyla kullanılabilecek geçerli ve güvenilir bir ölçek olduğu sonucuna ulaşıldı. A-GDÖ kullanılarak depresyon taraması yapılması, gebelerin ruhsal durumlarının A-GDÖ ile izlenmesi, bir değişiklik olduğunda depresyon için erken uyarı olarak algılanması ve ileri inceleme için bir psikiyatri uzmanına yönlendirme yapılmasının faydalı olacağı düşünülmektedir.

Anahtar Kelimeler: Depresyon, Gebe, Gebelik, Geçerlik, Güvenirlik, Tarama

INTRODUCTION

Depression is a common mental disorder in pregnant women. It has been reported that approximately 10% of pregnant women worldwide have depression (1). Depression has short and long term negative effects on pregnancy, fetus and after delivery baby (2). Early diagnosis of depression during pregnancy is important in order to prevent the negative consequences (3,4).

Depression screening in pregnant women is a topic that has come to the fore recently, and it is recommended to perform routine screenings with easy-to-use tools in prenatal controls (2, 5). With the help of self-report questionnaires that can be administered by people who are not experts in mental health, it is possible to identify pregnant women at risk of depression with low costs and using less resources (6). In particular, it is important to carry out screenings by health personnel in primary care and to include psychological evaluation in the follow-up of pregnant women (2, 7).

Although the validity and reliability studies of some depression scales in pregnant have positive results or some questions for screening depression during pregnancy have been found appropriate, no scale has been found in the literature that includes pregnancy-specific questions developed only for depression seen during pregnancy. There is a lack of approved, valid and reliable screening tools to screen for depression during pregnancy (2, 8). Because it may be difficult to distinguish normal somatic and emotional symptoms of pregnancy from depression symptoms, the use of unconfirmed measurement tools may yield inaccurate results (9). Reliable tools are needed to detect pregnancy depression.

In the study, it was aimed to develop the "Aydoğan-Depression Screening Scale for Pregnant" (A-DSP) to screen for depression during pregnancy, to test its validity and reliability, and to evaluate the suspicion of depression in pregnant women.

MATERIAL AND METHODS

Study Design and Study Group: The study is a methodological type study conducted on pregnant women aged 18 and over who applied to Eskişehir Osmangazi University Health Practice and Research Hospital Gynecology and Obstetrics Polyclinic between March 2020 and November 2021.

In validity and reliability studies, reaching 5-10 times the number of items or a sample of 300 people is considered good (10, 11). In our study, as the main sample, validity and reliability analyzes were performed on 369 pregnant women. Confirmatory factor analysis was performed in another group consisting of 308 pregnant women.

The ages of the women, who constituted the main sample, ranged between 18-43, with a mean of 30.49±4.73. Gestational weeks ranged from 4 to 40, with a mean of 23.15±9.52. 44.7% (n=165) were in the second trimester.

Data Collection Tools: Data were collected by a questionnaire. In the first part of the questionnaire, there are questions about the sociodemographic characteristics, questions about pregnancy and some factors that may be related to depression. In the second section, there is the A-DSP, which will be developed for the purpose of screening for depression in pregnant women, and the Edinburgh Postnatal Depression Scale (EDS) in the third section.

EDS was developed for the recognition of postpartum depression. The Turkish validity and reliability study was performed by Aydın et al. The cut-off point was suggested as 12.5 (12). Validity and reliability studies have been conducted on pregnant women in many countries. It has been used in many studies conducted to evaluate depression in pregnant women in Turkey.

Creating the A-DSP: In order to develop A-DSP, various psychiatry books, DSM, scales used for depression screening and diagnosis, depression scales used in research on depression in pregnant women, and publications specific to depression during pregnancy were examined. An 88-item question pool was created, including the diagnosis criteria of depression and the symptoms of depression in pregnant women.

Six experts (a psychiatrist, a psychologist, a measurement/evaluation expert, a gynecologist and two public health experts who are competent in scale development) reviewed the 88-item pool of questions. It was evaluated whether the items represented the features to be measured and whether they contained information about depression during pregnancy. Highly repetitive and misleading items were eliminated. In addition, the items were also reviewed in terms of language. After the corrections were made, 53 questions remained in the question pool.

A-DSP was designed as a self-report scale. In the A-DSP, which consists of positive and negative propositions, pregnant women were asked to think about their mental state in the last 1 week and to mark how often they experienced/thought/felt the expression in each item. The scale questions designed as an ordinal scale and designed as a 4-point Likert type are answered as "never", "sometimes", "often" and "always". Responses to negative items were never=1, sometimes=2, often=3, always=4; responses to positive items were scored in the opposite way. It was accepted that the risk of depression increased as the total score that could be obtained from the scale increased.

Language Suitability and Content Validity: Expert opinion was sought to evaluate the language suitability and content validity of the A-DSP. The 53-item form was submitted to the

opinion of 22 experts. The items were reviewed in terms of language. It was checked whether the items were understandable, whether there were any errors in their meaning, whether the items expressed the desired thing correctly and clearly. Experts evaluated each item according to the options of "necessary and sufficient", "necessary but insufficient" and "unnecessary". After the expert evaluation, suggested corrections were made for the items evaluated as "necessary but insufficient". The content validity ratio (CVR) was calculated for each item and the content validity index (CVI) for the overall scale was calculated. 15 items with a CVR lower than 0.42 were removed from the scale. Thus, 40 questions remained in the form and the smallest recalculated CVR was 0.45, and the CVI was 0.789. The content validity of the scale was considered to be sufficient (13).

Preliminary Study: Incomprehensible and erroneous questions were corrected with the preliminary study applied to 21 pregnant women. As a result, 7 items were removed from the scale.

Pilot Study: At this stage of the study, since the number of items in the A-DSP was 33, it was aimed to apply a questionnaire to at least 165 pregnant women for the pilot study by taking 5 times the number of items (11). Draft scale was applied to 200 pregnant women. Internal consistency analyzes and item analysis were performed. 11 items with an item-total correlation coefficient below 0.30 were excluded from the scale (14). As a result of the pilot study, A-DSP decreased to 22 items.

Validity and Reliability Analysis with the Main Sample: After the pilot study, the main sample (n=369) was surveyed again. To test the construct validity, exploratory factor analysis (EFA, it was done in a different group of 308 pregnant women) and confirmatory factor analysis (CFA) was performed, and discriminant validity was tested with the help of differences between groups. Assumptions were checked before performing factor analysis. For factor analysis in the literature, reaching 5-10 times the number of items or a sample of 300 people is considered good (10, 11). In line with these recommendations, a sample of 369 pregnant women for EFA and 308 pregnant women for CFA is considered sufficient. In addition, the Kaiser-Meyer-Olkin (KMO) test and Bartlett test of sphericity were used to assess the adequacy of the sample size and suitability for factorization. The correlation matrix was examined to determine whether there was singularity and multiple collinearity. The anti-image correlation matrix and Measures of Sampling Adequacy (MSA) values were also examined for suitability for factor analysis. Direct oblimin rotation were used in EFA. Factors with an eigenvalue greater than 1 were taken into account when deciding on the number of factors (15, 16). The factor load limit value was 0.32 (15). According to the results of EFA made with the 22-item version of A-DSP, it was seen that an item fell into the same sub-dimension with questions that didn't measure the same feature as itself. For this reason, that item was removed from the scale. EFA was performed again with the remaining 21 items. Lavaan 0.6-7 package was used in R program for CFA. Before starting CFA, sample size, normality, singularity, and multiple collinearity assumptions were checked. The MVN 5.8 package was used to test the multivariate normal distribution. Evaluation was made with the Q-Q chart and Mardia's multivariate normality test. In the Q-Q plot of A-DSP, it was seen that many data points deviated from multivariate normality (Figure 1).

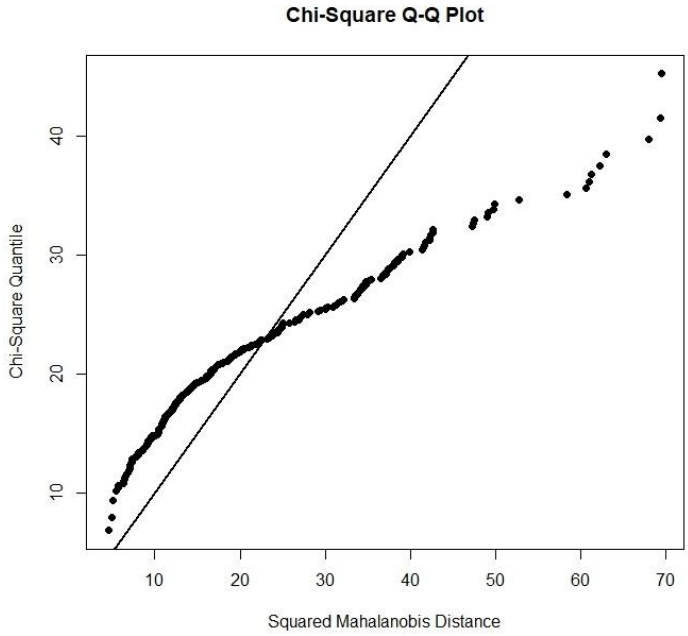

Figure 1. Q-Q Plot Obtained as a Result of Multivariate Normality Analysis

In addition, when Mardia's skewness and kurtosis values, the significance levels of these values and the statistical decision regarding the significance levels were examined, it was seen that multivariate normality was not achieved (Table 1).

Table 1. Mardia's Multivariate Normality Test Results

Test	Statistics	p value	Statistical decision
Skewness	4506.74	<0.001	No
Kurtosis	37.30	<0.001	No
MVN	-	-	No

Since the items of A-DSP were ordinal and multivariate normality could not be achieved, the diagonal weighted least squares method was used as the parameter estimation method. Robust versions of the goodness-of-fit indices obtained by adjusting for non-normal distributions were taken into account. Acceptable value for chi-square/degrees of freedom was considered as <5, for CFI and TLI (NNFI) as >0.95, for RMSEA and SRMR as <0.08 (17). The Spearman correlation coefficient between EDS and A-DSP was evaluated for concurrent criterion validity. Cronbach's Alpha reliability coefficient and item-total correlation coefficient were calculated for internal consistency. The item scores of the lower and upper 27% groups were compared for item discrimination. The test-retest method was used to evaluate stability, which is a component of reliability. The scale was applied to 26 pregnant women with an interval of 2 weeks, Pearson correlation coefficient and ICC value were calculated. ROC analysis was performed to determine the cut-off score corresponding to optimal sensitivity and optimal specificity of A-DSP. In the ROC analysis, groups with and without risk for depression were used according to the cut-off score of EDS, which is used as an equivalent criterion.

Data analysis was performed using SPSS (version 15.0) and R (version 4.0.3) statistical packages. Mann-Whitney U and Chi-square tests were used to compare the groups. Statistical significance level was accepted as $p<0.05$.

Ethical Approval: Ethics committee approval was obtained from Eskişehir Osmangazi University Non-Interventional Clinical Research Ethics Committee (25403353-050-99-E.38314, 26.03.2020).

RESULTS

Exploratory Factor Analysis: KMO test results were 0.934, and Bartlett's p-value was<0.001. The sample size was sufficient for factor analysis and the data were suitable for analysis. The correlation coefficient between all items was observed to be less than 0.8. Thus, it was concluded that there was no singularity and multiple collinearity in the data. The MSA values of the items in the anti-image correlation matrix were between 0.897 and 0.961. It was observed that the values outside the diagonal of the matrix were mostly small. Since all MSA values were above 0.5, it was concluded that the data could be factored.

As a result of factor analysis, it was seen that the scale consisted of 4 sub-dimensions and 21 items. Factor loadings ranged from 0.428 to 0.798. The total explained variance was 56.3% (Table 2).

Internal Consistency Reliability and Item Analysis: The Cronbach's Alpha coefficient of the 21-item final version of the A-DSP, was found to be 0.919. Item-total correlation coefficients ranged from 0.403 to 0.726. The Cronbach's Alpha value was found to be 0.860 for the first factor, 0.784 for the second factor, 0.698 for the third factor, and 0.765 for the fourth factor. All of the item-total correlation coefficients between each factor's own items were greater than 0.3 (Table 2). Internal consistency of the A-DSP and all factors was considered to be sufficient.

In order to evaluate item discrimination, the scores obtained from the A-DSP were ordered from high to low. The item medians of the lower and upper 27% groups were compared. A significant difference was found between the total scores of the upper 27% group and the lower 27% group from A-DSP and between the scores they received from each item ($p<0.001$ for each). It was accepted that each item of the A-DSP and the whole scale had item discrimination, and that it could distinguish pregnant women with and without depression risk.

Confirmatory Factor Analysis: CFA assumptions were checked and it was found that the sample size (308) was sufficient and there was no singularity and multicollinearity (all coefficients in the correlation matrix are less than 0.8).

The chi-square test value of the model is 475.099 ($p<0.001$). Among the goodness of fit indices obtained by CFA, the chi-square/degrees of freedom value of 2.56, CFI of 0.962 and TLI (NNFI) of 0.957 indicate a very good fit. The SRMR value of 0.060 and the RMSEA value of 0.071 indicate acceptable fit. It was found that sufficient model-data fit was achieved (17). It was determined that the standard regression coefficients (factor load) were sufficient (between 0.59-0.92, Figure 2). It was concluded that the A-DSP provided construct validity.

Criterion Validity: The total A-DSP scores of the pregnant women were between 21-70 (mean=36.15±10.11), and their EDS scores were between 0-26 (mean=8.40±5.54). A strong positive correlation was determined between EDS and A-DSP scores ($r=0.810$, $p<0.001$).

Discriminant Validity: It was found that those who scored 13 points (cut-off point) or higher on the EDS and who reported having a physician-diagnosed mental illness or depression scored higher on the A-DSP (Table 3). It was determined that the scale provided discriminant validity.

Table 2. The Final Factor Pattern of A-DSP

Factors	Items*	For All Scale		For Each Factor	
		Item-Total Correlation Coefficient	Factor Loading Values of Items	Cronbach Alpha if Item Deleted	Item-Total Correlation Coefficient
Factor 1: Low Energy **Initial Eigenvalue:** 8.239 **Common Factor Variance:** 39.234 **Cronbach's Alpha:** 0.860	1. It is physically and mentally difficult to devote myself to a job in my daily life.	0.566	0.798	0.841	0.621
	2. During pregnancy, my life energy decreased.	0.698	0.741	0.823	0.736
	3. I no longer enjoy the things I used to enjoy doing in my spare time before pregnancy.	0.603	0.652	0.842	0.615
	4. I feel that I do not have the strength to strive for something.	0.726	0.616	0.828	0.714
	5. I don't feel like doing anything.	0.590	0.602	0.845	0.589
	6. I have no desire to meet people.	0.472	0.581	0.859	0.494
	7. I'm not as cheerful as I used to be.	0.676	0.472	0.840	0.625
Factor 2: Pessimism **Initial Eigenvalue:** 1.257 **Common Factor Variance:** 5.986 **Cronbach's Alpha:** 0.784	8. My postpartum responsibilities scare me.	0.521	0.704	0.730	0.597
	9. I am hopeful for postpartum.	0.434	0.643	0.787	0.449
	10. I think that I will not be as productive as before in my life after birth.	0.653	0.624	0.704	0.676
	11. I'm afraid of not being able to take good care of my baby.	0.578	0.536	0.741	0.571
	12. I feel like everything will get worse as my pregnancy progresses.	0.646	0.447	0.752	0.536
Factor 3: Worthlessness-Guilt **Initial Eigenvalue:** 1.184 **Common Factor Variance:** 5.638 **Cronbach's Alpha:** 0.698	13. My life has no meaning.	0.444	0.708	0.661	0.451
	14. I think I have a negative impact on my baby's health.	0.523	0.684	0.607	0.523
	15. I think I am worthless from the perspective of the people around me.	0.533	0.669	0.606	0.524
	16. I feel guilty for things that went wrong during pregnancy.	0.484	0.541	0.653	0.456
Factor 4: Depressed Mood **Initial Eigenvalue:** 1.147 **Common Factor Variance:** 5.463 **Cronbach's Alpha:** 0.765	17. I feel like crying for no reason.	0.403	0.632	0.764	0.406
	18. I feel sad.	0.603	0.584	0.702	0.619
	19. I am having a happy pregnancy.	0.566	0.554	0.734	0.534
	20. I always think of bad possibilities regarding the pregnancy process.	0.605	0.536	0.713	0.563
	21. I think my mood is worse than other pregnant women.	0.659	0.428	0.698	0.609
Total Explained Variance: 56.321 **Total Cronbach's Alpha:** 0.919					

*Items 9 and 19 are reverse coded.

Test-Retest Reliability: The total scores from the first test ranged from 22 to 50 (mean=35.42±6.71). The total scores from the second test ranged from 21 to 55 (mean=34.53±8.11). The Pearson correlation coefficient between the total A-DSP scores obtained from the first test and the second test was 0.745 ($p<0.001$) and the ICC value was 0.845 (95% CI:0.655-0.931, $p<0.001$). It was concluded that A-DSP gave similar results in both measurements, had high reliability, was stable and didn't change over time.

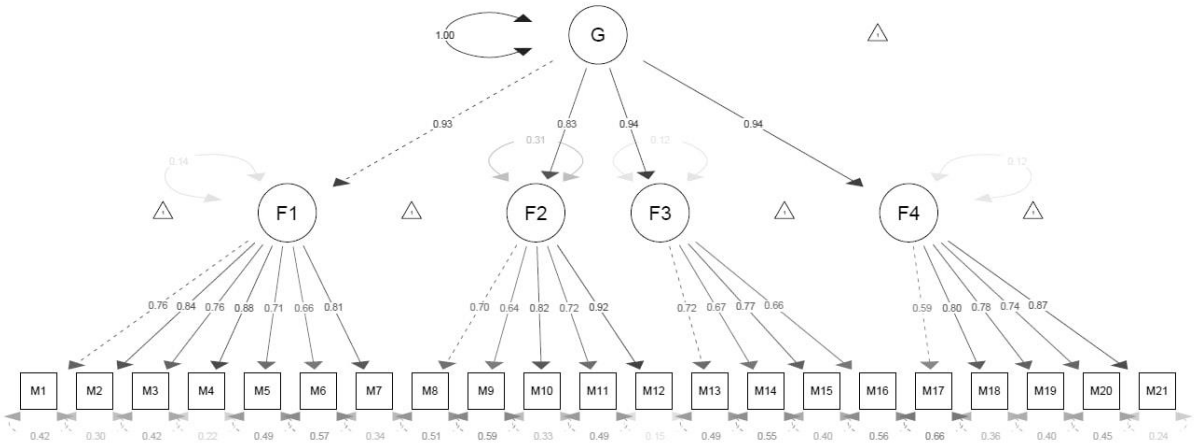

Figure 2. Path Diagram Showing the Model Structure and Standard Regression Coefficients

Table 3. Distribution of the A-DSP Scores of the Pregnant Women According to the EDS Scores and the Presence of Current Mental Illness

	n (%)	A-DSP Total Score		Test Statistic; p
		Mean±SD*	Median (minimum-maximum)	
EDS Score				
12 and below	282 (76.4)	32.33±6.99	31.50 (21.00-60.00)	**12.206;**
13 and above	87 (23.6)	48.55±8.63	47.00 (29.00-70.00)	**<0.001**
Current physician-diagnosed mental illness				
No	360 (97.6)	35.85±9.80	34.00 (21.00-70.00)	**3.046;**
Yes	9 (2.4)	49.44±13.68	53.00 (21.00-64.00)	**0.002**
Current physician-diagnosed depression				
No	365 (98.9)	36.02±10.04	34.00 (21.00-70.00)	**2.259;**
Yes	4 (1.1)	48.75±10.14	47.50 (39.00-61.00)	**0.024**
Total	369 (100.0)	36.1±10.11	34.0 (21.0-70.0)	

*Standard deviation

Cut-Off Score: In the ROC analysis, the area under the curve was found to be 0.932 (%95 CI: 0.905-0.959, p<0.001). The points where the sensitivity and specificity values were highest and closest to each other were examined. The optimal sensitivity (0.851) and specificity (0.876) values were found to be 40.5 cut-off points. In addition, the likelihood ratio (LR) value for this cut-off score was found to be 6.8.

When the EDS and A-DSP cut-off scores were examined, it was determined that 23.6% of the pregnant women according to the EDS and 29.5% according to the A-DSP were at risk of depression.

DISCUSSION

A-DSP was designed as a self-report scale based on the self-evaluation of pregnant women. Self-report scales are used to measure features that cannot be observed directly. Evaluation is made according to the person's statement. The fact that the individual answers the questions honestly affects the accuracy and reliability of the data obtained. A self-report-based screening test cannot replace clinical diagnosis, but it can show which pregnant women need further evaluation (18).

A comprehensive literature review was conducted to create the A-DSP. General information on depression, scales, and publications specific to depression during pregnancy were reviewed. Information on peripartum depression, where pregnancy depression was first defined, and information on postpartum depression were compiled. One of the scales used was the Pregnancy Depression Scale (PDS), which was created for the purpose of screening for depression in pregnant women. It was created by revising the Hamilton Depression Rating Scale using a structured clinical interview for DSM-4. It was determined that 7 items of the Hamilton Depression Rating Scale were associated with depression during pregnancy (depressed mood, feeling of guilt, decrease in work activities, psychomotor retardation, diurnal variation, fatigability, social withdrawal). It was stated that these 7 items forming the PDS predicted a major depressive episode during pregnancy (3). Items that question these symptoms are also found in A-DSP. Only diurnal variation is not included in the A-DSP. Unlike PDS and other depression scales used in

pregnancy, A-DSP questions were created using expressions specific to pregnancy.

It was seen that the A-DSP consisted of 4 factors. Because psychological characteristics have complex structures, it is generally not possible for scales measuring psychological characteristics to be unidimensional. Depression scales also measure the emotional, cognitive, somatic and perceptual symptoms of depression. Within the framework of these symptoms, it is expected that the scales will consist of sub-dimensions. It has been reported in many studies that depression scales consist of many sub-dimensions. Beck et al. defined the cognitive and somatic-affective dimensions of the Beck Depression Inventory. This structure was also confirmed in Turkey (19). It has been reported that the CES-D has a four-dimensional structure: depressive symptoms, positive affect, somatic symptoms, and difficulties in interpersonal relationships (20). Similarly, when the validity and reliability studies of depression scales on pregnant women are examined, it is seen that there are multidimensional structures. It has been reported that EDS, which was developed as one-dimensional, showed a three-factor structure including depression, anxiety and suicide in studies conducted in England and the Netherlands (21,22). In another study conducted in England, the existence of a two-factor structure, anxiety and depression, was mentioned in the first trimester (23). In a study conducted in France, it was reported that a two-factor structure was detected, including depression and other disorders including anxiety (24). In the validity and reliability study performed on Hungarian pregnant women, it was reported that EDS consisted of 3 factors (25). The multidimensional structure of A-DSP in our study is compatible with the literature.

There are different limits in the literature for the total variance explained by the scale. Having 50% or more of the total variance explained by a scale has been accepted as sufficient in many studies (15). It is considered sufficient that the total variance explained in the scales used in social areas is 50-60% (16). According to the data obtained as a result of EFA, the contribution of 21 items and 4 factors that make up A-DSP to the total variance is 56.3%. In other words, approximately 56.3% of the depression risks of pregnant women can be determined with the help of A-DSP. It can be said that the total variance level explained by A-DSP is sufficient.

The similarity between the measurement results of the newly developed test and the standard test, which is known to measure a feature correctly and proven validity and reliability, shows that the new scale provides criterion validity (26). In the hypothesis established in this direction, it was expected that the scores of the pregnant women in A-DSP and the scores they got in the EDS would show a positive and acceptable correlation. As expected, a strong positive correlation was found between the scores obtained from the two scales used in our study (r=0.810). The results obtained show that the A-DSP provided the criterion validity.

Reliability shows the ability of the scale to measure accurately and its invariance over time (14). In order to ensure reliability, the scale should be consistent, stable and sensitive. The Cronbach Alpha reliability coefficient is used to evaluate internal consistency. It shows the degree of consistency between the items of a scale and the whole scale. High values indicate that the scale items are self-consistent and that the scale measures a single feature. Although lower Cronbach Alpha values are accepted in scales with few questions, between 0.7-0.95 are generally accepted as reliable (14, 27). The Cronbach's Alpha coefficient of A-DSP was calculated as 0.860 for the first factor, 0.784 for the second factor, 0.698 for the third factor, 0.765 for the fourth factor, and 0.919 for the whole scale. A sufficient level of Cronbach's Alpha value for each factor and 21 questions that make up the whole scale shows that the questions are consistent. It can be interpreted that the internal consistency of the A-DSP is provided and it is quite reliable. The Cronbach's alpha value of the PDS, which was created to screen for depression in pregnant women using the Hamilton Depression Rating Scale, was found to be 0.81.

The reliability coefficient is affected by the sample size. In addition, the correlation coefficients between the items and the fact that the participants knew the purpose of the test before collecting data are among the factors affecting reliability (14). In our study, the sample size was sufficient and the correlation coefficients between the items were in the appropriate range. Before applying the questionnaire, the participants were informed about the purpose of the study, and the points that were curious or not understood by the participants were answered by the researcher and the participants were enlightened. For these reasons, it can be said that the reliability coefficient is calculated correctly without being affected by these factors.

Stability, which is a component of reliability, is evaluated with the test-retest method. In this method, which is based on applying the scale to the same people twice with a certain time interval and calculating the correlation coefficient between the two measurement results, the high correlation coefficient indicates that the measurement is stable (14, 26). Another coefficient calculated in the test-retest method is ICC. It is expected that the correlation coefficient and ICC value will be 0.70 and above (28). It was observed that there was a strong positive correlation between the scores obtained as a result of applying A-DSP to the same pregnant women at two-week intervals (r=0.745). In addition, the ICC value was calculated as 0.845. It was found that the scores obtained from A-DSP

did not change according to time, A-DSP was stable in repeated measurements, gave similar results, and provided test-retest reliability.

ROC analysis was performed to calculate the cut-off score of A-DSP. The fact that the area under the curve in the ROC analysis is close to 1 indicates that the test has high discrimination (29). In our study, the area under the curve was found to be 0.932. When deciding on the cut-off point, it is recommended to use the point where the sensitivity+selectivity value is the highest and the sensitivity and selectivity values are closest to each other (29). When evaluated according to these criteria, the cut-off score of A-DSP was 40.5. For this cut-off score, the sensitivity was 85.1% and the specificity was 87.6%. In other words, while the success of A-DSP to identify a pregnant woman at risk of depression is 85%, the success of identifying a pregnant woman without a risk of depression is 87%. In addition, the LR value for this cut-off score was found to be 6.8. The larger the LR value, the better distinguishing individuals who are truly at risk. It can be interpreted that A-DSP produced 6.8 true positive versus 1 false positive result (29).

Strengths and Limitations of the Study: This study is important because it's the first scale development study that includes pregnancy-specific questions designed only for the pregnancy period on depression. One of the strengths is that the study was carried out on a large sample.

The diagnostic criteria for depression in DSM and some normal symptoms during pregnancy are similar. For this reason, the diagnosis of depression can be made more than normal in the evaluations made according to the DSM criteria in pregnant women. Self-report scales developed according to DSM criteria may also indicate more cases than they actually are and produce erroneous results (30). In order to avoid such mistakes during the development of A-DSP, no questions were prepared that included somatic symptoms such as palpitations, weight gain, increased or decreased appetite, and decreased sexual desire, which overlapped with pregnancy symptoms. However, it was not possible to exclude all overlapping symptoms from the scale. Although changes in mood, symptoms of weakness and fatigue are expected symptoms of pregnancy, they are also among the most basic symptoms of depression. For this reason, items questioning these features are included in the A-DSP.

There were few cases of doctor-diagnosed depression in the study. EDS was used as the gold standard in the ROC analysis instead of clinical diagnosis. This limitation is one of the weaknesses of the study. In addition, this study was conducted on pregnant women who applied to only one medical school hospital. It would be useful to test the scale on larger groups for its generalizability.

CONCLUSION

As a result, it was seen that A-DSP is a valid and reliable scale that can be used to screen for depression in pregnant women in Turkish society. A-DSP consists of 21 items and 4 sub-dimensions. The total score that can be obtained varies between 21-84. It's accepted that the higher the score, the higher the risk of depression in pregnant. In addition, it's accepted that there is a suspicion of depression in pregnant women who score 41 and above. In line with this information, it was determined that 29.5% of the pregnant women had a suspicion of depression in this study.

It is thought that it would be beneficial to monitor the mental status of pregnant women using A-DSP, to perceive it as an early warning for depression when there is a change, to screen for depression using A-DSP, and to refer to a psychiatrist for further examination when necessary. Identifying pregnant women with suspected depression or an increased risk of depression during follow-up with the help of A-DSP will help to define the risk factors for pregnancy depression. It would be appropriate to study and test A-DSP in different parts of the society and in pregnant women with different characteristics.

REFERENCES

1. Woody C, Ferrari A, Siskind D, Whiteford H, Harris M. A systematic review and meta-regression of the prevalence and incidence of perinatal depression. Journal of Affective Disorders. 2017;219:86-92.
2. Breedlove G, Fryzelka D. Depression screening during pregnancy. Journal of Midwifery & Women's Health. 2011;56(1):18-25.
3. Altshuler LL, Cohen LS, Vitonis AF, Faraone SV, Harlow BL, Suri R, et al. The Pregnancy Depression Scale (PDS): a screening tool for depression in pregnancy. Archives of Women's Mental Health. 2008;11:277-85.
4. Campagne DM. The obstetrician and depression during pregnancy. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2004;116(2):125-30.
5. Su K-P, Chiu T-H, Huang C-L, Ho M, Lee C-C, Wu P-L, et al. Different cutoff points for different trimesters? The use of Edinburgh Postnatal Depression Scale and Beck Depression Inventory to screen for depression in pregnant Taiwanese women. General Hospital Psychiatry. 2007;29(5):436-41.
6. Bennett HA, Einarson A, Taddio A, Koren G, Einarson TR. Depression during pregnancy: overview of clinical factors. Clinical Drug Investigation. 2004;24:157-79.
7. Tran TD, Tran T, La B, Lee D, Rosenthal D, Fisher J. Screening for perinatal common mental disorders in women in the north of Vietnam: a comparison of three psychometric instruments. Journal of affective disorders. 2011;133(1-2):281-93.

8. Ji S, Long Q, Newport DJ, Na H, Knight B, Zach EB, et al. Validity of depression rating scales during pregnancy and the postpartum period: impact of trimester and parity. Journal of Psychiatric Research. 2011;45(2):213-9.
9. Bennett HA, Einarson A, Taddio A, Koren G, Einarson TR. Prevalence of depression during pregnancy: systematic review. Obstetrics & Gynecology. 2004;103(4):698-709.
10. Comrey AL, Lee HB. A first course in factor analysis. Psychology Press; 2013.
11. Osborne JW. Best practices in quantitative methods. Sage Publications; 2008.
12. Aydin N, Inandi T, Yigit A, Nalan Sahin Hodoglugil N. Validation of the Turkish version of the Edinburgh Postnatal Depression Scale among women within their first postpartum year. Social Psychiatry and Psychiatric Epidemiology. 2004;39:483-6.
13. Lawshe CH. A quantitative approach to content validity. Personnel Psychology. 1975;28(4):563-75.
14. Sürücü L, Maslakçı A. Validity and reliability in quantitative research. Business & Management Studies: An International Journal. 2020;8(3):2694-726.
15. Watson JC. Establishing evidence for internal structure using exploratory factor analysis. Measurement and Evaluation in Counseling and Development. 2017;50(4):232-8.
16. Williams B, Onsman A, Brown T. Exploratory factor analysis: A five-step guide for novices. Journal of Emergency Primary Health Care (JEPHC). 2010;8:1-13.
17. Hooper D, Coughlan J, Mullen MR. Structural equation modelling: guidelines for determining model fit. Electronic Journal of Business Research Methods. 2008;6(1):53-60.
18. Yonkers KA, Wisner KL, Stewart DE, Oberlander TF, Dell DL, Stotland N, et al. The management of depression during pregnancy: a report from the American Psychiatric Association and the American College of Obstetricians and Gynecologists. General Hospital Psychiatry. 2009;31(5):403-413.
19. Arkar H, Şafak C. Exploring dimensions of the Beck Depression Inventory in a clinical sample. Turkish Journal of Psychology. 2004;19(53):117-127.
20. Tatar A, Saltukoglu G. The adaptation of the CES-Depression Scale into Turkish through the use of confirmatory factor analysis and item response theory and the examination of psychometric characteristics. Bulletin of Clinical Psychopharmacology. 2010;20(3):213-227.
21. Jomeen J, Martin C. Replicability and stability of the multidimensional model of the Edinburgh Postnatal Depression Scale in late pregnancy. Journal of Psychiatric and Mental Health Nursing. 2007;14(3):319-24.
22. Brouwers EP, van Baar AL, Pop VJ. Does the Edinburgh Postnatal Depression Scale measure anxiety? Journal of Psychosomatic Research. 2001; 51(5):659-663.
23. Jomeen J, Martin CR. Confirmation of an occluded anxiety component within the Edinburgh Postnatal Depression Scale (EPDS) during early pregnancy. Journal of Reproductive and Infant Psychology. 2005;23(2):143-54.
24. Adouard F, Glangeaud-Freudenthal N, Golse B. Validation of the Edinburgh Postnatal Depression Scale (EPDS) in a sample of women with high-risk pregnancies in France. Archives of Women's Mental Health. 2005;8:89-95.
25. Töreki A, Andó B, Keresztúri A, Sikovanyecz J, Dudas RB, Janka Z, et al. The Edinburgh Postnatal Depression Scale: translation and antepartum validation for a Hungarian sample. Midwifery. 2013;29(4):308-15.
26. Heale R, Twycross A. Validity and reliability in quantitative studies. Evidence-Based Nursing. 2015;18(3):66-67.
27. Tavakol M, Dennick R. Making sense of Cronbach's alpha. International Journal of Medical Education. 2011;2:53-55.
28. Bujang MA, Baharum N. A simplified guide to determination of sample size requirements for estimating the value of intraclass correlation coefficient: a review. Archives of Orofacial Science. 2017;12(1):1-11.
29. Hajian-Tilaki K. (2013). Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. Caspian Journal of Internal Medicine. 2013;4(2):627-635.
30. Matthey S, Ross-Hamid C. (2011). The validity of DSM symptoms for depression and anxiety disorders during pregnancy. Journal of Affective Disorders. 2011;133(3):546-552.

RESEARCH ARTICLE

Cuneyd Yavas[1]
Nezih Hekim[1]
Lutfiye Karcioglu Batur[1]
Recep Eroz[2]
Ahmet Ozaydin[3]

[1] Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Biruni University, Istanbul, Türkiye
[2] Department of Medical Genetics, Medical Faculty, Aksaray University, Aksaray, Türkiye
[3] Department of Medical Genetics, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Türkiye

Corresponding Author:
Lutfiye Karcioglu Batur
mail: lbatur@biruni.edu.tr

Received: 03.10.2024
Acceptance: 23.03.2025
DOI:10.18521/ktd.1560608

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Biomarkers of Vitamin D Sufficiency: Vitamin D Metabolite Levels do not depend on 25-Hydroxyvitamin D2 levels in Healthy Turkish Individuals

ABSTRACT

Objective: Patient-specific factors may influence the adequate supplemental vitamin D dose. In this study, we evaluated the relationship between 25(OH)D_2 and free vitamin D levels and vitamin D deficiency in a healthy Turkish population.

Method: Blood samples were obtained from 92 healthy adults aged ≥18 years. Total 25(OH)D was determined by CMIA. Serum 25(OH)D_3 and D_2 levels were measured by LC-MS. Free 25(OH)D was calculated according to the Bikle method.

Results: In 54% of the participants, 25(OH)D_3 levels were below 20 ng/mL. Those with 20 ng/mL or higher had higher mean serum 25(OH)D_3 and free vitamin D levels (P<0.001). Mean 25(OH)D_2 concentration did not differ according to deficiency criteria. Serum 25(OH)D_2 levels were consistent regardless of free vitamin D concentrations.

Conclusions: Serum 25(OH)D_3 and free vitamin D concentrations measured by LC-MS indicate deficiency by influencing the total 25(OH)D_3 concentration. However, serum 25(OH)D_2 concentration did not differ between individuals and does not directly indicate deficiency.

Keywords: Vitamin D, 25-Hydroxyvitamin D_2, 25-Hydroxyvitamin D_3, free vitamin D.

D Vitamini Yetersizliğinin Biyobelirteçleri: Sağlıklı Türk bireylerde D Vitamini Metabolit Düzeyleri 25-Hidroksivitamin D2 Düzeylerine Bağlı Değildir

ÖZET

Amaç: Hastaya özgü faktörler yeterli ek D vitamini dozunu etkileyebilir. Bu çalışmada, sağlıklı bir Türk popülasyonunda 25(OH)D_2 ve serbest D vitamini düzeyleri ile D vitamini eksikliği arasındaki ilişki değerlendirilmiştir.

Yöntem: 18 yaş üstü 92 sağlıklı yetişkinden kan örnekleri alınmıştır. Toplam 25(OH)D CMIA ile belirlenmiştir. Serum 25(OH)D_3 ve D_2 seviyeleri LC-MS ile ölçülmüştür. Serbest 25(OH)D hesaplaması Bikle yöntemine göre yapılmıştır.

Bulgular: Katılımcıların %54'ünde 25(OH)D_3 seviyesi 20 ng/mL'nin altındaydı. 20 ng/mL veya daha yüksek olanlarda, ortalama serum 25(OH)D_3 ve serbest D vitamini seviyeleri daha yüksekti (P<0.001). Ortalama 25(OH)D_2 konsantrasyonu eksiklik kriterlerine göre fark göstermemiştir. Serum 25(OH)D_2 seviyeleri, serbest D vitamini konsantrasyonlarından bağımsız olarak tutarlıydı.

Sonuç: LC-MS ile ölçülen serum 25(OH)D_3 ve serbest D vitamini konsantrasyonları, toplam 25(OH)D_3 konsantrasyonunu etkileyerek eksikliği göstermektedir. Ancak, serum 25(OH)D_2 konsantrasyonu bireyler arasında fark göstermemiştir ve eksikliği doğrudan göstermez.

Anahtar Kelimeler: D vitamini, 25-Hidroksivitamin D_2, 25-Hidroksivitamin D_3, serbest D vitamini

INTRODUCTION

Vitamin D occurs predominantly in two forms: ergocalciferol (D_2) and cholecalciferol (D_3). Most vertebrates' skin produces around 80% of their D_3 by UVB irradiation of 7-dehydrocholesterol. In contrast, UVB radiation produces vitamin D_2 in plants and fungus (1). Vitamin D is essential for bone metabolism, among other metabolic and catabolic pathways, and functions in the congenital and adaptive immune systems that influence the cure, severity and mortality of various acute and chronic diseases and bacterial and viral illnesses (2-4). Multiple forms of vitamin D can play a key role in modulating immunity, including ergocalciferol (D_2), cholecalciferol (D_3), 25-hydroxyvitamin D_2 ($25(OH)D_2$), 25-hydroxyvitamin D_3 ($25(OH)D_3$), and 1,25-dihydroxyvitamin D_3 ($1,25\text{-}(OH)2D_3$) (5). The primary metabolic pathway for vitamin D physiology involves the formation of $25(OH)D_2$ and $25(OH)D_3$ from 25(OH)D (6). Through the bloodstream, vitamin D and its metabolites are transported predominantly attached to vitamin D binding protein (VDBP) (about 85%) and albumin (about 15%) (7). Upon reaching target cells, the vitamin D complex dissociates from either VDBP or albumin, allowing vitamin D to enter the cells and engage with nuclear vitamin D receptors (VDRn), which are present in various tissues and act as transcriptional factors (8).

Vitamin D insufficiency is commonly defined as serum $25(OH)D_3$ concentrations less than 20 nmol/L, while other recommendations and published research have varying cut-off values (9). It is often necessary to augment low serum $25(OH)D_3$ levels with ergocalciferol (D_2) or cholecalciferol (D_3), although their therapeutic equivalentity is debatable (10-12). Research indicates that taking D_3 orally increases levels of both free and total 25(OH)D more than using D_2 supplements (10, 13, 14). Factors unique to each patient may also influence how much extra vitamin D is needed (15) and for this reason, it's critical to validate vitamin D metabolite thresholds as well as which metabolite is clinically meaningful in order to recommend vitamin D supplementation. This study provides a comprehensive analysis by including both $25(OH)D_3$ and $25(OH)D_2$ levels in a larger cohort, allowing for a more detailed evaluation of vitamin D_2 status in healthy individuals. We present new findings on the potential role of $25(OH)D_2$ in vitamin D metabolism, which has been largely overlooked in previous studies. Owing to variations in patient reactions to vitamin D therapy and demographic variables, our goal was to look at the relationship between $25(OH)D_2$ and free vitamin D levels as well as vitamin D insufficiency in a cohort of Turkish adults who were otherwise in good health.

MATERIAL AND METHODS

Study Design: Ninety-two healthy adults, aged 18 or older, of both sexes (38 men and 54 women), who applied for a normal yearly check-up at the Biruni University Hospital's Check-up Unit, satisfied the research's requirements, and consented to take part in the investigation were included in the study. The following three requirements were met in order to be eligible for this prospective study: 1) no health condition, such as obesity, that might influence vitamin D concentrations; 2) no vitamin D supplementation during the previous two years; and 3) at least two generations of Turkish ancestry. The exclusion criteria included the following: 1) refusing to participate in the study; 2) not being of Turkish ethnic descent; 3) taking any kind of vitamin D supplement in the previous two years; 4) having a diagnosis of an infectious disease that is actively active (such as acute hepatitis, AIDS, or tuberculosis); 5) using steroids or their derivatives in the previous two years; 7) being under the age of eighteen, pregnant, or nursing.

Ethics committee permission for the study was obtained from Biruni University Ethics Committee (Ethical apporaval number: 2020/43-20). This study was conducted in full compliance with the Helsinki Declaration. After notifying all subjects about the study, formal permission forms were obtained from each.

In our previous study, we genotyped vitamin D binding protein in 51 patients (16). In this study, we added 41 more patients to the patients and performed a different statistical study and biochemical analysis of vitamin D_2 from a different perspective. In addition, the measurement differences between vitamin D_2 and D_3 levels were also revealed.

Collection of Blood Samples: Venous blood samples were taken and divided into two tubes: one for serum with a gel and the other for EDTA (Nest- UK). Blood samples collected in serum tubes at 4100 rpm and centrifuged (NF 800, Nuve, Turkey), the blood was split into two aliquots and stored at -80°C.

Metabolic Measurements of Vitamin D: 25(OH)D concentrations were evaluated using chemiluminescence microparticle immunoassay (CMIA). The Architect 25-OH Vitamin D kit (5P02, Abbott Diagnosis, USA) and i1000SR analyzer (Abbott Laboratories, USA) were used in the study. The 2011 IOM report on dietary reference intakes of 20 ng/ml was taken as the cut-off for vitamin D deficiency (17). To measure the albumin concentration of the samples, a Roche/Hitachi cobas C instrument was used in accordance with the manufacturer's instructions, which uses a colorimetric assay technique. Serum VDBP concentration was determined in accordance with the literature (18) and Quantikine kit for

monoclonal immunoassay measurement of human vitamin VDBP was performed using the manufacturer's instructions (R&D Systems, Cat No: DVDBP0, USA).

The analysis of 25(OH) D_3 and vitamin D_2 was conducted using liquid chromatography–mass spectrometry (LC-MS), employing an Agilent Infinity 1290 HPLC system (Agilent Technologies, Santa Clara, CA, USA). This system featured a binary pump (G4220A), column compartment (G1316C), and autosampler (G7167B), which were coupled to a 6470 triple quadrupole mass spectrometer (6470A, Agilent Technologies, Santa Clara, CA, USA). The process utilized a CE-in vitro diagnostic certified Jasem vitamin D LC-MS/MS analysis kit (Sem Laboratuar Cihazları Pazarlama Inc., Turkey).

For sample preparation, patient samples and serum-based calibrants/quality control materials were handled according to the kit's protocol. This procedure included a protein precipitation phase before injection into the system. The HPLC system was operated using the chromatographic parameters specified in the kit, and detection was performed with MS/MS using positive electronic spray ionization in multiple-reaction monitoring mode. For accurate measurement of analyte concentrations, the ratio of the peak area of vitamin D_2 (25(OH) D_3) to the internal standard (labeled stable isotope-d6 25(OH) D_3) was calculated. The Bikle Method, generally known as the following equation, was utilized to determine free 25(OH)D (19):

Free 25(OH)D = Total 25(OH)D/ (1 + (KALBx[ALB]) + (KDBPx[VDBP]))

Where [VDBP] is the concentration of vitamin D binding protein, [KALB] is the affinity constant for 25(OH)D with albumin, and [ALB] is the albumin concentration. Similarly, KDBP is the affinity constant for 25(OH)D_3 with vitamin D binding protein.

The authors' previously published work (16) examined VDBP gene polymorphisms using data on blood 25(OH) D_3, albumin, and VDBP concentrations of 51 people.

Statistical Analysis: A priori power analysis was conducted using G*Power 3.1.9.7 to determine the appropriate sample size for this study. The analysis was based on an expected effect size of 0.5, an alpha level of 0.05, and a statistical power of 95%. The results indicated that a minimum of 34 participants was required to achieve sufficient power. Since our study included 92 participants, we ensured adequate statistical power to detect significant effects.

The presentation of all the data was as mean ± standard deviation. Kolmogorov-Smirnov distance test was used to test the normality of all in-group variables. The numerical variables were compared to a normal distribution using an unpaired t-test with Welch correction. The correlation between 25-OH-Vit D_2 and 25-OH-Vit D_3 levels was assessed using Spearman's rank correlation coefficient due to the non-parametric nature of the data distribution. Statistical significance was defined as $P<0.05$. For all statistical analyses, GraphPad InStat ver. 3.06 (USA) was used.

RESULTS

Patient Characteristics: Ninety-two people had a mean age of 37.28 ± 15.23. Among the patients, 58.7% were female and 41.3% were male. Although there was a substantially greater 25(OH) D_3 in females compared to men, the mean concentration of total 25(OH) D_3, 25(OH)D_2, and 25(OH) D_3 as determined by LC-MS and free vitamin D did not differ significantly across genders (Table 1). Compared to males (17 ng/mL), women had mean 25(OH) D_3 concentrations of 22 ng/mL, which suggests that women had greater amounts of vitamin D_3. Men and women have similar free vitamin D levels (pg/mL), indicating that while women have greater total 25(OH) D_3, there is no discernible difference in free vitamin D levels between the sexes. Females show significantly higher levels of both 25(OH) D_3 and free vitamin D compared to males. This suggests that women in the sample have greater amounts of bioavailable (free) vitamin D in addition to having higher total vitamin D_3 levels.

Table 1. Vitamin D metabolite levels of healthy individuals according to the gender

	Total (n = 92)	Male (n = 38)	Female (54)	P value
Total 25(OH)D_3 (ng/ml)	19.45 ± 13.08	16.78 ± 8.19	21.02 ± 15.83	*0.413*
25(OH)D_2 (ng/ml)	2.95 ± 0.46	3.00 ± 0.50	2.90 ± 0.42	*0.375*
25(OH)D_3 (ng/ml)*	18.74 ± 11.85	15.98 ± 8.77	20.85 ± 13.54	*0.054*
Free vitamin D (pg/ml)	3.89 ± 2.45	3.24 ± 1.83	4.17 ± 2.56	*0.130*

*measured by LC-MS/MS

Comparison of Vitamin D Metabolites: Among the total participants, 54% had blood levels of total 25(OH) D_3 below 20 ng/mL, which led to a diagnosis of vitamin D insufficiency. Subjects with total 25(OH) D_3 levels equal to or greater than 20 ng/mL had significantly higher mean concentrations of 25(OH) D_3 (measured by LC-MS) and free vitamin D compared to those with levels

below 20 ng/mL (P < 0.001). However, there was no significant difference in the mean serum concentration of 25(OH) D_2 between the two groups, based on the criteria for vitamin D deficiency (Table 2).

Table 2. Vitamin D metabolite levels of healthy individuals compared with the total 25- Hydroxyvitamin D_3 levels

	Total 25(OH)D_3 <20ng/ml (n = 50)	Total 25(OH)D_3≥ 20ng/ml (n = 42)	P value
25(OH)D_2 (ng/ml)	2.91 ± 0.48	2.99 ± 0.44	*0.266*
25(OH)D_3 (ng/ml)*	10.63 ± 5.36	28.40 ± 10.07	***<0.001***
Free vitamin D (pg/ml)	2.29 ± 1.31	5.82 ± 2.08	***<0.001***

*measured by LC-MS/MS

Additionally, 30.4% of all participants had free vitamin D levels under 2 pg/mL. Individuals with serum free vitamin D levels of 2 pg/mL or higher showed significantly higher mean concentrations of both total 25(OH) D_3 and 25(OH) D_3, compared to those with free vitamin D levels below 2 pg/mL (P < 0.001), as determined by LC-MS. Nevertheless, no significant differences were observed in the mean serum 25(OH)D_2 levels across subjects when categorized by free vitamin D levels (Table 3).

Table 3. Vitamin D metabolite levels of healthy individuals compared with the free vitamin D levels

	Free vitamin D < 2 pg/ml (n = 28)	Free vitamin D ≥ 2 pg/ml (n = 64)	P value
Total 25(OH)D_3 (ng/ml)	7.50 ± 2.59	23.75 ± 13.0	***<0.001***
25(OH)D_2 (ng/ml)	2.93 ± 0.41	2.94 ± 0.40	*0.902*
25(OH)D_3 (ng/ml)*	6.18 ± 2.88	23.41 ± 10.80	***<0.001***

*measured by LC-MS/MS

The scatter plot analysis demonstrates the relationship between serum 25-OH-Vit D_2 and 25-OH-Vit D_3 levels. The Spearman correlation coefficient (r= 0,03062) was calculated as (p= 0,7695), indicating a weak correlation between the two variables (Figure 1). The trendline with confidence intervals suggests that higher levels of 25-OH-Vit D_3 are not strongly associated with variations in 25-OH-Vit D_2 levels.

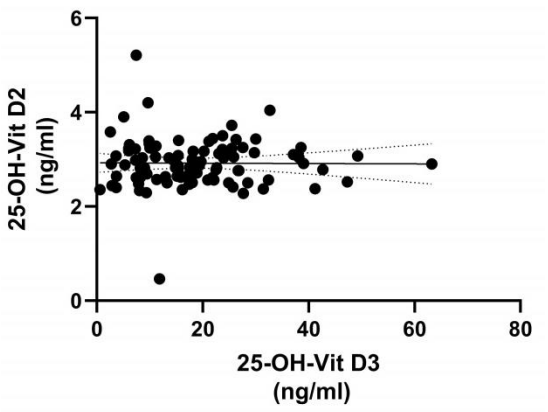

Figure 1. Scatter plot showing the correlation between serum 25-hydroxyvitamin D_2 (25-OH-Vit D_2) and 25-hydroxyvitamin D3 (25-OH-Vit D_3) levels. Each dot represents an individual patient sample. The correlation was assessed using Spearman's rank correlation coefficient (r = 0,03062, p = 0,7695). The dotted lines indicate the confidence interval of the regression trendline.

DISCUSSION

Serum total 25(OH)D is a widely used biomarker to assess vitamin D reserves and estimate human vitamin D status as it detects both forms of 25(OH)D_2 and 25(OH) D_3 (16). We looked into the relationship between the levels of 25(OH)D_2 and of free vitamin D and vitamin D insufficiency in a healthy Turkish population since the type of vitamin D (D_2 or D_3) might impact the dosage response of 25(OH) D_3 to vitamin D_3. The mean concentration of 25(OH) D_2 in serum did not differ among individuals according to Vitamin D deficiency criteria and to concentration of free vitamin D, and therefore does not directly indicate vitamin D deficiency. However, the mean concentration of 25(OH) D_3 measured by LC-MS and free vitamin D affects the total 25(OH) D_3 concentration measured by automated systems and may indicate vitamin D deficiency. These findings may point to gender-specific variations in vitamin

D exposure or metabolism, such as variations in vitamin D binding proteins or increased sun exposure (Table 1).

Over the previous 20 years, a number of sensitive and focused commercial tests have been created (16). The variations in the cross-reactivity of antibodies with epimers and/or metabolites, as well as the processes of vitamin D extraction, deproteinization, and purification, account for the variations among these tests. Even with these technological advancements, measuring 25(OH) D_3 concentration precisely and accurately remains difficult due to the presence of various hydrophobic vitamin D metabolites and fluctuating ratios of 25(OH) D_2 to 25(OH) D_3 in the bloodstream. These metabolites also have low free quantities in serum due to their ability to bind to lipids, albumins, and vitamin D binding protein (VDBP). Therefore, the challenges are attributable to the accuracy and sensitivity of assays which might result in discrepancies across different testing methodologies. Of these methods, automated immunoassays account for 90% of routine 25(OH)D testing because of their low manual labor requirements, high throughput, and automated sample handling (20). All immunoassays should assess D_2 and D_3 metabolites similarly (with equimolar reactivity), although detection of 25(OH) D_2 and 25(OH) D_3 largely depends on the antibody specificity. Immunoassays that are capable of detecting 25(OH) D_2 are unable to distinguish it from 25(OH) D_3 (21). Because it is significantly more sensitive than automated methods but also more costly, isotope-dilution LC-MS/MS is now the gold standard for 25(OH) D_3 testing (22). In the current investigation, we additionally assessed 25(OH) D_3 using LC-MS/MS and compared the results with the overall levels of 25(OH) D_3 determined by automated CMIA. The results demonstrate that there was consistency in the 25(OH) D_3 levels across the two techniques of assessment.

Several studies have demonstrated that vitamin D_2 in equimolar doses is less effective at increasing blood 25(OH) D_3 levels compared to vitamin D_3 (23-25). Additionally, research suggests that vitamin D_3 metabolites exhibit a stronger affinity for VDBP and interact differently with the vitamin D receptor compared to vitamin D_2 metabolites. Furthermore, the 25-hydroxylation rate of vitamin D_3 is higher than that of vitamin D_2 (26). Although the majority of commercially available vitamin D supplements are in the D_3 form, vitamin D_2 is still present in certain dietary sources and fortified foods. As a result, individuals may have varying contributions of 25(OH)D_2 to their total vitamin D levels, depending on their dietary intake and metabolism. Furthermore, evaluating both 25(OH)D_3 and 25(OH)D_2 provides a more comprehensive understanding of vitamin D metabolism. Previous studies have primarily focused on total 25(OH)D_3 levels, overlooking the potential role of 25(OH)D_2 in maintaining overall vitamin D status (27-29). Given these considerations, this study aims to explore the presence and significance of 25(OH)D_2 in healthy individuals, contributing to a more detailed assessment of vitamin D homeostasis across different populations. One possible reason for the higher production of 25(OH)D_3 over 25(OH)D_2 could be the increased hydroxylation efficiency of vitamin D_3. In our current study, 25(OH)D_3, rather than 25(OH) D_2, seems to be the primary contributor to overall 25(OH)D_3 levels. A possible explanation for the difference between 25(OH)D_2 and 25(OH)D_3 may be the lower affinity of vitamin D_2 metabolites for VDBP, resulting in a shorter half-life and quicker clearance from the bloodstream (30). Additionally, it has been shown that 1,25$(OH)_2D_2$ undergoes a more rapid inactivation phase during 24-hydroxylation, whereas 1,25,24$(OH)_3D_3$ requires further steps for deactivation. These findings suggest that 25(OH)D_3 remains physiologically active for a longer period, thereby playing a direct role in sustaining vitamin D levels (3). Research by Shieh et al. comparing high doses of vitamin D_2 and D_3 found that vitamin D_3 increased both total and free 25(OH)D levels more effectively than vitamin D_2 (13). Our results, based on 25(OH)D_3 levels measured by LC-MS and free vitamin D levels calculated using the Bikle method, align with the literature and point to vitamin D deficiency, rather than low 25(OH)D_2 levels, as the likely issue. Moreover, 25(OH)D_3 and free vitamin D appear to be better markers of vitamin D bioactivity than 25(OH)D_2, making them the more reliable indicators of physiological vitamin D sufficiency. In our Turkish research population, we also discovered that females had greater levels of 25(OH)D_3 than males. Similar results have also been noted in Indian (31), and Norwegian (32) sample populations, but not in Saudi (33) populations, despite the anthropometric, ethnic, and geographic disparities in the populations. These findings may reflect physiological differences between males and females, such as variations in PTH levels, or cultural factors, like differences in sun exposure, or possibly a combination of both. Previous studies have indicated that variations in total 25(OH)D levels and VDBP gene polymorphisms may account for the differences in free and bioavailable 25(OH)D levels among healthy Turkish individuals. This points to the significant role of genetic factors in influencing vitamin D metabolite levels (16). 25(OH)D_3, 25(OH)D_2 and free vitamin D levels were compared between vitamin D deficient and non-deficient groups. It is generally observed that the deficient group exhibited significantly lower 25(OH)D_3 levels and lower free vitamin D levels, possibly in combination with 25(OH)D_2 (Table 2). Conversely, the non-deficient group presents higher levels of

these metabolites, particularly 25(OH)D_3, reflecting normal vitamin D status. This underscores the role of vitamin D in maintaining overall health and suggests that 25(OH)D_3 is the key form linked to sufficient vitamin D levels.

Our study has some limitations. First, the sample size was relatively small. Second, although 50 participants had total 25(OH)D_3 levels below 20 ng/mL, indicating a considerable proportion of subjects with lower total 25D levels, the sample may not fully represent broader trends. Third, this study did not include a follow-up after vitamin D supplementation, which could have provided additional insights into distinguishing vitamin D deficiency. Additionally, the healthy participants were not severely deficient in vitamin D.

CONCLUSION

Despite these limitations, the study provides valuable insights into comparing free vitamin D and 25(OH)D_2 levels with 25(OH) D_3 levels in healthy Turkish individuals. Our findings indicate that 25(OH)D_3 and free vitamin D play a more significant role in determining vitamin D metabolite levels than 25(OH)D_2. Further research is necessary to explore whether variations in vitamin D metabolite levels are linked to specific vitamin D dosages and supplements, and to better understand the influence of dietary, sex, ethnic, and endocrine factors—such as PTH—on these physiological interactions.

Statement of Ethics: The study was approved by Biruni University Non-Interventional Research Ethics Committee (Approval No: 2020/43-20). Written informed consent was obtained from all participants.

Conflict of Interest Statement: The authors declare that there was no conflict of interest.

Funding Sources: This research received no external funding.

Data Availability Statement: The data of serum 25(OH)D_3, albumin and VDBP concentrations of 51 individuals were used for a previously published study of the authors in which VDBP gene polymorphisms were investigated [19].

REFERENCES

1. Wang S. Epidemiology of vitamin D in health and disease. Nutr Res Rev. 2009;2(22):188-203
2. Zelzer S, Prüller F, Curcic P, Sloup Z, Holter M, Herrmann M, et al. Vitamin D Metabolites and Clinical Outcome in Hospitalized COVID-19 Patients. Nutrients. 2021;7(13):
3. Ramasamy I. Vitamin D Metabolism and Guidelines for Vitamin D Supplementation. Clin Biochem Rev. 2020;3(41):103-26
4. Nikolac Gabaj N, Unic A, Miler M, Pavicic T, Culej J, Bolanca I, et al. In sickness and in health: pivotal role of vitamin D. Biochem Med (Zagreb). 2020;2(30):020501
5. Al-Thagfan, S S, Alolayan SO, Ahmed S, Emara M M, Awadallah MF, et al. Impacts of deficiency in vitamin D derivatives on disease severity in adult bronchial asthma patients. Pulm Pharmacol Ther. 2021;70):102073
6. Jenkinson C. The vitamin D metabolome: An update on analysis and function. Cell Biochem Funct. 2019;6(37):408-23
7. Bikle D. Vitamin D: production, metabolism and mechanisms of action. Europe PMC; 2015.
8. Haussler MR., Whitfield GK, Kaneko I, Haussle CA, Hsieh D, Hsieh JC, t al. Molecular mechanisms of vitamin D action. Calcif Tissue Int. 2013;2(92):77-98
9. Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr. 2020;11(74):1498-513
10. Glendenning P, Chew GT, Inderjeeth CA, Taranto M, Fraser WD, et al. Calculated free and bioavailable vitamin D metabolite concentrations in vitamin D-deficient hip fracture patients after supplementation with cholecalciferol and ergocalciferol. Bone. 2013;2(56):271-5
11. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney, R. P., ... & Weaver, C. M. (et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;7(96):1911-30
12. Holick MF, Biancuzzo RM, Chen TC, Klein EK., Young A, Bibuld D, et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab. 2008;3(93):677-81
13. Shieh A, Chun RF, Ma C, Witzel S, Meyer B, Rafison B, et al. Effects of High-Dose Vitamin D2 Versus D3 on Total and Free 25-Hydroxyvitamin D and Markers of Calcium Balance. J Clin Endocrinol Metab. 2016;8(101):3070-8
14. Romagnoli E, Mascia ML, Cipriani C, Fassino V, Mazzei F, D'Erasmo E., ... et al. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. J Clin Endocrinol Metab. 2008;8(93):3015-20
15. Vieth R, Chan PC, MacFarlane GD. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. Am J Clin Nutr. 2001;2(73):288-94
16. Karcıoğlu Batur L, Özaydın A, Maviş ME, Gürsu GG, Harbige L, Hekim N.. Vitamin-D Binding Protein Gene Polymorphisms and Serum 25-Hydroxyvitamin-D in a Turkish Population. Metabolites. 2021;10(11):

17. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;1(96):53-8
18. A M Mondul, S J Weinstein, J Virtamo & D Albanes . Influence of vitamin D binding protein on the association between circulating vitamin D and risk of bladder cancer. Br J Cancer. 2012;9(107):1589-94
19. Powe CE, Ricciardi C, Berg AH, Erdenesanaa D, Collerone G, Ankers E, et al. Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship. J Bone Miner Res. 2011;7(26):1609-16
20. Galior K, Ketha H, Grebe S, Singh RJ, et al. 10 years of 25-hydroxyvitamin-D testing by LC-MS/MS-trends in vitamin-D deficiency and sufficiency. Bone Rep. 2018;8):268-73
21. Ferrari D, Lombardi G, Banfi G,. Concerning the vitamin D reference range: pre-analytical and analytical variability of vitamin D measurement. Biochem Med (Zagreb). 2017;3(27):030501
22. Stepman HC, Vanderroost A, Van Uytfanghe K, & Thienpont LM, et al. Candidate reference measurement procedures for serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 by using isotope-dilution liquid chromatography-tandem mass spectrometry. Clin Chem. 2011;3(57):441-8
23. Tripkovic L, Wilson LR, Hart K, Johnsen S, De Lusignan S, Smith CP, et al. Daily supplementation with 15 mug vitamin D(2) compared with vitamin D(3) to increase wintertime 25-hydroxyvitamin D status in healthy South Asian and white European women: a 12-wk randomized, placebo-controlled food-fortification trial. Am J Clin Nutr. 2017;2(106):481-90
24. Tripkovic L, Lambert H, Hart K, Smith CP, Bucca G, Penson S, . et al. Comparison of vitamin D2 and vitamin D3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis. Am J Clin Nutr. 2012;6(95):1357-64
25. Heaney RP, Recker RR, Grote J, Horst RL, Armas L, A et al. Vitamin D(3) is more potent than vitamin D(2) in humans. J Clin Endocrinol Metab. 2011;3(96):E447-52
26. Horst R, Prapong S, Reinhardt T, Koszewski N, Knutson J, Bishop C.Comparison of the relative effects of 1,24-dihydroxyvitamin D(2) [1, 24-(OH)(2)D(2)], 1,24-dihydroxyvitamin D(3) [1,24-(OH)(2)D(3)], and 1,25-dihydroxyvitamin D(3) [1,25-(OH)(2)D(3)] on selected vitamin D-regulated events in the rat. Biochem Pharmacol. 2000;5(60):701-8
27. Zhu T, Zhao J, Zhuo S, Hu Z, Ouyang S, Wunier Y,. et al. High Fat Diet and High Cholesterol Diet Reduce Hepatic Vitamin D-25-Hydroxylase Expression and Serum 25-Hydroxyvitamin D3 Level through Elevating Circulating Cholesterol, Glucose, and Insulin Levels. Mol Nutr Food Res. 2021;65(21):2100220.
28. Hanel A, Veldhuizen C, Carlberg C,. Gene-regulatory potential of 25-hydroxyvitamin D3 and D2. Front Nutr. 2022;9:910601.
29. Hasan M, Oster M, Reyer H, Wimmers K, Fischer DC,. Efficacy of dietary vitamin D3 and 25 (OH) D3 on reproductive capacities, growth performance, immunity and bone development in pigs. Br J Nutr. 2023;130(8):1298-1307.
30. Houghton LA, Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr. 2006;4(84):694-7
31. Sanghera DK, Sapkota BR, Aston CE, Blackett PR. Vitamin D Status, Gender Differences, and Cardiometabolic Health Disparities. Ann Nutr Metab. 2017;2(70):79-87
32. Johnson LK, Hofsø D, Aasheim ET, Tanbo T, Holven KB, Andersen LF, et al. Impact of gender on vitamin D deficiency in morbidly obese patients: a cross-sectional study. Eur J Clin Nutr. 2012;1(66):83-90
33. Abudawood M, Tabassum H, Ansar S, Almosa K, Sobki S, Ali MN, et al. Assessment of gender-related differences in vitamin D levels and cardiovascular risk factors in Saudi patients with type 2 diabetes mellitus. Saudi J Biol Sci. 2018;1(25):31-36.

RESEARCH ARTICLE

Emel Akbas[1]
Nagihan Ege[2]
Dursun Atik[1]
Emel Caliskan[1]
Sukru Oksuz[1]
Idris Sahin[1]
Elif Cihadiye Ozturk[3]

[1] Duzce Üniversity, Medical Faculty, Deparment of Medical Microbiology, Duzce, Türkiye
[2] Duzce Ataturk State Hospital, Deparment of Medical Microbiology, Duzce, Türkiye
[3] Arel Üniversity, Medical Faculty, Deparment of Medical Microbiology, Istanbul, Türkiye

Corresponding Author:
Sukru Oksuz
mail: droksuz@gmail.com

Received: 30.09.2024
Acceptance: 23.03.2025
DOI:10.18521/ktd.1558505

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Investigation of Isoniazid, Rifampicin and Second Generation Antibiotic Resistance Genes in Rifampicin-Resistant *Mycobacterium tuberculosis* Complex Strains Isolated at Düzce University Between 2004-2021

ABSTRACT

Objective: To determine the gene patterns causing antibiotic resistance in *M. tuberculosis* complex (MTBC) strains using molecular methods.

Methods: Nineteen rifampicin-resistant MTBC strains isolated between 2004 and 2021 were included. The species of these strains with MTBC genotype and the gene pattern causing rifampicin resistance with MTBDR plus genotype were analysed.

Results: Nineteen of the isolates were identified as *M. tuberculosis*/*canetti* by the MTBC genotype method. Seven of these isolates were genotypically resistant to rifampicin. One of the resistant isolates had deletion in WT8 and WT6 bands, one had deletion in WT8 band, one had deletion in WT7 band and rpoBMUT2A mutation, and four had deletion in WT8 band and rpoBMUT3 mutation. Seven of the resistant isolates were genotypically INH resistant. Five of them had katGMUT1 mutation with deletion in katGWT band and two of them had only INH AMUT3B mutation. Of the 10 multidrug-resistant MTBC isolates, nine were genotypically resistant to none of the second-generation drugs using the GenoType MTBDR sl ver 2.0 method. However, one isolate could not be evaluated with this assay.

Conclusions: The presence of MDR-TB and RR-TB is an important challenge especially in TB control, which increases the need for molecular methods. Although it still has not replaced culture, there is a need for the use and development of new molecular methods that will benefit us in TB treatment and control.

Keywords: Drug Resistance, *Mycobacterium tuberculosis* Complex, Multi-Drug Resistance Tuberculosis.

Düzce Üniversitesi'nde 2004-2021 Yılları Arasında İzole Edilen Rifampisin Dirençli *Mycobacterium tuberculosis* Kompleks Suşlarında İzoniazid, Rifampisin ve İkinci Nesil Antibiyotik Direnç Genlerinin Araştırılması

ÖZET

Amaç: *M. tuberculosis* complex (MTBC) suşlarında antibiyotik direncine neden olan gen paternlerini moleküler yöntemlerle belirlemek.

Yöntem: 2004-2021 yılları arasında izole edilen rifampisine dirençli on dokuz MTBC suşu çalışmaya dahil edilmiştir. Bu suşların MTBC genotipine sahip türleri ve MTBDRplus genotipi ile rifampisin direncine neden olan gen paterni analiz edildi.

Bulgular: İzolatların on dokuzu MTBC genotip yöntemi ile M. tuberculosis/canetti olarak tanımlanmıştır. Bunlardan yedisi genotipik olarak rifampisine dirençliydi. Dirençli izolatlardan birinde WT8 ve WT6 bantlarında delesyon, birinde WT8 bandında delesyon, birinde WT7 bandında delesyon ve rpoBMUT2A mutasyonu ve dördünde WT8 bandında delesyon ve rpoBMUT3 mutasyonu vardı. Dirençli izolatların yedisi genotipik olarak INH dirençliydi. Bunların beşinde katGWT bandında delesyon ile katGMUT1 mutasyonu ve ikisinde sadece inh AMUT3B mutasyonu. Çok ilaca dirençli 10 MTBC izolatından dokuzu GenoType MTBDR sl ver 2.0 yöntemi kullanılarak ikinci nesil ilaçların hiçbirine genotipik olarak dirençli bulunmamıştır. Bir izolat bu test ile değerlendirilememiştir.

Sonuç: ÇİD-TB ve RR-TB varlığı özellikle TB kontrolünde önemli bir zorluktur ve bu durum moleküler yöntemlere olan ihtiyacı artırmaktadır. Halen kültürün yerini almamış olsa da, TB tedavisi ve kontrolünde bize fayda sağlayacak yeni moleküler yöntemlerin kullanılmasına ve geliştirilmesine ihtiyaç vardır.

Anahtar Kelimeler: İlaç Direnci, *Mycobacterium tuberculosis* Kompleksi, Çoklu İlaca Dirençli Tüberküloz

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* complex (MTBC), is the most common infectious disease causing death with an annual mortality rate of 1.5 million deaths worldwide. Although the global incidence of TB has been on a downward trend since 2000, the emergence and spread of drug-resistant TB strains has significantly impacted efforts to control and eradicate the disease (1). The World Health Organisation has reported an estimated half a million cases of MDR-TB in recent years, of which 8.5% were MDR-TB (2). Currently, the advent of multidrug resistance (MDR) is a major burden on the global tuberculosis control program. The spread of MTB-MDR is rising throughout the world in both fresh tuberculosis cases and patients cured of MTB with the commonly used drugs (RIF and INH). RIF-resistance (≥90% cases) in MTB-infected patients have been classified as a main biomarker for drug resistance detection (3).

Early detection of MTBC isolates and accurate drug susceptibility testing (DST) are vital to prevent transmission of MDR-TB strains. Due to the long duration of DST with culture-based methods, various probe-based and sequence analysis-based molecular methods have been developed today to provide results in a short time and with high accuracy and to detect the mutation associated with resistance. Probe-based methods include GeneXpert MTB/RIF test (Cepheid, USA) using molecular probes and MTBDRplus and MTBDR sl (Hain LifeScience GmbH, Germany) using line probes (1). Sequence analysis based methods include Sanger sequencing, pyrosequencing and next generation sequencing. Sequence analysis-based methods are used to obtain the sequences of wild-type isolates or mutants, while probe-based methods are used to detect the presence of mutations (4).

The GenoType MTBDRplus test, based on PCR-based reverse hybridisation, is one of the most widely used commercial molecular tests (5). While this test detects MTBC species and RIF and INH resistance, the GenoType MTBDR sl test can detect resistance to secondary drugs as well as MTBC.

The *Mycobacterium tuberculosis* complex is a group of Mycobacteria that comprises of *M. tuberculosis, M. africanum, M. bovis, M. caprae, M. canettii, M. africanum, M. microti, M. pinnipedii* and *M. caprae*. Additionally, two novel species (*M. oris* and *M. mungi*) are also referred to as MTBC (6). GenoType MTBC test (Hain LifeScience GmbH, Germany) is a molecular method based on reverse hybridisation for typing MTBC members. It is the oldest and probably the most widely used molecular test to differentiate the causative agents of tuberculosis (7).

MATERIAL AND METHODS

This study was carried out with the approval of Düzce University Faculty of Medicine, Non-Interventional Health Research Ethics Committee with the decision dated 18.10.2021.

In our study, resistant strains selected among 854 MTBC isolates isolated from various clinics between 2004 and 2021 at the Tuberculosis Unit of the Medical Microbiology Laboratory of Düzce University Health Application and Research Centre were included.

Culture and species identification of the isolates were performed according to standard mycobacteriological procedures. Anti-TB drug susceptibilities of the isolates were performed on a BACTEC MGIT 320 (Becton Dickinson, USA) according to the standard procedure recommended by the manufacturer. Drug concentrations include streptomycin (SM) 1.0 μg/mL, INH 0.1 μg/mL, RIF 1.0 μg/mL and 5.0 μg/mL for EMB. All strains were sub-cultured in skimmed milk stock medium and stored at -20 °C until molecular studies were performed. Positive MGIT tubes with simultaneous growth were stored at +4 ºC (1,4-5).

In order to revitalise the strains before the commencement of the study, LJ medium was inoculated from the stored positive MGIT bottles and stock media and incubated at 37 ºC. Equivalent MGIT liquid medium was inoculated simultaneously and placed in the BACTEC MGIT 320 device. Weekly growth controls were performed. DNA isolation for PCR was performed for the isolates in which growth was observed (1,4).

MTBC subspecies determination was performed by GenoType MTBC (Hain LifeScience GmbH, Germany) test and rpoB gene mutation for RIF resistance, katG gene for high level INH resistance, promoter region of inhA gene for low level INH resistance were analysed by GenoType MTBDRplus (Hain LifeScience GmbH, Germany) test. In addition, the molecular resistance pattern against second-line fluoroquinolones (FLQ) (gyrA and gyrB genes) and second-line injectable drugs (SLID) (rrs and eis genes) was investigated in 10 MDR-TB isolates by GenoType MTBDR sl VER 2.0 (Hain LifeScience GmbH, Germany) (7).

Statistical Analyses: IBM SPSS 22.0 package programme was used for statistical analysis of the data. The relationships between categorical variables were analysed using Chi-square and Fisher's Exact tests. $p<0.05$ was considered statistically significant.

RESULTS

Of the 854 MTBC strains phenotypically tested for antibiotic susceptibility, 24 (2.8%) were found to be RIF resistant. Of these strains, 14 (1.6%) were determined to be MDR-TB with combined RIF+INH resistance. Three of the 24 rifampicin-resistant MTBC strains could not be resuscitated by passages and 21 of them were included in the study.

Of the 21 patients with phenotypic rifampicin resistance, 7 (33.3%) were female and

14 (66.7%) were male with a mean age of 45.0±17.1 years (Table 1).

Table 1. Demographic characteristics of patients from whom rifampicin-resistant MTBC strains were isolated

Cinsiyet	n	%
Female	7	33.3
Male	14	66,7
Total	**21**	**100**

When the distribution of the isolates included in the study was analysed according to the years, MDR-TB was not detected in eight of the eighteen years. Of the MDR-TB isolates, 2 (9.5%) each were isolated in 2007, 2010, 2011, 2012, 2014, 2021; 4 (19%) in 2009 and 5 (23.8%) in 2016. Although there was no statistical difference between the years, the highest number of resistant strains was found in 2016 (p=0.066) (Figure 1).

Twelve (57.1%) of the rifampicin-resistant MTBC isolates were positive by EZN staining, while nine (42.9%) were negative by EZN staining (Figure 2).

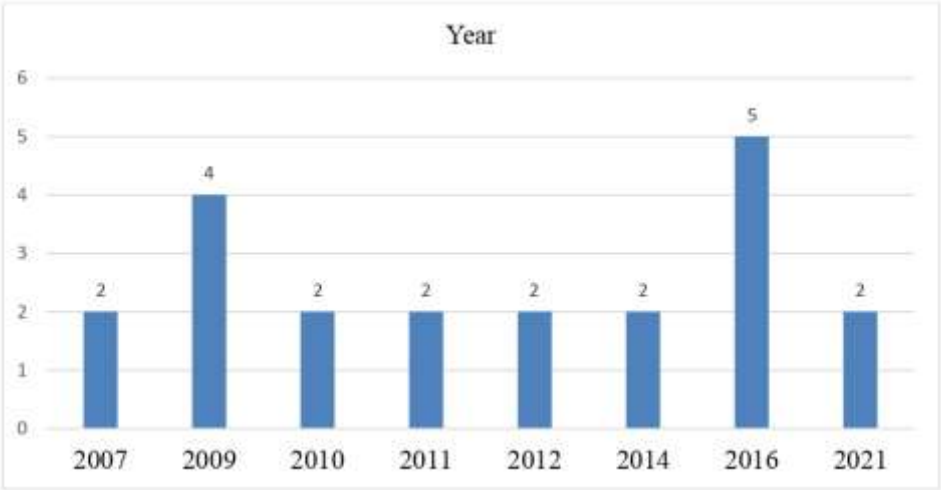

Figure 1. Distribution of rifampicin-resistant MTBC strains according to years.

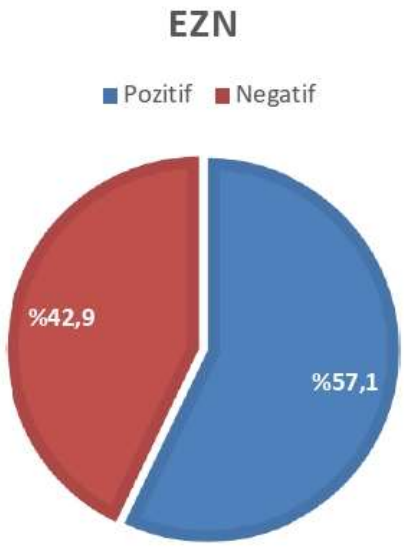

Figure 2. EZN positivity of rifampicin-resistant MTBC strains.

f the 21 rifampicin-resistant MTBC isolates, nine (42.9%) were resistant to streptomycin (SM) and seven (33.3%) were resistant to ethambutol (EMB). While nine (42.9%) of the isolates were resistant to RIF alone (RR-TB), 12 (57.1%) were MDR-TB with RIF+INH co-resistance. There was no significant difference between RIF and INH co-resistance and the others (p=0,482). (Table 2).

Table 2. Drug susceptibility results of rifampicin resistant MTBC strains by MGIT SIRE method.

Anti-TB Drug (n=21)	Resistance		p
	n	%	
RIF+SM *	9	42.9	p=0.482
RIF+EMB**	7	33.3	
RIF only	9	42.9	
RIF+INH	12	57.1	

*RIF+SM: Rifampicin +Sreptomycin,
**RIF+EMB: Rifampicin+ Ethambutol

Genotypic drug susceptibilities of phenotypically RIF 21 resistant MTBC isolates were investigated by GenoType MTBDRplus method. However, two isolates were excluded from the study because they did not meet the evaluation criteria of this test (Figure 3).

When the genotypic resistance status of 19 isolates detected with the Genotype MTBDRplus kit was evaluated, genotypic RIF resistance was detected in seven of the isolates (36.8%). In four of these resistant isolates (57.1%), deletion in the WT8 region and rpoBMUT3 mutation were found together. In the other isolates, one (14.3%) had deletion in WT8 and WT6 bands and one (14.3%) had deletion in WT8 band. In one (14.3%) isolate, deletion in WT7 band and rpoB MUT2A mutation were observed together.

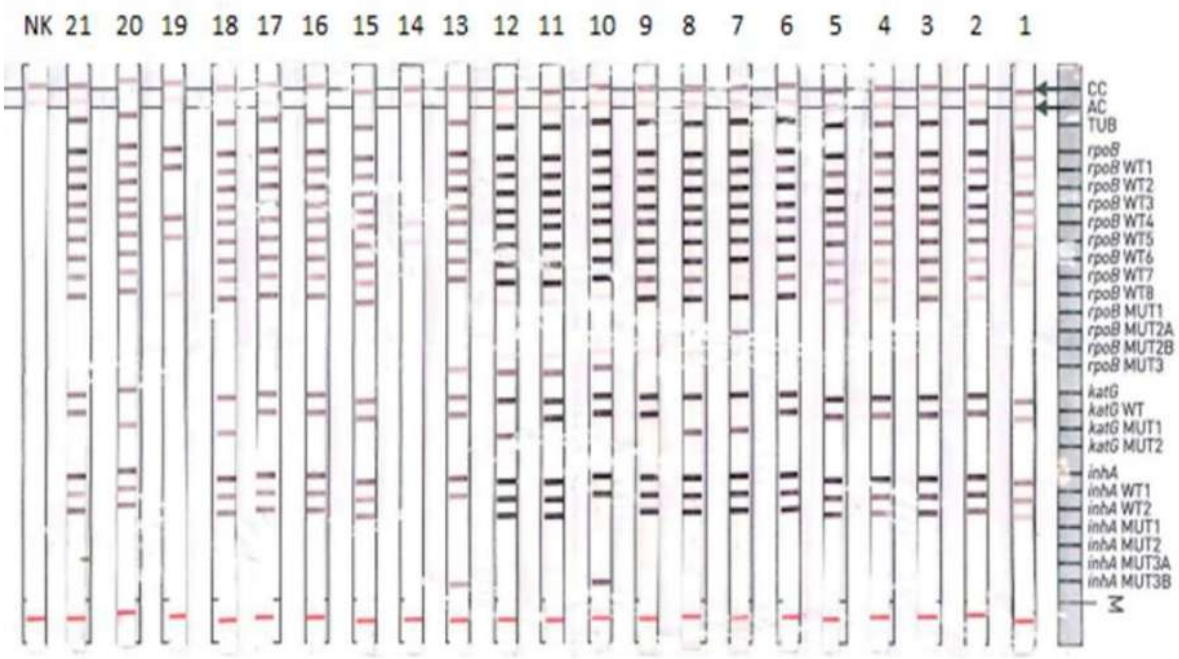

Figure 3. GenoType MTBDR plus test results

Seven (36.8%) of the 19 isolates were genotypically resistant to INH by Genotype MTBDRplus kit, while 12 (63.2%) were susceptible. Five (71.4%) of the resistant patients had katG WT band deletion and katGMUT1 mutation together and two (28.6%) had inhA MUT3B mutation (Table 3).

Table 3. Genotype MTBDRplus test results

MTBDR drug name/Resistance gene-mutation site		**Number**	**%**
Genotype MTBDRplus-RIF	Resistant	7	36.8
	Sensitive	12	63.2
Pozitive WT band	WT8/WT6	1	14.3
	WT8	1	14.3
	WT7/ rpoBMUT2A	1	14.3
	WT8/ rpoBMUT3	4	57.1
Genotype MTBDRplus-INH	Resistant	7	36.8
	Sensitive	12	63.2
Pozitive WT band	katGWT/ katGMUT1	5	71.4
	ınhAMUT3B	2	28.6

Eight of the 19 isolates detected with the Genotype MTBDRplus kit were identified as RR-TB and 11 as MDR-TB. Genotypic RIF resistance was detected in 25% (2/8) of RR-TB isolates with Genotype MTBDRplus kit. Moreover, RIF resistance was genotypically detected in 45.5% (5/11) of MDR-TB isolates by Genotype MTBDRplus kit.

While 12.5% (1/8) of RR-TB isolates were phenotypically INH resistant, genotypically INH resistance was detected with GenoType MTBDRplus kit. Genotypic INH resistance was observed in 54.5% (6/11) of MDR-TB isolates. No significant difference was observed between the presence of RIF and INH resistance in terms of test results ($p>0.05$) (Table 4).

Nine of the 10 MDR-TB isolates evaluated were genotypically resistant to none of the second generation drugs by Genotype MTBDR sl ver 2.0 method. One of these isolates could not be interpreted because it did not meet the evaluation criteria of this test (Figure 4).

Table 4. Comparison of genotypic RIF and INH resistance in RR-TB and MDR-TB isolates

Genotypic Susceptibility Profiles		RR-TB		MDR-TB		p*
		n	%	n	%	
Genotype MTBDRplus-RIF	Resistant	2	25	5	45.5	0.633
	Sensitive	6	75	6	54.5	
Pozitive WT band	WT8/WT6	-	-	1	20	0.714
	WT8	1	50.0	0	0	
	WT7/rpoBMUT2A	0	-	1	20	
	WT8/ rpoBMUT3	1	50	3	60	
Genotype MTBDRplus-INH	Resistant	1	12.5	6	54.5	0.147
	Sensitive	7	87.5	5	45.5	
Pozitive WT band	katGWT/katGMUT1	1	100	4	66.7	0.495
	ınhAMUT3B	-	-	2	33.3	

*Chi-square analysis was performed

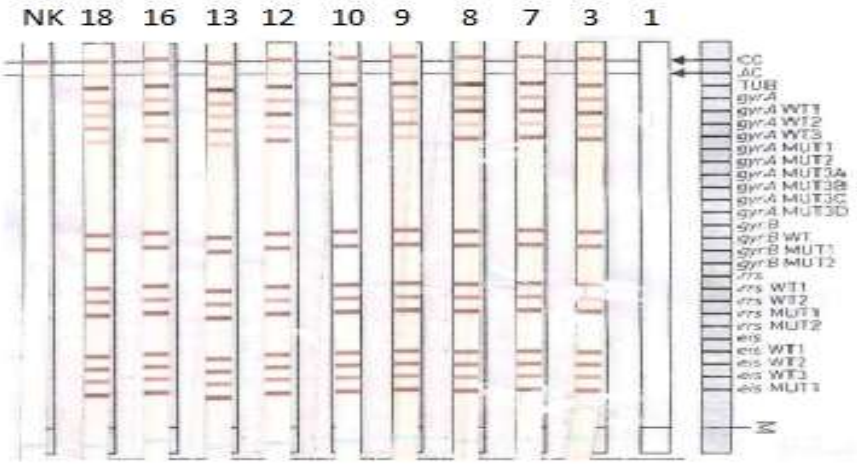

Figure 4. Genotype MTBDR sl ver 2.0 test results.

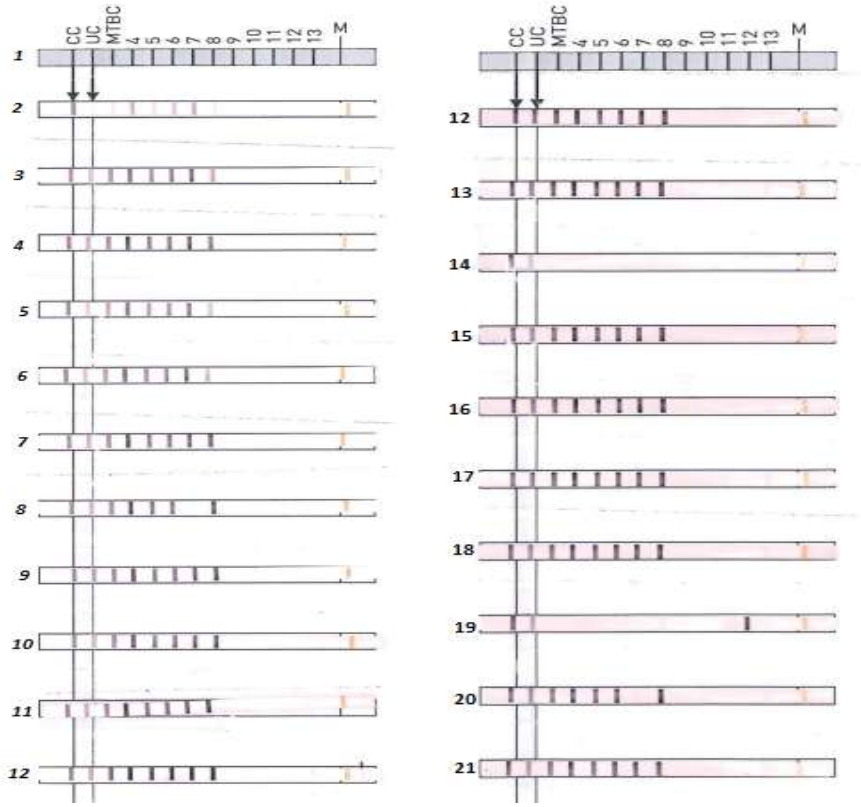

Figure 5. GenoType MTBC test results

When 21 rifampicin-resistant isolates were evaluated by Genotype MTBC method, 19 (90.4%) were found to be *M. tuberculosis*/*canetti*. However, two of them could not be evaluated because they did not meet the interpretation criteria of this test (Figure 5).

DISCUSSION

According to the WHO data, it is estimated that approximately 440,000 RR-TB/MDR-TB cases and 25,000 MDR-TB cases occur annually and 150,000 MDR-TB cases die each year (8). Similarly, the Turkish Tuberculosis Control Report stated that the rate of MDR-TB was 2.6% in new cases and 9.9% in previously treated cases. In the same report, eight (4.5%) of 176 MDR-TB cases in 2018 were identified as MDR-TB cases (9). In a study conducted by Kumar et al. (10) including 164 MTBC isolates, 13.4% of the strains were found to have MDR-TB. Similarly, Yazıcı et al.(11) at Akdeniz University reported that 68 (6.9%) of 974 MTBC isolates were MDR-TB. In a study conducted by Yılmaz et al.(12) in Erzurum, where 419 MTBC isolates were evaluated, the rate of MDR-TB was found to be 3.6% and their results were within the average of Turkey. In our study, 14 (1.6%) of 854 MTBC isolates were found to have MDR-TB. It was observed that the rate of MDR-TB was lower in our region compared to the data in the world and in our country.

Tuberculosis is generally more common in males (8). Liu et al.(13) analysed 139 MDR-TB cases in China and found that 99 (71.4%) were male, 40 (28.6%) were female and the mean age was 51 years. Soeroto et al.(14) analysed 492 cases in a study conducted in Indonesia, where the prevalence of MDR-TB is high, and found that MDR-TB was more common in patients aged <45 years. In a study conducted by Apoorva et al.(15), which included 452 MTBC specimens, 283 of the patients were male (62.3%) and 169 were female (37.2%) and 42.1% were in the 40-59 age group. Of the 12 MDR-TB strains detected in our study, four (33.3%) were female and eight (66.7%) were male patients and the mean age was 43 years. When the data we determined and the literature were evaluated together, it was determined that the age of MDR-TB incidence in our study was similar to the literature and it was more common in males.

Rifampicin is an important first-line anti-TB drug. RIF resistance is an important factor in determining the treatment regimen and prognosis of TB. Therefore, more attention has been paid to the mechanisms of rifampicin resistance (16). Mutations related to RIF resistance are mostly located in the rifampicin resistance determining region (RRDR) of the rpoB gene. This makes RIF more advantageous than other drugs in the application of genotypic-based drug susceptibility testing. Mutations related to RIF resistance are mostly mutations in codons 516, 526 and 531 of the rpoB gene. In a multicentre study conducted by Campbell et al.(17) with 314 clinical samples, mutations in the rpoB gene region were detected in 97% of 174 strains known to be phenotypically RIF resistant. The detected mutations were found between codons 507-533 defined as the RRDR region (17). In a study conducted by Javed et al.(18) on 53 MDR-TB strains in Pakistan using Genotype MTBDRplus test, 42 (79.2%) isolates were genotypically RIF resistant. Among these, the most common mutation was found to be S531L pattern with rpoMUT3 mutation in 34 (64.1%) isolates. Of these 34 isolates, 32 (60.3%) had deletion in the WT8 band, one (1.8%) had deletion in the WT3/WT4 bands and one (1.8%) had deletion in all WT bands. Additionally, five (9.4%) of the other isolates showed rpoMUT1 mutation with WT3/WT4 band deletion. WT7 band deletion was found in two (3.7%) isolates. One (1.8%) isolate showed different mutation patterns with deletion in WT5/WT6 band. In the study of Kumar et al. (19) involving 442 RR-TB isolates, MTBDRplus rifampicin resistance was found to be highest in the combination of both WT8 and MUT3 with 60.6%, and this was shown to include the 530-533 codon. Sağlam et al.(20) at Uludağ University reported 11 (84.6%) of 13 phenotypically RIF resistant strains had mutations in the rpoB gene. They further highlighted that three of these isolates had WT5 band deletion in the rpoB gene region and two of these three isolates had S531L gene pattern with both WT5 band deletion and rpoB MUT3 mutation, while the other three isolates had rpoB WT2 band deletion.

In our study, seven (36.8%) of 19 phenotypically RIF resistant isolates were genotypically RIF resistant by Genotype MTBDRplus test. Deletion in the WT8 band region was detected in six (85.7%) of these isolates. Four of these isolates (57.1%) had a mutation in the rpoB MUT3 gene region and were found to have the S531L gene pattern at codon 530-533. In one (14.2%) isolate, deletion of the WT6 band was observed together with WT8. In one (14.2%) of the seven isolates in which RIF resistance was genotypically determined, a mutation was observed in the rpoB MUT2A gene region with deletion in the WT7 band, and this isolate had the H526Y gene pattern at codon 526-529. When compared with various studies conducted in our country and in the world, the RIF resistance found in our study was genotypically lower. The fact that the resistance we found was most frequently detected in the S531L region was considered to be compatible with the literature.

Additionally, genotypic RIF resistance was observed in five (50%) of 10 MDR-TB isolates in this study. Genotypic resistance was observed in two (22.2%) of the nine isolates with phenotypic RIF resistance alone. This suggests that genotypic RIF resistance in MDR-TB isolates may be more

likely to develop due to gene mutation compared to isolates with RIF resistance alone.

The proportion of isoniazid-resistant TB cases is increasing globally. Mutations in the katG gene play a very prominent role in mediating INH resistance (21). The most common S315T resistance pattern has been reported to be associated with moderate or high levels of resistance to INH (22). A strong correlation between this mutation and the transmission dynamics of MDR-TB and MDR-TB has been previously reported (23). In a recent systemic review on INH resistance, it was shown that 64% and 19% of all INH resistance was associated with katG 315 and inhA-15 mutations, respectively (24). In a study conducted by Javed et al.(18) on 53 MDR-TB isolates phenotypically using Genotype MTBDRplus method in Pakistan, 38 (71.7%) isolates were genotypically INH resistant. In all but one of these isolates (37/53; 69.8%), S315T gene pattern was detected in the katG gene, and in 21 (39.6%) of these, WT band deletion was additionally reported. In the same study, four (7.5%) isolates were found to have mutations in the inhA promoter, and two different resistance patterns were detected: C-15T (MUT1-WT) in three (5.6%) and C-15T and T-8C (MUT1-MUT3A) in one (1.9%). In the study of Kumar et al.(25) involving 442 RR-TB isolates, 11.7% had inh A resistance pattern while 90% had fold G resistance pattern and the most common mutation for fold G was shown as Mut1 mutation and WT deletion in which point mutations occurred at codon 315. In our study, INH resistance patterns were evaluated in 19 phenotypically resistant RR-TB isolates and 10 MDR-TB isolates by Genotype MTBDRplus method. Six of 10 MDR-TB isolates (60%) were genotypically INH resistant. Four of them (40%) had S315T1 gene pattern showing deletion in katG WT band and katG MUT1 gene mutation. Two of the isolates (20%) had T8-A gene pattern with INHA-MUT3B gene mutation. INH resistance was detected genotypically in the katG gene region in a strain that was not phenotypically INH resistant. In our study, the rates of both phenotypic and genotypic INH resistance in MDR-TB strains and the fact that genotypic resistance was mostly seen as S315T1 pattern were evaluated in accordance with the literature. In our study, although there was no phenotypic resistance in one strain, genotypic resistance pattern was observed, which is important in terms of showing the incompatibility between the two tests.

Conventional DST for extensively drug-resistant MTBC strains is performed sequentially. This is a long and laborious two-step procedure starting with culture and first-line drug testing, with the need for further drug testing in case of multidrug resistance. A systematic review to evaluate Genotype MTBDR sl, which is considered to be the only commercially available molecular test for second-line anti-TB drug resistance, showed that it has good accuracy in detecting resistance to FQs, amikacin and capreomycin. However, it is not a suitable choice for kanamycin and ethambutol due to poor sensitivity (26). In our study, nine of 10 phenotypically resistant MDR-TB isolates were susceptible to second generation anti-TB drugs by Genotype MTBDR sl test. One isolate could not be evaluated because it did not meet the interpretation criteria of this test. Considering that the rate of MDR-TB is low in our country, our results were considered to be compatible with the literature. However, since phenotypic susceptibility testing could not be performed on the isolates, the inability to comment on genotypic resistance concordance was considered a limitation in our study.

MTBC consists of a genetically homogenous group. In this group, *M. tuberculosis subsp. tuberculosis* is the most common species causing tuberculosis in humans, while *M. bovis subsp. bovis, M. bovis subsp. caprae, M. canettii, M. africanum* and *M. microti* are the second most common species (6). The distribution and frequency of MTBC strains and sub-strains causing tuberculosis vary in different parts of the World (27). The origins of 19 MTBC isolates included in our study were analysed by Genotype MTBC test and all of them were identified as *M. tuberculosis*.

In conclusion,diagnosis of MTBC and drug susceptibility tests usually takes a long time with traditional methods. Newly developed molecular methods are very advantageous in terms of providing accurate and rapid results in both diagnosis and determination of drug susceptibility. The presence of MDR-TB and RR-TB is an important challenge especially in TB control, which increases the need for molecular methods. Although it has not still replaced culture, there is a need for the use and development of new molecular methods that will benefit us in TB treatment and control. Moreover, the disadvantage of molecular methods is the incompatibility with phenotypic resistance in cases where resistance develops by other mechanisms. We think that our study will contribute to the existing literature in this sense.

Conflict of interest: The authors declare no conflict of interest.

Supporting Institution: Düzce University Research Fund, Project No:2022.04.01.1277

REFERENCES

1. Javed H, Bakuła Z, Pleń M, Hashmi HJ, Tahir Z, Jamil N, et al. Evaluation of Genotype MTBDRplus and MTBDRsl Assays for Rapid Detection of Drug Resistance in Extensively Drug-Resistant Mycobacterium tuberculosis Isolates in Pakistan. Front Microbiol. 2018; 9:1–10.

2. G lobal tuberculosis report 2020: executive summary. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.
3. Niyam D, Shruchi S. Comparative Analysis of Microscopic and Real-time Polymerase Chain Reaction-based Methods for the Detection of Multidrug Resistance in Mycobacterium tuberculosis. Journal of Preventive, Diagnostic and Treatment Strategies in Medicine. 2024;3(2):71-75.
4. Winn, W, Allen, S, Janda, W, Koneman, E, Procop G, Schreckenberger P, and Woods, G. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th Edition, Lippincott Williams and Wilkins, New York, 2006.
5. Maharjan E, Pant ND, Neupane S, Amatya J, Shrestha B. Use of Genotype MTBDRplus Assay for Diagnosis of Multidrug-Resistant Tuberculosis in Nepal. International Scholarly Research Notices. 2017; Article ID 1635780, 5 pages.
6. Rastogi N, Legrand E, Sola C. The mycobacteria: an introduction to nomenclature and pathogenesis. Rev Sci Tech Int Des Epizoot. 2001;20 (1):21–54.
7. Loiseau C, Brites D, Moser I, Coll F, Pourcel C, Robbe-Austerman S. et al. Revised Interpretation of the Hain Lifescience GenoType MTBC To Differentiate Mycobacterium canettii and Members of the Mycobacterium tuberculosis complex. Antimicrob Agents Chemother. 2019;63(6). e00159-19
8. Global tuberculosis report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
9. Türkiye'de Verem Savaşı 2020 Raporu, TC.Sağlık Bakanlığı Halk Sağlığı Müdürlüğü.. Ankara; 2021.
10. Maurya AK, Nag VL, Kant S, Singh Kushwaha RA, Dhole TN. Genotypic Characterization of rpoB, katG and inhA Gene of Multi Drug Tuberculosis Isolates from Extra Pulmonary Tuberculosis. Biomed Biotechnol Res J. 2017;1(2):129-33
11. Yazısız H, Hırçın Cenger D, Yazısız V, Kılıç L AS. Mycobacterium tuberculosis kompleks izolatlarında birinci basamak anti-tüberküloz ilaçlara direnç trendleri: Türkiye'den üçüncü basamak bir hastane çalışması. Tuberk Toraks. 2019;67(92):101.
12. Yılmaz A, Afşin D. Investigation of the Sensitivity of Mycobacterium Tuberculosis Strains Isolated from Various Clinical Samples in Eastern Turkey to Major Anti-tuberculosis Drugs. J Contemp Med. 2021;30;11:92–6.
13. Liu Q, Lu P, Martinez L, Yang H, Lu W, Ding X, et al. Factors affecting time to sputum culture conversion and treatment outcome of patients with multidrug-resistant tuberculosis in China. BMC Infect Dis. 2018;18(1):114.
14. Soeroto AY, Pratiwi C, Santoso P, Lestari BW. Factors affecting outcome of longer regimen multidrug-resistant tuberculosis treatment in West Java Indonesia: A retrospective cohort study. PLoS One. 2021;16(2):e0246284.
15. Gota A, Shenoy VP, Kamath A. Evaluating Diagnostic Utility of geneXpert Ultra (Mycobacterium tuberculosis/Rifampicin), Microscopy and Liquid Culture to Isolate Mycobacterium tuberculosis and Nontuberculous Mycobacteria among Pulmonary Tuberculosis Suspects. J Prev Diagnostic Treat Strateg Med. 2023;2(4):236-42.
16. Xu G, Liu H, Jia X, Wang X, Xu P. Mechanisms and detection methods of Mycobacterium tuberculosis rifampicin resistance: The phenomenon of drug resistance is complex. Tuberculosis. 2021;128:102083.
17. Campbell PJ, Morlock GP, Sikes RD, Dalton TL, Metchock B, Starks AM, et al. Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2011;55(5):2032–41.
18. Javed H, Bakuła Z, Pleń M, Hashmi HJ, Tahir Z, Jamil N, et al. Evaluation of Genotype MTBDRplus and MTBDRsl Assays for Rapid Detection of Drug Resistance in Extensively Drug-Resistant Mycobacterium tuberculosis Isolates in Pakistan. Front Microbiol. 2018;9:2265.
19. Das PK, Ganguly SB, Mandal B. Genetic Mutations at Rifampicin Resistance-Determining Region of RpoB Gene in Conferring Resistance to Isolated Multidrug-Resistant Tuberculosis Strains. Biomed Biotechnol Res J. 2018;2:295-9
20. Sağlam Ds. Mycobacterium tuberculosis Suşlarında Anti-Tüberküloz İlaçlara Direncin Moleküler Yöntemlerle Araştırılması.Uzmanlık Tezi, Bursa Uludağ Üniversitesi; 2016.
21. Unissa AN, Subbian S, Hanna LE, Selvakumar N. Overview on mechanisms of isoniazid action and resistance in Mycobacterium tuberculosis. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis. 2016;45:474–92.
22. van Soolingen D, de Haas PEW, van Doorn HR, Kuijper E, Rinder H, Borgdorff MW. Mutations at amino acid position 315 of the katG gene are associated with high-level resistance to isoniazid, other drug resistance, and successful transmission of Mycobacterium tuberculosis in the Netherlands. J Infect Dis. 2000;182(6):1788–90.
23. Ano H, Matsumoto T, Suetake T, Nagai T, Tamura Y, Takamatsu I, et al. Relationship between the isoniazid-resistant mutation katGS315T and the prevalence of MDR-/XDR-TB in Osaka, Japan. Int J Tuberc Lung Dis. 2008;12(11):1300–5.

24. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. PLoS One. 2015;10(3):e0119628.
25. Das PK, Ganguly SB, Mandal B. Early Diagnosis of Smear-Negative Childhood Pulmonary Tuberculosis and Its Substantial Yield in Gastric Lavage/Aspirates through Cartridge-Based Nucleic Acid Amplification Test (Xpert Mycobacterium tuberculosis/Rifampicin Assay). Biomed Biotechnol Res J. 2019;3(4):258-63
26. Feng Y, Liu S, Wang Q, Wang L, Tang S, Wang J, et al. Rapid diagnosis of drug resistance to fluoroquinolones, amikacin, capreomycin, kanamycin and ethambutol using genotype MTBDRsl assay: a meta-analysis. PLoS One. 2013;8(2):e55292.
27. Duffy SC, Venkatesan M, Chothe S, Poojary I, Verghese VP, Kapur V, et al. Development of a Multiplex Real-Time PCR Assay for Mycobacterium bovis BCG and Validation in a Clinical Laboratory. Microbiol Spectr. 2021;9(2):e0109821.

RESEARCH ARTICLE

Muhammet Fethi Saglam[1]
Emrah Uguz[1]
Kemal Esref Erdogan[1]
Huseyin Unsal Ercelik[2]
Murat Yucel[2]
Cevat Ahmet Sert[3]
Fatih Yamac[4]
Erol Sener[1]

[1] Ankara Yıldırım Beyazıt University Faculty of Medicine, Department of Cardiovascular Surgery, Ankara, Türkiye
[2] Ankara Bilkent City Hospital, Ankara, Türkiye
[3] Ankara Etlik City Hospital, Ankara, Türkiye
[4] Sincan Training and Research Hospital, Ankara, Türkiye

Corresponding Author:
Muhammet Fethi Sağlam
mail:dr.m.fethisaglam@gmail.com

Received: 02.01.2025
Acceptance: 24.03.2025
DOI:10.18521/ktd.1611953

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Artificial Intelligence in Patient Communication: Performance of GPT-3.5 and GPT-4 in Coronary Bypass Surgery

ABSTRACT

Objective: This study aims to evaluate the ability of GPT-3.5 and GPT-4 to provide accurate, comprehensible, and clinically relevant responses to common patient questions about coronary bypass surgery.

Methods: A cross-sectional study was conducted at Ankara Yıldırım Beyazıt University Bilkent City Hospital with 80 cardiovascular surgery specialists. Participants rated the responses of GPT-3.5 and GPT-4 to 10 common patient questions about coronary bypass surgery based on four criteria: accuracy, understandability, clinical appropriateness, and overall evaluation. Statistical analysis included independent t-tests, Cronbach's Alpha reliability analysis, and Cohen's d effect size calculation.

Results: GPT-4 significantly outperformed GPT-3.5 across all metrics. The mean scores for GPT-4 were higher in accuracy (3.02 vs. 1.77), understandability (2.99 vs. 1.81), clinical appropriateness (2.96 vs. 1.78), and overall evaluation (2.98 vs. 1.77) ($p<0.05$ for all). Cronbach's Alpha values indicated good internal consistency (≥ 0.69 for all metrics), and Cohen's d effect sizes demonstrated large differences (1.54 to 1.65).

Conclusions: GPT-4 shows superior potential compared to GPT-3.5 in answering patient questions about coronary bypass surgery. Despite its strengths, occasional inaccuracies and incomplete responses highlight the need for further refinement. Future research should integrate patient feedback and evaluate the real-world clinical impact of these models to optimize their application in healthcare.

Keywords: Coronary Bypass Surgery, Artificial İntelligence, GPT-3.5, GPT-4, Patient Communication.

Hasta İletişiminde Yapay Zeka: Koroner Bypass Cerrahisinde GPT-3.5 ve GPT-4'ün Performansı

ÖZET

Amaç: Bu çalışma, GPT-3.5 ve GPT-4'ün koroner bypass cerrahisiyle ilgili yaygın hasta sorularına doğru, anlaşılır ve klinik olarak uygun yanıtlar verme yeteneğini değerlendirmeyi amaçlamaktadır.

Yöntem: Ankara Yıldırım Beyazıt Üniversitesi Bilkent Şehir Hastanesi'nde 80 kalp ve damar cerrahisi uzmanı ile kesitsel bir çalışma yürütülmüştür. Katılımcılar GPT-3.5 ve GPT-4'ün koroner bypass cerrahisi ile ilgili 10 yaygın hasta sorusuna verdiği yanıtları dört kritere göre değerlendirmiştir: doğruluk, anlaşılabilirlik, klinik uygunluk ve genel değerlendirme. İstatistiksel analiz bağımsız t-testlerini, Cronbach Alfa güvenilirlik analizini ve Cohen's d etki büyüklüğü hesaplamasını içermektedir.

Bulgular: GPT-4 tüm ölçütlerde GPT-3.5'ten önemli ölçüde daha iyi performans göstermiştir. GPT-4 için ortalama puanlar doğruluk (3,02'ye karşı 1,77), anlaşılabilirlik (2,99'a karşı 1,81), klinik uygunluk (2,96'ya karşı 1,78) ve genel değerlendirme (2,98'e karşı 1,77) açısından daha yüksekti (tümü için $p<0,05$). Cronbach's Alpha değerleri iyi bir iç tutarlılık (tüm ölçütler için $\geq 0,69$) ve Cohen's d etki büyüklükleri büyük farklılıklar (1,54 ila 1,65) göstermiştir.

Sonuç: GPT-4, koroner bypass cerrahisi ile ilgili hasta sorularını yanıtlamada GPT-3.5'e kıyasla üstün potansiyel göstermektedir. Güçlü yönlerine rağmen, zaman zaman ortaya çıkan yanlışlıklar ve eksik yanıtlar daha fazla iyileştirme ihtiyacının altını çizmektedir. Gelecekteki araştırmalar, hasta geri bildirimlerini entegre etmeli ve sağlık hizmetlerinde uygulamalarını optimize etmek için bu modellerin gerçek dünyadaki klinik etkilerini değerlendirmelidir.

Anahtar Kelimeler: Koroner Bypass Cerrahisi, Yapay Zeka, GPT-3.5, GPT-4, Hasta İletişimi

INTRODUCTION

Coronary artery disease is one of the leading causes of cardiovascular morbidity and mortality worldwide, contributing to millions of deaths each year (1, 2). Coronary bypass surgery is one of the most effective surgical treatment methods to ensure adequate blood flow to the heart muscle by replacing blocked or narrowed coronary vessels with healthy vessels. However, this surgical procedure carries serious risks and can cause physical, psychological and social difficulties for the patient. Therefore, answering patient questions accurately and clearly before, during and after this surgery is critical for patient satisfaction and treatment success (3-5).

The rapid development of artificial intelligence (AI) technologies has led to significant changes in the field of healthcare. AI-based systems such as big language models have been used in a wide range of applications, from answering patient questions to clinical decision support systems. For example, GPT series models stand out as potential tools to support healthcare professionals with their text generation and natural language processing capabilities (6-8). However, the performance of these systems in terms of accuracy, comprehensibility and clinical relevance has not yet been sufficiently investigated (9-11).

In the literature, there are various studies on the use of big language models in healthcare. For example, Wang et al. (2024) (12), in their study examining the capacity of language models to provide accurate answers to general patient questions about surgical procedures, emphasized that these models are particularly effective for answers containing general health information. However, the adaptability of model performances to specific clinical domains is still unclear and studies on this topic are limited (13-15). There is no study evaluating the performance of large language models for patient questions in coronary bypass surgery. This emphasizes the originality of our study and its contribution to the literature.

The aim of this study is to evaluate the performance of GPT-3.5 and GPT-4 models in answering patient questions about coronary bypass surgery. In the study, the models were compared according to the criteria of accuracy, understandability and clinical relevance. This evaluation, based on expert opinions, provides important data to better understand the potential of large language models in the field of patient communication and clinical support and to contribute to the development of these models.

MATERIAL AND METHODS

This is a cross-sectional study designed to evaluate the performance of GPT-3.5 and GPT-4 models in responding to patient questions about coronary bypass surgery. The study aims to examine the potential of artificial intelligence models in patient communication and clinical decision support systems. The study was conducted at Ankara Yıldırım Beyazıt University Bilkent City Hospital Cardiovascular Surgery clinic and 80 Cardiovascular Surgery specialists with at least 5 years of professional experience and expertise in coronary bypass surgery participated in the study. Participants were academicians, clinicians, or professionals working in both positions. Incomplete or incorrectly completed forms and participants with less than 5 years of professional experience were excluded from the analysis. This study received ethical approval from the Ankara Bilkent City Hospital 1nd Clinical Research Ethics Committee on 23.11.2024 with decision number TABED 1-24-679. The study was reviewed for ethical considerations and unanimously approved.

During the data collection process, the 10 most frequently asked questions about coronary bypass surgery were determined and these questions were sent to the participants via Google Form. Participants rated the answers provided by the GPT-3.5 and GPT-4 models according to four main criteria: accuracy, which refers to the scientific accuracy of the answer; understandability, which refers to how easily the answer can be understood by the patient; clinical relevance, which refers to the validity of the answer in terms of clinical practice; and overall score, which refers to the overall evaluation of the answer. Each criterion was scored using a Likert scale from 1 (inadequate) to 5 (excellent). The list of common questions asked by patients regarding coronary bypass surgery is provided in Table 1. These questions were utilized to assess the performance of the AI models in providing accurate, understandable, and clinically appropriate responses.

Statistical Analysis: Data for this study were analyzed using IBM SPSS Statistics 29 software. During the data preparation and cleaning phase, any records with missing, erroneous, or outlier values were removed from the analysis. Descriptive statistics were utilized to provide fundamental information about the demographics and professional experiences of the participants. This included calculating the distribution of categories and average years of professional experience among participants. Independent t-tests were conducted to evaluate the differences in mean scores between the GPT-3.5 and GPT-4 models. These tests determined whether the differences were statistically significant, with all tests maintaining a significance level of $p<0.05$. Reliability analysis was performed using Cronbach's Alpha coefficient to assess the internal consistency of the measurement tools. Cronbach's Alpha values are interpreted as follows: values below 0.7 indicate acceptable reliability, values between 0.7 and 0.9 indicate good reliability, and values above 0.9 indicate excellent reliability. Effect size analysis was conducted using Cohen’s d

to quantify the magnitude of differences between the models on each metric. Cohen's d values are interpreted as follows: values of 0.2 or below indicate small effects, values around 0.5 indicate medium effects, and values of 0.8 or above indicate large effects.

Table 1. Common Questions Asked by Patients Regarding Coronary Bypass Surgery

Question Number	Question
1	Is it absolutely necessary for me to have coronary bypass surgery? Are there alternative treatments?
2	Is this surgery generally successful? What complications might occur during or after the procedure?
3	What are the risks of coronary bypass surgery? Is there any life-threatening danger?
4	How should I prepare before the surgery? What should I pay attention to?
5	Where will you take the veins to replace the blocked arteries? Will it cause other problems in my body?
6	How is coronary bypass surgery performed, and how long does it take?
7	Is the heart connected to a machine during the surgery? Does this procedure have any harm?
8	Will I stay in intensive care after the surgery? How long will I remain in the hospital?
9	How long will it take for me to recover after coronary bypass surgery? When can I return to my daily life?
10	Do I need to make lifestyle changes after the surgery? How should I continue my daily life?

RESULTS

According to Table 2, 33.75% of the participants are academics, 40.00% are both clinicians and academics, and 26.25% are solely clinicians. The average years of professional experience are 13.7 for academics, 16.5 for clinicians, and 12.9 for those who are both. This diversity in professional backgrounds provides a robust foundation for the comprehensive evaluation of the AI models.

As shown in Table 3, the GPT-4 model significantly outperforms GPT-3.5 across all primary performance metrics, including accuracy, understandability, clinical suitability, and overall score. The statistical measures, including T-statistics and P-values, indicate substantial differences, suggesting the superior efficacy of the GPT-4 model in handling clinical queries. Table 4 details the reliability of the evaluations, assessed through Cronbach's Alpha. The values obtained suggest high internal consistency across the measurements, with all metrics showing alphas above 0.70, indicating reliable assessments of the models' performances

Table 2. Demographics and Professional Experience of Participants by Category

Category	Count	Percentage	Average Years of Experience
Academics	27	33.75%	13.7 years
Both Clinician and Academic	32	40.00%	12.9 years
Clinicians	21	26.25%	16.5 years

Table 3. Comparative Performance of GPT-3.5 and GPT-4 Models on Key Performance Metrics

Metric	GPT-3.5 Mean	GPT-4 Mean	T-Statistic	P-Value
Accuracy	1.77	3.02	-9.97	<0.05
Understandability	1.81	2.99	-9.48	<0.05
Clinical Suitability	1.78	2.96	-11.14	<0.05
Overall Score	1.77	2.98	-10.57	<0.05

Table 4. Reliability Analysis Across Core Performance Metrics

Metric	Cronbach's Alpha
Accuracy	0.72
Understandability	0.69
Clinical Suitability	0.74
Overall Score	0.71

In Table 5, the effect sizes (Cohen's d) are presented, illustrating large effect sizes for all considered metrics. These substantial effect sizes highlight the practical significance of the performance differences between the models, with GPT-4 not only statistically outperforming GPT-3.5 but also showing considerable improvements that are likely to be clinically relevant.

Table 5. Effect Size Analysis Between Models Across Principal Performance Metrics

Metric	Cohen's d
Accuracy	1.65
Understandability	1.56
Clinical Suitability	1.54
Overall Score	1.59

The comparative performance of GPT-3.5 and GPT-4 models on key performance metrics, including accuracy, understandability, clinical suitability, and overall score, is illustrated in Figure 1. The figure highlights the significant differences in performance between the two models, with GPT-4 consistently achieving higher scores.

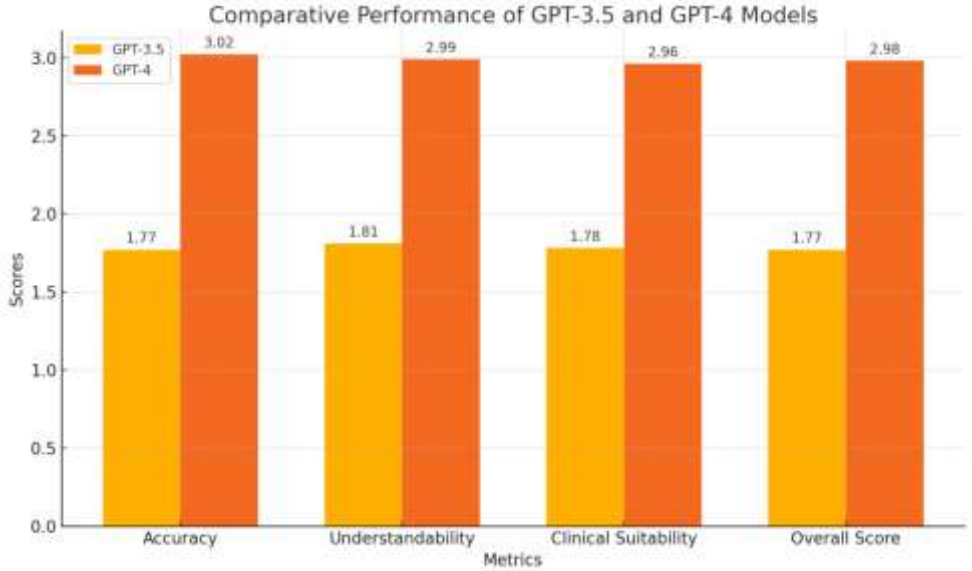

Figure 1. Comparative performance of GPT-3.5 and GPT-4 models across key performance metrics, including accuracy, understandability, clinical suitability, and overall score.

DISCUSSION

This study is one of the first to comparatively evaluate the performance of GPT-3.5 and GPT-4 models in responding to patient questions about coronary bypass surgery based on accuracy, understandability, clinical appropriateness, and overall evaluation criteria. The findings revealed that the GPT-4 model performed significantly better than GPT-3.5 across all metrics. These results align with existing literature on the use of large language models in healthcare and extend the understanding of their potential applications.

Accuracy is a critical metric for AI systems, especially in healthcare. Lewine et al. (2024) noted that GPT-3.5 demonstrated high accuracy in general knowledge but had limitations in clinical contexts (16). In this study, GPT-4 showed significant superiority in accuracy compared to GPT-3.5, particularly in providing specific clinical information about coronary bypass surgery. This can be attributed to GPT-4's updated knowledge base and advanced natural language processing capabilities. However, both models occasionally provided inaccurate or incomplete responses, consistent with Liu et al. (2022), who highlighted the potential for AI systems to falter in complex clinical scenarios (17).

Understandability plays a crucial role in patient communication, as it directly impacts patient engagement and comprehension of medical procedures. Bajva et al. (2021) (18) emphasized the importance of AI systems using simple and clear language to enhance patient satisfaction. In this study, GPT-4 achieved significantly higher scores in understandability compared to GPT-3.5, likely due to its advanced language generation capabilities that produce more fluent and patient-friendly responses. However, the occasional use of technical jargon, making responses less accessible to patients, aligns with Al Kuwaiti et al. (2023) (19), who argued for further optimization of language models to better cater to patient needs.

Clinical appropriateness extends beyond accuracy, focusing on the relevance and applicability of the information in a clinical context. Maleki et al. (2024) (20) emphasized that AI systems in surgical domains must prioritize clinical appropriateness to be reliable tools for healthcare professionals. In this study, GPT-4 outperformed GPT-3.5 in this metric, demonstrating better alignment with clinical contexts. Nevertheless, some responses were either incomplete or lacked contextual depth, highlighting the need for more specific training data to enhance model performance in niche clinical areas.

The overall evaluation metric combines all individual metrics to provide a comprehensive assessment of the models' performance. GPT-4 scored significantly higher than GPT-3.5, reflecting its superior performance across the other three metrics. As noted by Liu et al. (2024) (21), AI

systems that offer user-friendly and human-like responses can play a vital role in patient communication. However, further improvements are necessary to ensure that AI models meet diverse patient needs comprehensively.

This study's strengths include being the first to evaluate AI models' responses to patient questions about coronary bypass surgery and its reliance on expert evaluations. However, several limitations must be acknowledged. First, the study is based solely on expert opinions, excluding direct feedback from patients. Second, the AI models were evaluated using a specific dataset, which limits the generalizability of the results. Furthermore, the cross-sectional design of the study does not allow for the assessment of the models' performance over time as they continue to evolve.

CONCLUSIONS

This study demonstrates the potential of AI-based large language models as tools for patient communication in coronary bypass surgery. GPT-4 outperformed GPT-3.5 in accuracy, understandability, clinical appropriateness, and overall evaluation criteria. However, limitations such as occasional inaccuracies and incomplete responses remain evident in both models. Future research should involve larger cohorts of patients and experts, evaluate the models' impact on real-world patient outcomes, and train these systems on more specific clinical datasets. This study represents a significant step forward in exploring the effective use of AI systems in healthcare delivery.

Declarations

Funding: Not applicable.

Competing Interests: The authors declare no competing interests.

Authors Contributions: All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Muhammet Fethi Sağlam, Emrah Uguz, Kemal Eşref Erdoğan, Hüseyin Ünsal Erçelik, Murat Yücel, Cevat Ahmet Sert, Fatih Yamaç and Erol Şener. The first draft of the manuscript was written by Muhammet Fethi Sağlam, Emrah Uguz, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics Approval: This study received ethical approval from the 1st Scientific and Ethical Review Committee for Medical Research (TABED) of Ankara Bilkent City Hospital on 23.10.2024, with decision number TABED 1-24-679. The study was reviewed and unanimously approved in terms of ethical considerations.

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

Consent to publish: Not applicable.

Availability of Data and Materials: The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Acknowledgements: Not applicable.

REFERENCES

1. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. Journal of the American College of Cardiology. 2020;76(25):2982-3021.
2. Vogel B, Acevedo M, Appelman Y, Bairey Merz CN, Chieffo A, Figtree GA, et al. The Lancet women and cardiovascular disease Commission: reducing the global burden by 2030. Lancet (London, England). 2021;397(10292):2385-438.
3. Powell R, Scott NW, Manyande A, Bruce J, Vögele C, Byrne-Davis LM, et al. Psychological preparation and postoperative outcomes for adults undergoing surgery under general anaesthesia. The Cochrane database of systematic reviews. 2016;2016(5):Cd008646.
4. Açıkel MET. Evaluation of Depression and Anxiety in Coronary Artery Bypass Surgery Patients: A Prospective Clinical Study. Brazilian journal of cardiovascular surgery. 2019;34(4):389-95.
5. Aburuz ME, Maloh H. Preoperative anxiety and depressive symptoms predicted higher incidence of delirium post coronary artery bypass graft surgery. 2024.
6. Alowais SA, Alghamdi SS, Alsuhebany N, Alqahtani T, Alshaya AI, Almohareb SN, et al. Revolutionizing healthcare: the role of artificial intelligence in clinical practice. BMC medical education. 2023;23(1):689.
7. Yuan M, Bao P, Yuan J, Shen Y, Chen Z, Xie Y, et al. Large language models illuminate a progressive pathway to artificial intelligent healthcare assistant. Medicine Plus. 2024:100030.
8. Guo RX, Tian X, Bazoukis G. Application of artificial intelligence in the diagnosis and treatment of cardiac arrhythmia. 2024;47(6):789-801.
9. Ali S, Abuhmed T, El-Sappagh S, Muhammad K, Alonso-Moral JM, Confalonieri R, et al. Explainable Artificial Intelligence (XAI): What we know and what is left to attain Trustworthy Artificial Intelligence. Information fusion. 2023;99:101805.
10. Coleman JJ, Owen J, Wright JH, Eells TD, Antle B, McCoy M, et al. Using Artificial Intelligence to Identify Effective Components of Computer-Assisted Cognitive Behavioural Therapy. Clinical psychology & psychotherapy. 2024;31(6):e70023.

11. Ismail AMA. Chat GPT in Tailoring Individualized Lifestyle-Modification Programs in Metabolic Syndrome: Potentials and Difficulties? Annals of biomedical engineering. 2023;51(12):2634-5.
12. Wang D, Zhang S. Large language models in medical and healthcare fields: applications, advances, and challenges. Artificial Intelligence Review. 2024;57(11):299.
13. Samant S, Bakhos JJ, Wu W, Zhao S, Kassab GS, Khan B, et al. Artificial Intelligence, Computational Simulations, and Extended Reality in Cardiovascular Interventions. JACC Cardiovascular interventions. 2023;16(20):2479-97.
14. Krajcer Z. Artificial Intelligence for Education, Proctoring, and Credentialing in Cardiovascular Medicine. Texas Heart Institute journal. 2022;49(2).
15. Biswas SS. Role of Chat GPT in Public Health. Annals of biomedical engineering. 2023;51(5):868-9.
16. Levine DM, Tuwani R, Kompa B, Varma A, Finlayson SG, Mehrotra A, et al. The diagnostic and triage accuracy of the GPT-3 artificial intelligence model: an observational study. The Lancet Digital Health. 2024;6(8):e555-e61.
17. Liu J, Wang C. Utility of ChatGPT in Clinical Practice. 2023;25:e48568.
18. Bajwa J, Munir U, Nori A, Williams B. Artificial intelligence in healthcare: transforming the practice of medicine. Future healthcare journal. 2021;8(2):e188-e94.
19. Al Kuwaiti A, Nazer K, Al-Reedy A, Al-Shehri S, Al-Muhanna A. A Review of the Role of Artificial Intelligence in Healthcare. 2023;13(6).
20. Maleki Varnosfaderani S, Forouzanfar M. The Role of AI in Hospitals and Clinics: Transforming Healthcare in the 21st Century. 2024;11(4).
21. Liu C-L, Ho C-T, Wu T-C, editors. Custom GPTs enhancing performance and evidence compared with GPT-3.5, GPT-4, and GPT-4o? A study on the emergency medicine specialist examination. Healthcare; 2024:MDPI.

RESEARCH ARTICLE

Ozgur Agdogan[1]

[1] Tekirdağ Namık Kemal University Faculty of Medicine, Department of Plastic Reconstructive and Aesthetic Surgery, Tekirdağ, Türkiye

Corresponding Author:
Ozgur Agdogan
mail:drozguragdogan@hotmail.com

Received: 04.12.2024
Acceptance: 26.03.2025
DOI:10.18521/ktd.1594423

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Modified Reverse Cross Finger Flap: Reticulodermo-Adipofascial Flap

ABSTRACT

Objective: The reverse cross finger flap is a modified crossed finger flap with the advantages of the heterodigital island flap. Local flap reconstruction techniques have been used predominantly to repair small to medium-sized finger defects. This flap is considered suitable for all fingers except the thumb. The subdermal plexus provides the main blood supply of this flap. The aim of this study is to present the characteristics and results of patients treated in our clinic.

Methods: In our clinic, 11 patients with exposed bone, tendon, nerve or vessels on the dorsum of the finger; skin and soft tissue defects; and the need for reconstruction after tumoral mass excision were retrospectively investigated with the 'Modified Reverse Cross Finger Flap: Reticulodermo-Adipofascial Flap' technique. Results were evaluated.

Results: The mean age of the patients was 57.2 years. 7 patients had a finger dorsal defect after trauma, 2 patients had a finger dorsal defect after tumoral mass excision, 1 patient had a finger dorsal defect after chronic wound excision, and 1 patient had a finger dorsal defect after burn scar revision. Modified Reticulodermo-Adipofascial Reverse Cross Finger Flap technique was applied to these defects. No flap loss was observed in any patient. No inclusion cyst was seen in the flap area. 2 patients had minimal, mild joint motion limitation at the distal interphalangeal joint. Wound site appearance and functional gain were acceptable and good.

Conclusions: We prepared a stronger flap by incorporating only the reticular dermis into the adipofascial tissue. We also prevented the damage of the subdermal vascular plexus by couring the reticular dermis. In previous applications, the design of the reverse cross finger flap was planned as dermoadipofascial after the epidermis was de-epithelialized or as adipofascial after the epidermis and dermis were de-epithelialized. This flap design prevents the formation of cysts originating from hair, oil and sweat glands. In addition, this flap design prevents cyst formation and contributes to the blood supply of the flap.

Keywords: Finger Dorsal Defect, Reverse Reticulodermo-Adipofascial, Cross Finger Flap.

Modifiye Ters Çapraz Parmak Flebi: Retikülodermo-Adipofasyal Flep

ÖZET

Amaç: Ters çapraz parmak flebi, heterodijital ada flebinin avantajlarına sahip modifiye edilmiş bir çapraz parmak flebidir. Lokal flep rekonstrüksiyon teknikleri, ağırlıklı olarak küçük ve orta boyuttaki parmak defektlerini onarmak için kullanılmıştır. Bu flebi başparmak hariç diğer parmaklar için uygun görülmüştür. Subdermal pleksus, bu flebin ana kanlanmasını sağlar. Bu çalışmanın amacı kliniğimizde tedavi edilen hastaların özellikerini ve sonuçlarını sunmaktır.

Yöntem: Kliniğimizde el parmak dorsumunda ekspoze kemik, tendon, sinir veya damarlar olan; cilt ve yumuşak doku defekti olan; tümöral kitle eksizyonu sonrası rekonstrüksiyon gereği olan ‘Modifiye Ters Çapraz Parmak Flebi: Retikülodermo-Adipofasyal Flep’ tekniği yapılan 11 hasta retrospektif olarak araştırıldı. Sonuçları değerlendirildi.

Bulgular: Hastaların yaş ortalamaları 57,2 idi. 7 Hastada travma sonrası el parmak dorsalinde defekt, 2 hastada tümöral kitle eksizyonu sonrası el parmak dorsalinde defekt, 1 hastada kronik yara eksizyonu sonrası el parmak dorsalinde defekt ve 1 hastada yanık skarı revizyonu sonrası el parmak dorsalinde defekti mevcut idi. Bu defektlere Modifiye Retikülodermo-Adipofasyal Ters Çapraz Parmak Flebi tekniği uygulandı. Hiçbir hastada flep kaybı görülmedi. Flep uygulanan bölgede inklüzyon kisti görülmedi. 2 hastada distal interfalangeal eklemde minimal, hafif eklem hareket kısıtlılığı mevcut idi. Yara yeri görünümü ve fonksiyonel kazanç kabul edilebilir ve iyi idi.

Sonuç: Sadece retiküler dermisi adipofasyal dokuya dahil ederek daha güçlü bir flep hazırlandı. Retiküler dermisi kouryarak subdermal vasküler pleksusun hasarlanmasını da önlemiş olduk. Daha önceki uygulamalarda ters çapraz parmak flebi tasarımı; epidermis de-epitelize edildikten sonera dermoadipofasyal şeklinde planlanmış yada epidermis ve dermis de-epitelize edildikten sonra adipofasyal şeklinde tasarlanmış. Bu flep dizaynı; kıl, yağ ve ter bezi kaynaklı kistlerinin oluşma ihtimalini engel olmaktır. Ayrıca bu flep dizaynı kist oluşumuna engel olurken flebin kanlanmasına da katkı sağlamaktır.

Anahtar Kelimeler: Parmak Dorsal Defekti, Ters Retikülodermo-Adipofasyal, Çapraz Parmak Flebi.

INTRODUCTION

Dorsal finger defects always pose a challenge for the reconstructive surgeon. Because there are not many regional options. Local flap reconstruction techniques have been used predominantly to close small and medium-sized finger defects (1).

Although various flaps have been described for adequate coverage of dorsal finger defects, these flaps may not result in cosmetic and functional satisfaction due to volume mismatch (2). Tube flaps harvested from the chest, abdomen, groin and forearm are reliable for adequate closure of dorsal finger wounds; however, these flaps will be bulky and offer poor cosmetic and functional results (3). To minimize the morbidity of the donor finger, the reverse cross finger flap was planned from the second, third, fourth and fifth fingers (2).

The reverse cross finger flap is a modified crossed finger flap that has the advantages of the heterodigital island flap (4). The skin connection helps to preserve the distal viability of the flap while reducing pedicle movement and preventing venous congestion (4,5). The flap is made to fit the boundaries of the functional phalanx unit exactly (4,6).

This flap is supplied by the branches of the dorsal digital arteries of the radial or ulnar side of the finger and the appropriate digital arteries. The dorsal digital artery cannot be used as the axial vessel of dorsal digital flaps due to its short diameter (1). In addition, the continuity of the digital artery is preserved. These vessels are known to be fixed on the proximal and middle phalanges. The subdermal plexus provides the main blood supply of this flap.

Indications for the reverse cross finger flap; reconstruction of the eponychial skin fold; coverage of an exposed extensor tendon near the interphalangeal joint (IP); reconstruction of exposed sterile matrix nail bed defects; covering a crushed, repaired or grafted extensor tendon without a paratenon; boutonniere deformity with poor quality skin over the proximal interphalangeal joint (PIP) after burn or avulsion injury; repair of the nail bed, germinal matrix and skin around the fingers after complete avulsion; and correcting the finger deformity in selected cases (7,8). This flap is also among the options after the excision of tumoral masses on the hand finger's dorsal surface. It should be noted that the hand is exposed to intense sun exposure, but the formation of basal cell carcinoma in the hand is relatively rare. (9).

Extensive dorsal skin loss and injuries to adjacent fingers constitute contraindications (7). Vasospastic conditions such as Raynaud disease, diabetes mellitus and Buerger disease may represent absolute contraindications (8). Pre-existing disability problems such as Dupuytren's contracture, rheumatoid arthritis and advanced age pose an increased risk for the outcome of the cross finger flap (8). The most basic prerequisite for soft tissue repair in the hand is to cleanse the wound from infection, remove all dead tissue and provide a stable skeletal structure (10). The dorsum of the finger contains a thin skin with a small amount of subcutaneous tissue underneath (11,4). The flap is planned to be approximately 1 cm longer and approximately 4 to 5 mm wider than the defect (7,2). Better cosmetic results are achieved by covering the flap with full-thickness skin grafts instead of split-thickness skin grafts (11).

The basic principle is to preserve the length of the finger, provide a sensory skin cover and provide functional improvement as much as possible (2). One of the most important criteria is whether the bone is exposed or not (2). The superiority of this flap has been proven in terms of sensitivity, durability, efficiency and reliability in terms of returning the patient to their profession (8).

The aim of this study is to present the characteristics and results of patients treated in our clinic.

MATERIAL AND METHODS

Our study was approved by Tekirdağ Namık Kemal University Non-Interventional Clinical Research Ethics Committee. Between 01.06.2017-30.10.2024, 11 patients who applied to our clinic and underwent 'Modified Reverse Cross Finger Flap: Reticulodermo-Adipofascial Flap' technique were retrospectively analyzed. Patients with open wounds, defects, tumoral masses and defects after mass excision with exposed bone, tendon, nerve or vessels on the dorsum of the finger and who underwent 'Modified Reverse Cross Finger Flap: Reticulodermo-Adipofascial Flap' technique in our clinic were examined. The number of patients who underwent this technique, gender, dominant hand, occupation, smoking, defect causative factor, defect localization, defect size, graft donor area, flap loss, joint function loss, inclusion cyst formation in the flap and wound appearance are indicated in the table. This flap was compared with previous methods. We also presented two case examples from our case series.

Surgical Technique: This flap includes all tissues from the reticular dermis to the paratenon of the extensor tendons. De-epithelialization is one of the most important technical details of this flap. During de-epithelialization, we de-epithelialized the epidermis and the superficial papillary dermis together. We included the reticular dermis, which is in the deep plane, in the adipofascial tissue. In other words, we designed a reticulodermo-adipofascial flap.

Case 1: A 68-year-old male patient had a 2x3 cm mass on the dorsal side of the middle phalanx of the right hand 2nd finger and a 1x1 cm hard hyperkeratotic mass fixed to the base on the DIPJ. The masses were benign and fixed to the base. The case was started under regional

anesthesia and tourniquet with loop guidance. The masses were resected with sufficient margins to include the tendon sheath. A 3x4 cm flap was designed from the dorsal side of the 3rd finger. The skin including the epidermis and papillary dermis was de-epithelialized. The reticular dermis is preserved. An incision was made for the reticuloadipofascial flap. The flap was incised on the defect side. The reticuloadipofascial flap was elevated in a way that the paratenon of the extensor tendon was protected. It was transferred to the defect area by rotating it 180 degrees. A tourniquet was opened. Bleeding control was performed. A full-thickness skin graft wider than the defect area was taken from the medial aspect of the right arm to cover the 2nd and 3rd fingers. The graft was de-fatted. Holes were opened in the graft. The graft was adapted to cover both the flap donor area and the transferred flap. Both fingers were fixed to each other with fixation sutures from the proximal and distal sides. A dressing with plenty of furacyn ointment was applied instead of a tie-over dressing. A short arm splint was applied. The dressing was removed on the 5th postoperative day. It was observed that the graft was adapted and the flap circulation was good. Flap separation was performed under local anesthesia on the 20th postoperative day. Wound follow-up was performed for another week. Hand rehabilitation was started at the end of the 4th postoperative week. Our case example and results are shown in figure 1.a-j.

Figure 1.a. Right hand 2nd finger middle phalanx dorsal 2x3 cm mass and 1x1 cm hard hyperkeratotic base fixed mass on DIPJ.

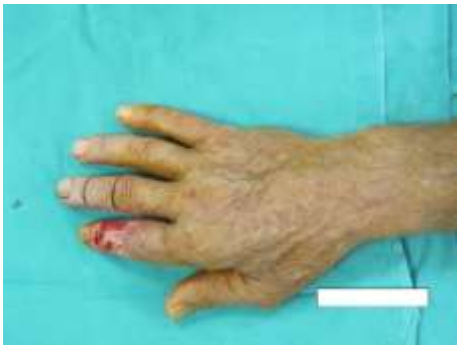

Figure 1.b. Exposed tendon and bone after mass excision.

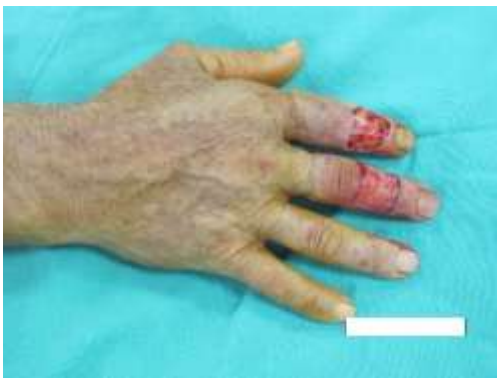

Figure 1.c. 3x4 cm flap design from dorsal 3rd finger; skin de-epidelization including epidermis and papillary dermis.

Figure 1.d. Reticuloadifascial flap elevation.

Figure 1.e. Transfer of reticulodermo-adifascial flap to defect area by rotating it 180 degrees.

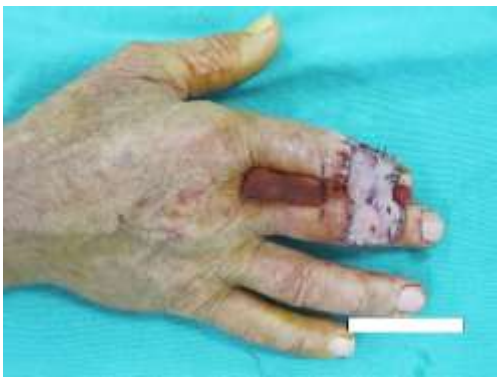

Figure 1.f. Repair of flap donor area and flap surface with full thickness skin graft.

Figure 1.g. Postoperative 3rd week view.

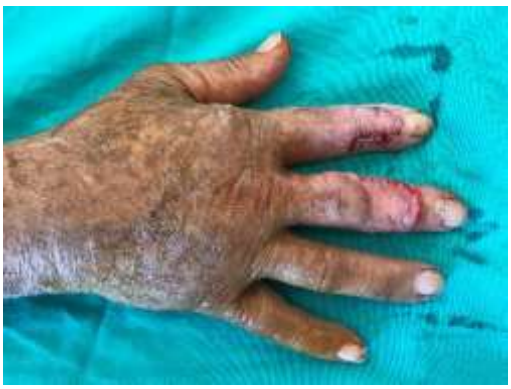

Figure 1.h. View after separation of flap in 2nd session operation.

Figure 1.i. Dorsal view of the fingers 1 month after flap separation.

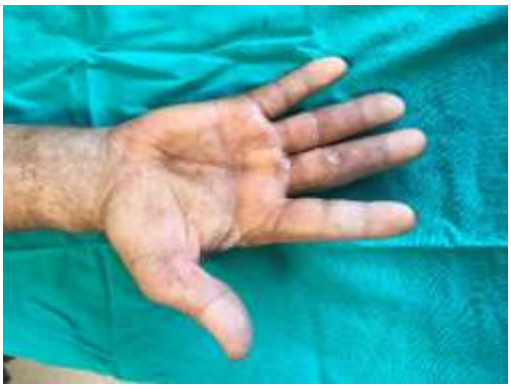

Figure 1.j. Volar view of the fingers 1 month after flap separation.

Case 2: A 43-year-old male patient had a crush and defective injury to his right hand fingers with a high-energy cutting tool. There was a 2x4 cm defect on the dorsal side of the middle phalanx of the 2nd finger, including the nail bed. The nail bed, bone tissue and extensor tendon were exposed. The extensor tendon was damaged. The defect extended to the DIPJ. There was skin damage on the dorso-ulnar side of the proximal phalanx of the 1st finger, nail and nail bed damage on the 3rd finger, and nail and nail bed damage in addition to the skin defect on the DIPJ and radial side of the distal phalanx of the 4th finger. There was no injury on the dorsal middle phalanx of the 3rd finger. Reconstruction was planned for the 2x4 cm defect including the dorsal middle phalanx of the 2nd finger and the nail bed. The case was started under regional anesthesia and tourniquet with loop guidance. The damaged extensor tendon was repaired. A 3x5 cm flap was designed from the dorsal side of the 3rd finger. The skin including the epidermis and papillary dermis was de-epithelialized. The reticular dermis is preserved. An incision was made for the reticuloadipofascial flap. The defect side of the flap was incised. The reticuloadipofascial flap was elevated in a way that the paratenon of the extensor tendon was protected. It was transferred to the defect area by rotating it 180 degrees. The tourniquet was released. Bleeding control was performed. A full thickness skin graft wider than the defect area was taken from the medial right arm to cover the 2nd and 3rd fingers. The graft was de-fatted. Holes were opened in the graft. The graft was adapted to cover both the flap donor area and the transferred flap. Both fingers were fixed to each other with fixation sutures from the proximal and distal sides. A generous furacyn ointment dressing was applied instead of a tie-over dressing. A short arm splint was applied. The dressing was removed on the 5th postoperative day. It was observed that the graft had adapted and the flap circulation was good. Flap separation was performed under local anesthesia on the 20th postoperative day. Wound follow-up was performed for another week. Hand rehabilitation was started at the end of the 4th postoperative week. Our case example and results are shown in figure 2.a-j.

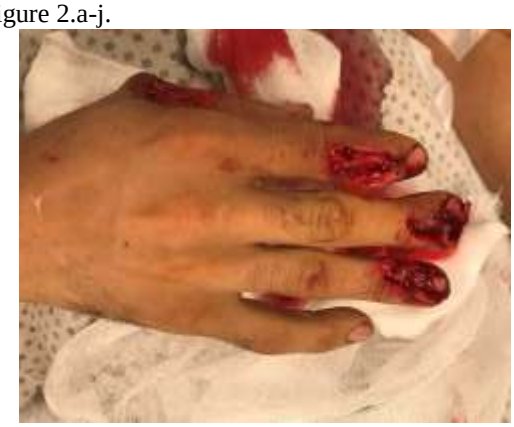

Figure 2.a. 2. A 2x4 cm defect including the nail bed on the dorsal side of the middle phalanx of the

2nd finger; Skin damage on the dorso-ulnar side of the proximal phalanx of the 1st finger; Nail and nail bed damage on the 3rd finger; Skin defect and nail and nail bed damage on the radial side of the DIPJ and distal phalanx of the 4th finger.

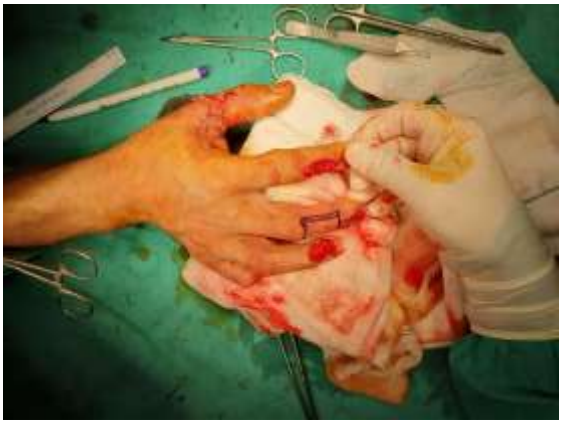

Figure 2.b. 2. A 2x4 cm defect including the nail bed on the dorsal side of the middle phalanx of the 2nd finger; Nail bed, bone tissue and extensor tendon exposed; Extensor tendon damaged; The defect extends to the DIPJ.

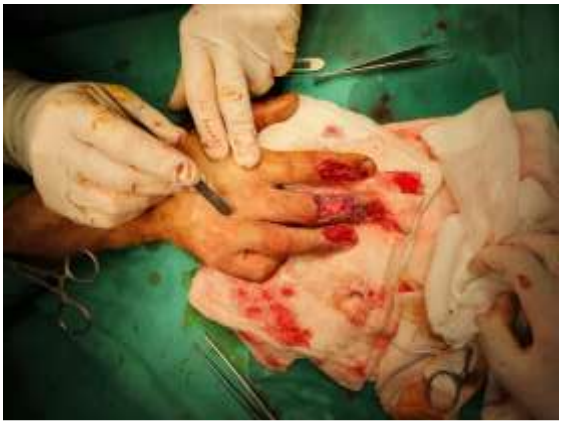

Figure 2.c. 3. A 3x5 cm flap design from the dorsal side of the 3rd finger; skin de-epidelization including epidermis and papillary dermis.

Figure 2.d. Elevation of reticuloadipfascial flap.

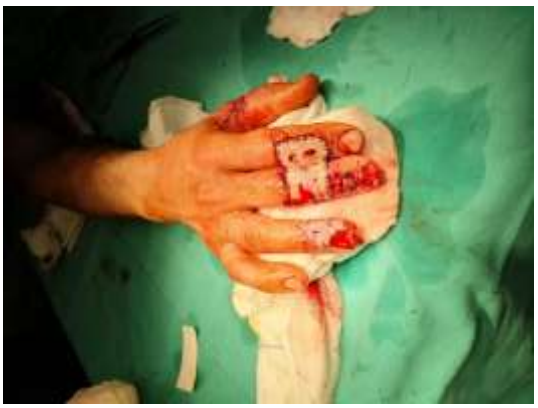

Figure 2.e. Transfer of reticulodermo-adifascial flap to defect area by rotating it 180 degrees.

Figure 2.f. Repair of flap donor area and flap surface with full thickness skin graft.

Figure 2.g. Postoperative 3rd week view.

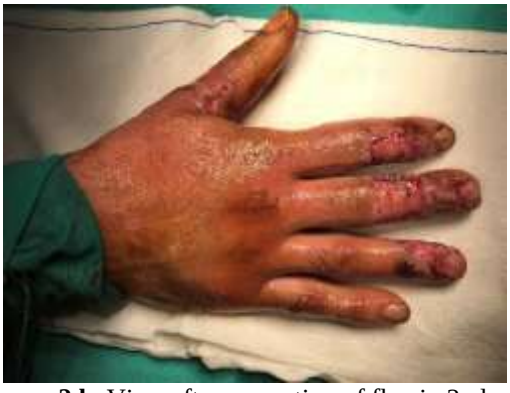

Figure 2.h. View after separation of flap in 2nd session operation.

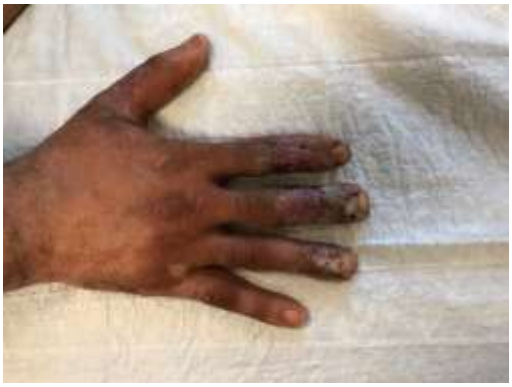

Figure 2.i. Dorsal view of fingers 1 month after flap separation.

Figure 2.j. Volar view of fingers 1 month after flap separation.

RESULTS

The characteristics of the patients, defect location, causes, size and postoperative findings are given in table 1. No flap loss was observed in any patient. No inclusion cyst was observed in the flap application area. There was minimal, mild joint movement restriction in DIPJ in 2 patients. Wound appearance and functional gain were acceptable and good.

Table 1. Characteristics of patients, defect location, causes, size and postoperative findings.

Number of patients	11
Gender	Female:3 Male:8
Dominant hand	Right hand:10 Left hand:1
Occupation	Farmer:7 Manual worker:2 Civil servant:1 Student:1
Smoking	Smoking + :8 Smoking - :3
Defect causative factor	Trauma:7 Tumoral mass:2 Chronic wound:1 Burn scar:1
Defect localization	Right hand 2nd finger middle phalanx dorsum:4 Right hand 2nd finger distal phalanx dorsum and DIPJ:2 Right hand 3rd finger middle phalanx dorsum:2 Right hand 4th finger middle phalanx dorsum:1 Left hand 2nd finger middle phalanx dorsum:1 Left hand 3rd finger middle phalanx dorsum and DIPJ:1
Defect size	Smallest: 2x2 cm Largest: 3x4 cm
Graft donor area	Right arm medial: 9 Left arm medial: 2
Flap loss	none
Joint function loss	2 Patients have slight limitation in DIPJ
Inclusion cyst formation in the flap	No
Wound appearance	Good

DISCUSSION

The reverse de-epithelialized crossed finger flap, a modification of the traditional crossed finger flap, provides good coverage. It has been found to be a reliable option for reconstructing dorsal defects of the finger. The reverse cross finger flap is also a very useful flap for open wounds on the dorsum of the finger, especially in the presence of exposed bone and tendon; and for nail bed defects that cannot be closed with other techniques.

The cross finger flap was first described by Gurdinin in 1950 and by Pangman in 1951 as a soft tissue cover for covering defects on the volar surfaces of single fingers (12).

Atasoy reported four patients with nail bed defects treated with axial pattern adipofascial flaps

after the reverse dermis flap described by Pakiam (2). Voche and Merle described the axial pattern homodigital subcutaneous flap for the treatment of dorsal finger defects (13). The most important disadvantages of this procedure are the distortion of the pedicle after transposition of the flap over the defect and the fact that it can only cover two thirds of the defect. The axial patterned adipofascial cross finger flap described by El-Khatib has the disadvantages of long dissection time and sacrificing the digital artery (14). Al-Qattan modified the existing technique by turning the flap proximally from the same finger (15).

Elhoda infiltrated isotonic saline for easy dissection of the flap (4). We did not find this necessary since we easily dissected with a loop.

A separation surgery should be performed on the 10th to 14th postoperative day; thus, joint stiffness, which is an important complication, will be prevented (16).

In the reverse cross finger flap technique described by Atasoy; the skin is elevated as a full-thickness skin flap. Then, the adipofascial tissue is transferred to the defect area as a reverse cross finger flap. The elevated full-thickness skin flap is adapted same place.

It is difficult to de-epithelialize only the epidermis and there is a possibility that the epidermis may remain in the flap. The reason we included the papillary dermis in de-epithelialization is to prevent the formation of hair, sebaceous and sweat gland cysts. By including the reticular dermis in the flap, we have prepared a stronger flap. We have also contributed to the blood supply of the flap from the deep dermal plexus. By keeping the reticular dermis intact, we have also prevented damage to the subdermal vascular plexus.

In previous applications, the reverse cross finger flap was designed as dermoadipofascial by de-epithelializing the epidermis or adipofascial by de-epithelializing the epidermis and dermis. Our aim in designing this flap is to prevent the formation of hair, sebaceous and sweat gland cysts and to contribute to the blood supply of the flap while preventing this.

As Karthikeyan stated, the possibility of inclusion cysts after the flap transferred to the defect area after de-epithelialization is a disadvantage (12). We aimed to prevent this disadvantage by modifying this flap and including only the reticular dermis in the adipofascial tissue. He drew attention to the possibility of partial necrosis of the dermal flap and the fact that graft harvesting was not optimum over the adipofascial flap (12). In the study conducted by Coşkunfirat, the skin flap was elevated in a zigzag shape, then the adipofascial flap was elevated and transferred to the defect area (17). They did not provide any information about the viability of the skin flaps elevated in a zigzag shape. Therefore, we did not use a dermal flap. We also did not use the skin graft taken from the flap. We covered the flap donor area and the flap with a healthy full-thickness skin graft of the same properties taken from the medial arm.

The primary goal of soft tissue reconstruction in the hand is to restore normal or near-normal motion of the fingers. This requires thin, flexible skin that allows the underlying tendons to glide freely. Although an adipofascial flap with skin graft is a thin flap, authors have obtained poor results in adipofascial flap reconstructions in the hand (10). The skin graft contracts significantly, limiting movement and eliminating the perceived advantages of the thin flap. To prevent this and to prevent the scarring effect of the skin graft on the adipofascial flap, we left the reticular dermis over the adipofascial tissue intact. We reduced secondary contraction by repairing the donor area and the flap with a full-thickness skin graft. This contributed to our functional gain in the finger.

CONCLUSION

Modified reverse cross finger flap: The reticulodermo-adipofascial flap is a satisfactory option for repairing defects on the dorsal aspect of the finger with the advantages of minimal donor site deformity, flexibility, ease, feasibility, a functional finger joint, ideal blood supply, flap design in correct plan and prevention of cystic lesions.

REFERENCES

1. Jiao H , Ding X , Liu Y , Zhang H , Cao X. Clinical experience of multiple flaps for the reconstruction of dorsal digital defects. Int J Clin Exp Med. 2015;8(10):18058-65.
2. Ekinci Y, Gürbüz K. Is the cross-finger flap a good option at the extensor zone defect?. Jt Dis Relat Surg. 2020;31(2):267-72.
3. Gürbüz K, Uzun E, Ozan F. Covering the Dorsal Finger Defect with Reverse Cross Finger Flap. J Clin Anal Med. 2016;7(2):262-4.
4. Elhoda MSA, Keshk TF, El Gamal AS, Taalab AA. A comparison between reversed cross-finger flap and Quaba flap in reconstruction of proximal dorsal digital defects. Egyptian J Surgery. 2021;39:622–631.
5. Kim DH, Seo KB, Lee SH, Lee HJ, Kang HJ. Reverse digital artery crossfinger flap for reconstruction of failed finger replantation. J Orthop Surg (Hong Kong). 2019;27:2309499018816773.
6. Fejjal N, Belmir R, El Mazouz S, Gharib N, Abbassi A, Belmahi A. Reversed cross fi nger subcutaneous fl ap: A rapid way to cover fi nger defects. Indian J Plast Surg. 2008:41(1):55-7
7. Atasoy E. The Reverse Cross Finger Flap. J Hand Surg Am. 2016;41(1):122e128.

8. Ahmed MM, Rao AL. Review of cross-finger flaps: Indications and modifications. International journal of science and research (IJSR). 2017;6(3):2299-2303.
9. Tabarestani A , Khlopas A, Wright T , Kim J. Basal Cell Carcinoma of the 2nd Digit Treated With the Reverse Cross-Finger Flap: A Case Report. Cureus. 16(7):e65105.
10. Das De S, Sebastin SJ. Considerations in Flap Selection for Soft Tissue Defects of the Hand. Clin Plastic Surg. 2109;46(3):393–406.
11. Sleem EA, Saied SM, Mohamed KA, Elsayed GY. A Comparison between Reversed Cross Finger Flap and Reversed Island Homo-digital Flap in Reconstruction of Distal Dorsal Digital Defects. The Egyptian Journal of Hospital Medicine. 2023;90(2):2567-73.
12. Karthikeyan G, Renganathan G, Subashini R. Versatility and modifications of the cross-finger flap in hand reconstruction. International journal of scientific study. 2017; 5(6):35-46
13. Voche P, Merle M. The homodigital subcutaneous flap for cover of dorsal finger defects. Br J Plast Surg. 1994;47:435-9.
14. El-Khatib H. Adipofascial axial pattern cross-finger flap. Plast Reconstr Surg. 1996;97:850-3.
15. Al-Qattan MM. The adipofascial turnover flap for coverage of the exposed distal interphalangeal joint of the fingers and interphalangeal joint of the thumb. J Hand Surg Am. 2001;26:1116-9.
16. Shao X, Chen C, Zhang X, Yu Y, Ren D, Lu L. Coverage of fingertip defect using a dorsal island pedicle flap including both dorsal digital nerves. J Hand Surg Am. 2009;34:1474-81.
17. Coşkunfirat OK, Velldedeoğlu HV, Bozdoğan N, Kurtay A, Demir Z. El Parmaklarindaki Yumuşak Doku Defektlerinin Onariminda Adipofasyal Ters Çevirme Flebi. Türk Plast Cer Derg. 1997;5(1).

REVIEW

Nevena G. Ianova[1,2,3]

[1] Department of Urology and General Medicine, Faculty of Medicine, Medical University of Plovdiv, Bulgaria
[2] St Karidad MHAT, Karidad Medical Health Center, Cardiology, Plovdiv, Bulgaria
[3] Department of "Internal Diseases, Social Medicine, Physiotherapy and Rehabilitation, Disaster Medicine", Faculty of Medicine, University "Prof. Dr. Asen Zlatarov", Burgas, Bulgaria

Corresponding Author:
Nevena G. Ianova
mail:nevenai@yahoo.com

Received: 04.12.2024
Acceptance: 26.03.2025
DOI:10.18521/ktd.1594423

Konuralp Medical Journal
e-ISSN1309–3878
konuralptipdergi@duzce.edu.tr
konuralptipdergisi@gmail.com
www.konuralptipdergi.duzce.edu.tr

Genetic Testing in Primary Care – Myth or Reality?

ABSTRACT

The Swiss scientist Friedrich Miescher first identified deoxyribonucleic acid (DNA) in 1869, marking the beginning of genetic research. Subsequent studies led to the discovery of DNA's composition and structure, culminating in Watson and Crick's 1953 model of its three-dimensional, double-helical structure. DNA's functional units, genes, encode proteins essential for biological processes, and variations in DNA sequences are classified as polymorphisms or mutations based on their population frequency. Advances in genetic research have facilitated the development of cytogenetic, biochemical, and molecular tests, enabling the precise analysis of genetic material. These tests provide valuable information for personalized medicine, particularly in pharmacogenomics and predictive medicine.

Once considered an exclusive domain of specialized medicine, genetic testing is now becoming an integral component of clinical practice. Technological advancements, declining costs, and increased understanding of DNA's role in disease susceptibility have contributed to its growing accessibility. Genetic testing holds significant potential in primary care, offering insights into disease predisposition, optimizing drug therapy, and enabling early interventions. However, despite its promise, the integration of genetic testing into routine medical practice remains a challenge due to concerns related to clinical utility, ethical considerations, and the need for physician education in genetics.

A key question persists: does genetic testing offer practical benefits for routine patient care, or does it remain largely theoretical? This review aims to explore the role of genetic testing in primary care, assessing its potential advantages while addressing challenges that may hinder its widespread adoption. By evaluating the current state of genetic testing, this analysis seeks to determine whether it represents a transformative tool in modern medicine or an evolving field with yet-to-be-fulfilled promises.

Keywords: DNA, Genetic Testing, Primary Care, General Practitioner.

Birinci Basamak Sağlık Hizmetlerinde Genetik Testler – Mit mi Gerçek mi?

ÖZET

İsviçreli bilim insanı Friedrich Miescher, 1869 yılında deoksiribonükleik asidi (DNA) keşfederek genetik araştırmaların temelini atmıştır. Sonraki çalışmalar DNA'nın bileşimi ve yapısını ortaya çıkarmış, bu süreç 1953 yılında Watson ve Crick'in DNA'nın üç boyutlu çift sarmallı yapısını tanımlamasıyla sonuçlanmıştır. DNA'nın işlevsel birimleri olan genler, biyolojik süreçler için gerekli proteinleri kodlamakta olup, DNA dizilimlerindeki değişiklikler popülasyon içindeki sıklıklarına bağlı olarak polimorfizm veya mutasyon olarak sınıflandırılmaktadır. Genetik araştırmalardaki ilerlemeler, sitogenetik, biyokimyasal ve moleküler testlerin gelişmesini sağlamış ve genetik materyalin hassas analizini mümkün kılmıştır. Bu testler, özellikle farmakogenomik ve prediktif tıp alanlarında kişiye özel tıbbi yaklaşımlar geliştirilmesine katkı sunmaktadır.

Bir zamanlar yalnızca uzmanlık gerektiren bir alan olarak görülen genetik testler, günümüzde klinik pratiğin ayrılmaz bir parçası haline gelmektedir. Teknolojik ilerlemeler, test maliyetlerinin azalması ve DNA'nın hastalık duyarlılığı üzerindeki rolünün daha iyi anlaşılması, genetik testlerin erişilebilirliğini ve klinik önemini artırmıştır. Genetik testler, hastalık yatkınlığı hakkında bilgi sağlama, ilaç tedavisinin bireyselleştirilmesi ve erken müdahale fırsatları açısından birincil sağlık hizmetlerinde büyük bir potansiyele sahiptir. Ancak, klinik fayda, etik kaygılar, veri gizliliği, sağlık politikaları ve hekimlerin genetik konularındaki eğitimi gibi zorluklar, bu testlerin rutin tıbbi uygulamalara entegrasyonunu sınırlayan önemli faktörlerdir.

Bu bağlamda, önemli bir soru ortaya çıkmaktadır: Genetik testler gerçekten hasta bakımında pratik faydalar sunmakta mıdır, yoksa büyük ölçüde teorik bir alan olarak mı kalmaktadır? Bu derleme, birincil sağlık hizmetlerinde genetik testlerin rolünü incelemeyi, potansiyel avantajlarını değerlendirmeyi ve yaygın kullanımını engelleyebilecek bilimsel, etik ve pratik zorlukları ele almayı amaçlamaktadır. Mevcut durumu analiz ederek, genetik testlerin modern tıpta dönüştürücü bir araç olup olmadığını veya hala tam anlamıyla gerçekleştirilememiş bir alan mı olduğunu belirlemeye çalışmaktadır.

Anahtar Kelimeler: DNA, Genetik Test, Birinci Basamak Sağlık Hizmetleri, Pratisyen Hekim.

INTRODUCTION

In 1869, the Swiss scientist Friedrich Miescher was the first to bring to the global scientific community's attention the existence of deoxyribonucleic acid (DNA). His research involved white blood cells, through which he successfully isolated a substance previously unknown to science. This substance, located in the cell nucleus, was named "nuclein," a term later replaced by "nucleic acid" and subsequently "deoxyribonucleic acid." Miescher conducted a chemical analysis and discovered that nuclein contained a high amount of phosphorus, leading him to recognize it as a novel molecule. Initially, he hypothesized that nuclein might play a role in hereditary transmission, although he later abandoned this idea (1).

Although Miescher's name faded into obscurity, Russian biochemist Phoebus Levene continued investigating the composition of nuclein (2). Through hydrolysis and the decomposition of nucleic acids from yeast, Levene proposed in 1919 that nucleic acids are composed of a series of nucleotides, each containing one of four nitrogenous bases, a sugar molecule, and a phosphate group. He was also the first scientist to identify the carbohydrate components of DNA-deoxyribose and RNA-ribose. He is credited with identifying the presence of the bases adenine, guanine, cytosine, and thymine in DNA, as well as uracil replacing thymine in RNA. Additionally, he proposed a tetranucleotide structure consisting of repeating units of four nucleotides arranged in a specific order (adenine, guanine, cytosine, thymine, and so forth), a hypothesis that was later refuted by other scientists. Nevertheless, his polynucleotide model remains valid today, as does his discovery that nucleotides are linked through bonds between the phosphate group of one nucleotide and the sugar of the next. This sequence forms the sugar-phosphate backbone, which constitutes the foundation of the DNA molecule. An interesting historical fact is that Levene believed the structure he proposed was too simple to serve as the carrier of genetic information. This notion somewhat delayed the acceptance of DNA as the molecule responsible for genetic material (3).

In 1944, Oswald Avery became the first scientist to make the groundbreaking discovery that DNA, and not proteins, is the carrier of genetic information and a fundamental component of genes and chromosomes (4). The next significant contribution to uncovering the structure of DNA came from American biochemist Erwin Chargaff, who was influenced by Avery's work. In 1950, Chargaff reported that the ratio and quantities of the four bases in the DNA double helix are constant but vary between different species of organisms (5). However, he was unable to explain the specific relationships between the bases, namely, that adenine pairs with thymine and cytosine pairs with guanine within the molecular structure of DNA (2).Chargaff's base-pairing rule, combined with the contributions of English researchers Rosalind Franklin and Maurice Wilkins through X-ray crystallography-a technique for determining the three-dimensional atomic structure of molecules-formed the foundation for the discovery of the **three-dimensional, double-helical model of DNA**, presented by Watson and Crick in 1953. Their model revealed that the bases are connected by hydrogen bonds, the strands are antiparallel and complementary, and the helix is predominantly right-handed (2).

The functional units of DNA are genes, which encode the synthesis of specific proteins essential for the structure and function of the cell and determine hereditary traits (6).

The continuous human endeavor to decode the genetic information embedded in DNA culminated in 1990 with the initiation of one of the most ambitious scientific projects of our time-the **Human Genome Project**, which aimed to sequence the entire human genome (i.e., determine the exact sequence of DNA bases). In 2000, the first reference genome was announced, leaving 8% of heterochromatic regions unanalyzed. However, in April 2022, the **Telomere-to-Telomere (T2T) Consortium** declared the completion of the sequencing process, providing information on the sequence of 3.055 billion base pairs, excluding the Y chromosome (7).

It is estimated that the human genome contains between 20,000 and 25,000 genes (8). Each individual has a unique genome, except for monozygotic twins. This uniqueness is attributed to the presence of **single nucleotide polymorphisms (SNPs)**-variations in which a single nucleotide, such as thymine, is replaced by another, or **short tandem repeats (STRs)** (9). It is estimated that there is one SNP for every 2.0 kilobase pairs, with over 1.4 million identified (10).

DNA variants with a frequency greater than 1% in the population are classified as SNPs, while those with a frequency below 1% are considered mutations (11). These genomic changes may have no impact on the synthesis of normal proteins, but in some cases, they can lead to pathological alterations in function and, consequently, be associated with disease development (12).

Advances in science and technological achievements now enable the use of genetic information for the diagnosis and treatment of diseases, forming the foundation of what is known as **genomic medicine**. This has the potential to entirely transform the way medicine is practiced (13).

Genetic testing, once considered a niche of specialized medicine, is rapidly entering clinical practice due to increased accessibility from decreasing test costs and a growing understanding

of the relationship between DNA changes and disease development. However, an important question arises: does genetic testing have practical applications in routine medical practice, or does it still reside in the realm of promises and theoretical possibilities?

The aim of this review is to analyze the role of genetic testing in primary care, exploring both its potential benefits and the challenges associated with its implementation.

Foundations of Genetic Testing: The primary goal of genetic testing is to identify changes in an individual's genetic material. The information obtained can indicate a disease or a predisposition to one and can be used to develop therapeutic or preventive strategies. It may also assist in family planning, career choices, or future professional development. The material analyzed for genetic testing can include blood, buccal mucosa, hair, skin, or other tissues.

Types of Tests: Depending on the genetic material or its product being analyzed, genetic testing can be categorized into the following types (14) (Table 1):

Table 1. Type of Genetic Testing

Type Genetic Testing	Description	Common Techniques	Clinical Applications
1. Cytogenetic	Examines entire chromosomes to detect structural and numerical abnormalities.	**-Karyotyping:** Microscopic observation of stained chromosomes. **-Fluorescent in situ hybridization (FISH):** Uses fluorescent molecules to detect genetic anomalies (insertions, deletions, translocations, and amplifications). .	DiGeorge syndrome, Chronic myelogenous leukemia (CML), B-cell lymphoma**.**
2. Biochemical	Measures protein levels, enzyme activity, and metabolic products encoded by specific genes.	**-Enzyme activity assays. -Metabolic product measurement. -Protein structural analysis.**	Detection of enzyme deficiencies, metabolic disorders, and structural protein abnormalities.
3. Molecular	Investigate DNA sequence variations, genetic variants, and mutations.	**-Whole-genome sequencing (WGS):** Analyzes the entire DNA sequence. **-Next-generation sequencing (NGS):** High-throughput DNA sequencing. **- Exome sequencing:** Focuses on protein-coding regions. **-Targeted gene analysis:** Uses polymerase chain reaction (PCR) and hybridization methods to detect specific mutations.	Identification of disease-causing mutations, SNPs, and genetic predispositions.

In addition to diagnosing diseases or risk assessment, genetic tests have found applications in **pharmacogenomics**, driven by the accumulation of data on the human genome and technological advancements. Numerous studies explore the impact of genetic variants on the distribution of drugs within the body (**pharmacokinetics**) and the sensitivity or response to treatment (**pharmacodynamics**). These findings are critical for **personalized medicine** (15).

The goal of pharmacogenomic testing is to optimize and maximize therapeutic efficacy while minimizing side effects and toxicity, based on the patient's individual genotype. Examples of medications with established genotype-related effects or risks include **warfarin**, **clopidogrel**, **abacavir**, **statins**, and others. This knowledge facilitates an individualized, lifelong treatment approach (16).

Another potential application of genetic testing in primary care lies in the field of **predictive medicine**. It can serve as a valuable tool for the early identification of patients at high risk for common diseases. Examples include testing for **BReast CAncer gene 1 (BRCA1)** and **BRCA2** mutations in women, which are associated with an increased risk of breast and ovarian cancers. Approximately 60% of women carrying such mutations will develop breast cancer during their lifetime. For BRCA1, the risk of developing ovarian cancer is estimated to be 39%–58%, while for BRCA2, the risk ranges between 13% and 29% (17). Information from these tests can guide decisions regarding targeted preventive measures,

such as regular screening or prophylactic surgery (18).

Additionally, **Apolipoprotein E (APOE)** genotypes have been associated with risks of dementia, Alzheimer's disease, and cardiovascular diseases (19). Such knowledge can inform early preventive interventions, including the implementation of dietary and cognitive strategies (20).

An important area where genetic screening tests can be conducted in primary care settings is **reproductive medicine**. Prenatal and carrier tests are particularly valuable for couples planning to start a family. These tests can identify the risk of severe hereditary diseases such as **cystic fibrosis** (21) or **thalassemia** (22).

Despite the great potential genomics holds, the expectations and opportunities for its implementation remain a process that is advancing at a slower pace (23). It is therefore crucial to identify the main barriers hindering its integration and to seek optimal solutions, particularly in primary care, where conditions are most favorable for the application of genetic testing in these areas. This is due to the close and long-term relationship with patients, familiarity with their medical and family histories, and broader opportunities for risk management and disease prevention.

As such, general practitioners can play a central role not only in identifying suitable patients but also in coordinating efforts with other healthcare professionals across the different levels of the healthcare system, including geneticists.

Challenges Faced by Primary Care Providers: The main barriers to the integration of genetic medicine into routine patient care can be categorized into the following areas, as identified in a systematic analysis of 38 publications (24):

1. **Knowledge:** The most frequently reported issue by participants in the studies was a **lack of general knowledge** in the field of genetics and the resulting lack of confidence (25,26), as well as insufficient training in clinical genetics (27). Other commonly noted deficits included inadequate preparation for obtaining a family history (28) and the necessary information required to collect (29).
2. **Skills:** The lack of **confidence** in possessing the necessary qualifications for genetic counseling was the most frequently cited issue in 16 of the 38 studies. A U.S.-based survey of 1,763 primary care physicians found that only 28.8% felt qualified to provide genetic counseling (30). Additionally, the inability to apply recommendations from guidelines regarding the interpretation of family history (31) and uncertainty in determining familial relationships between patients were also commonly identified problems (32).

Other studies reported that physicians lacked the skills to **interpret genetic test results** (33). For instance, a Canadian study of 341 primary care physicians revealed that only 15% felt capable of interpreting genetic results, and fewer than 10% were adequately informed about genetic testing (30). In 14 of the 38 studies, physicians admitted to lacking confidence in ordering genetic tests and struggled to explain the limitations of such tests to their patients, particularly concerning false-positive or false-negative results.

Moreover, 10 of the 38 studies reported difficulties in **assessing genetic risk**, including for specific conditions such as cancer (30). In a study conducted among 860 primary care physicians in the U.S., 38.3% felt uncomfortable conducting screening tests, providing preventive recommendations, or determining which patients should be referred for further consultation (34).

Another commonly identified barrier was the lack of awareness of available **educational resources** related to genetics and how to access them (26,35,36). Many physicians also expressed uncertainty about when and how to refer patients to a genetic specialist, a challenge cited in multiple studies (33,34).

These challenges highlight the need for targeted education and training programs to equip primary care providers with the knowledge and skills required for the effective application of genetic medicine in routine practice.

3. Ethical, Legal, and Social: The potential for patient **distress** and **anxiety** associated with genetic risk was frequently highlighted by physicians in 16 of the reviewed publications (25,35). Physicians also reported challenges related to the emotional reactions of patients upon receiving genetic test results (37). Identifying carrier status for a pathogenic gene could lead individuals to perceive themselves as unhealthy, even if no symptoms are present (38).

Another deterrent cited was the possibility of discovering **incidental findings** during testing, which could cause stress about the potential development of diseases in the future (39).

A significant concern raised in 15 of the 38 publications was the fear of social or insurance **discrimination**. For example, in a study of 1,251 primary care physicians, 80% expressed concern that patients with positive test results might face discrimination from insurers (30). Similar findings were reported in a study of 1,222 California physicians, where 75% shared the same concerns, noting that fear of discrimination could lead patients to decline genetic testing (40).

These challenges underscore the need for robust ethical guidelines, legal protections, and patient education to address concerns surrounding genetic testing and promote its adoption in clinical practice.

A significant barrier is the loss of **confidentiality** and the potential disclosure of genetic risk information to family members related

to the patient. In a study conducted among U.S. physicians**,** 53% reported that they would not be able to guarantee confidentiality to their patients after receiving genetic test results (30). Similarly, 61% of Swiss primary care physicians identified this issue as a major reason limiting their use of genetic testing for breast cancer due to its implications for other family members (41).

The use of **prenatal tests** has also presented challenges. Physicians expressed concerns that the results might negatively impact the emotional course of the pregnancy, lead to unnecessary medical interventions, or drive parents toward a desire for a "perfect" child. This could, in turn, result in social inequality and stigma against individuals with genetic disorders (41,42).

These findings highlight the ethical complexities and societal implications associated with genetic testing, emphasizing the need for careful consideration and policy development to address these barriers.

4. Organizational: The lack of access to **genetic services**, including consultations with genetic specialists, is the most frequently cited barrier by physicians (28,37,43,44). The reasons for this vary across studies and include inconvenient locations of genetic centers, lack of transportation in remote areas, absence of regulations for referrals to other levels of the healthcare system, and limited availability of genetic tests (30,32,34,44,45).

Time constraints are another significant factor reported by physicians. The need to take a detailed family history is often mentioned as a restrictive reason (32), as is the time required to explain genetic test results (31). In some studies, results indicated that the long turnaround time for prenatal test results impacted subsequent decisions regarding treatment (28).

These organizational challenges highlight the need for improved infrastructure, streamlined processes, and time-efficient solutions to facilitate the integration of genetic services into primary care.

A challenge highlighted by primary care physicians is their **perception of their role in providing genetic-related services** (41,46), with some reporting uncertainty about what their role entails (27,33,35,43). In several studies, it is noted that genetics is not perceived as an integral part of primary care and is considered to have less relevance at this level of the healthcare system (25,35,36,37,44,57,48). Many physicians believe that genetics falls under the domain of specialists and requires specific expertise (38,44,48).

The **cost** of genetic tests is frequently cited as a significant barrier to their integration into primary care, compounded by the lack of reimbursement for ordering such tests and the subsequent need for patient counseling (35).

The perception of genetics as a peripheral aspect of primary care responsibilities has also been identified as a reason for difficulties in integrating it into routine activities (26,45,49,50). In some studies, physicians reported challenges with incorporating family history into electronic health records (EHRs) (33).

These findings suggest that addressing misconceptions about the role of primary care physicians in genetics and providing clearer guidelines and resources may facilitate the integration of genetic services into primary care practice.

5. Scientific Evidence on Genetic Tests and Testing: Although genetic tests provide opportunities to identify diseases or assess the risk of their development, physicians perceive the **lack of therapeutic options** for certain conditions as a barrier to the broader application of these tests in practice (34,39,41,48). Additionally, some physicians believe that treatment approaches would not differ regardless of the availability of genetic test results (25,36,45,47,48).

In some studies, the insufficient **accuracy of genetic tests** is highlighted as a barrier to their use, particularly regarding false-positive results in cancer testing. Furthermore, 45% of physicians in one study believed that the risk of cancer development remains unclear even after genetic testing (30).

Similar findings were reported in another systematic review published in 2003, illustrating the lack of significant progress in integrating genetic testing into the routine activities of primary care physicians. Nonetheless, many physicians expressed a willingness to receive training in genetics, even though they did not consider it a priority (50).

These findings underscore the need for improved accuracy of genetic tests, development of actionable therapeutic strategies, and educational efforts to enhance the integration of genetics into primary care.

CONCLUSION

Despite significant progress in genetic research, both scientific and technological, the implementation of genetic testing as part of the routine activities of primary care physicians is advancing at a slow pace and currently holds limited significance. This lack of focus is attributed to various factors, with the most prominent being insufficient knowledge in this specialized field, a lack of confidence and experience in taking family histories, and challenges in ordering and interpreting test results, which often require subsequent genetic counseling. The high cost of genetic testing and the lack of reimbursement further restricts the broader adoption and use of these modern methods in routine patient care at this level of the healthcare system.

The outlined challenges can serve as a foundation for developing strategies to train general practitioners, equipping them with the necessary knowledge and skills in this promising field.

Additionally, national policies aimed at improving access to innovative testing methods could help unlock the potential of genetics to transform healthcare.

REFERENCES

1. Dahm R. Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. Hum Genet. 2008;122(6):565-81.
2. Pray L. Discovery of DNA structure and function: Watson and Crick. Nature Education. 2008;1(1):100.
3. Levene PA. The structure of yeast nucleic acid: IV. Ammonia hydrolysis. J Biol Chem. 1919;40(2):415-24.
4. Rijkers G, Dekker K, Berbers G. Oswald Avery: Pioneer of bacterial vaccines and the first to discover the function of DNA. Cureus. 2024;16(10):e71465.
5. Chargaff E. Chemical specificity of nucleic acids and mechanism of their enzymatic degradation. Experientia. 1950;50(4):368-76.
6. National Research Council (US) Committee on Mapping and Sequencing the Human Genome. Washington (DC): National Academies Press (US); 1988.
7. Nurk S, et al. The complete sequence of a human genome. Science. 2022;376:44-53.
8. International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature. 2004;431(7011):931-45.
9. Brown TA. Genomes. 2nd ed. Oxford: Wiley-Liss; 2002.
10. Sachidanandam R, Weissman D, Schmidt SC, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. Nature. 2001;409(6822):928-33.
11. Karki R, Pandya D, Elston RC, et al. Defining "mutation" and "polymorphism" in the era of personal genomics. BMC Med Genomics. 2015;8:37.
12. Sripichai O, Fucharoen S. Genetic polymorphisms and implications for human diseases. J Med Assoc Thai. 2007;90(2):394-8.
13. Williams MS. Early lessons from the implementation of genomic medicine programs. Annu Rev Genomics Hum Genet. 2019;20:2.1-2.23.
14. Genetic Alliance; District of Columbia Department of Health. Understanding genetics: A District of Columbia guide for patients and health professionals. Washington (DC): Genetic Alliance; 2010.
15. Sadée W, Dai Z. Pharmacogenetics/genomics and personalized medicine. Hum Mol Genet. 2005;14:R207-14.
16. Cavallari LH. Tailoring drug therapy based on genotype. J Pharm Pract. 2012;25(4):413-6.
17. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic. Version 3.2024.
18. King M, Levy-Lahad E, Lahad A. Population-based screening for BRCA1 and BRCA2: 2014 Lasker Award. JAMA. 2014;312(11):1091-2.
19. Haan MN, Mayeda ER. Apolipoprotein E genotype and cardiovascular diseases in the elderly. Curr Cardiovasc Risk Rep. 2010;4(5):361-8.
20. Genin E, Hannequin D, et al. APOE and Alzheimer disease: a major gene with semi-dominant inheritance. Mol Psychiatry. 2011;16(9):903-7.
21. Elson J, Drakeley A, Achilli C, Canham N, Kulke C. The use of expanded carrier screening in reproductive medicine. BJOG. 2024;131(10):e81-5.
22. Cousens NE, Gaff CL, Metcalfe SA, Delatycki MB. Carrier screening for beta-thalassaemia: a review of international practice. Eur J Hum Genet. 2010;18(10):1077-83.
23. Manolio TA, Chisholm RL, et al. Implementing genomic medicine in the clinic: the future is here. Genet Med. 2013;15(4):258-67.
24. Mikat-Stevens NA, et al. Primary-care providers' perceived barriers to integration of genetics services: a systematic review of the literature. Genet Med. 2015;17(3):169-76.
25. Robins R, Metcalfe S. Integrating genetics as practices of primary care. Soc Sci Med. 2004;59:223-33.
26. Huang QR, Trevena L, McIntosh J. GPs' experience and attitudes toward new genetics: barriers and need. Aust Fam Physician. 2004;33:379-80.
27. Aalfs CM, Smets EM, de Haes HC. Referral for genetic counselling during pregnancy: limited alertness and awareness about genetic risk factors among GPs. Fam Pract. 2003;20:135-41.
28. Carroll JC, Cappelli M, Miller F, et al. Genetic services for hereditary breast/ovarian and colorectal cancers - physicians' awareness, use, and satisfaction. Community Genet. 2008;11(1):43-51.
29. Al-Habsi H, Lim JN, Chu CE, Hewison J. Factors influencing the referrals in primary care of asymptomatic patients with a family history of cancer. Genet Med. 2008;10(10):751-7.
30. Freedman AN, Wideroff L, Olson L, et al. US physicians' attitudes toward genetic testing for cancer susceptibility. Am J Med Genet A. 2003;120A(1):63-71.
31. Wood ME, Stockdale A, Flynn BS. Interviews with primary care physicians regarding taking and interpreting the cancer family history. Fam Pract. 2008;25(5):334-40.

32. Iredale R, Jones L, Gray J, Deaville J. 'The edge effect': an exploratory study of some factors affecting referrals to cancer genetic services in rural Wales. Health Place. 2005;11(3):197-204.
33. Houwink EJ, van Luijk SJ, Henneman L, et al. Genetic educational needs and the role of genetics in primary care: a focus group study with multiple perspectives. BMC Fam Pract. 2011;12:5.
34. Vig HS, Armstrong J, Egleston BL, et al. Cancer genetic risk assessment and referral patterns in primary care. Genet Test Mol Biomarkers. 2009;13(6):735-41.
35. Bathurst L, Huang QR. A qualitative study of GPs' views on modern genetics. Aust Fam Physician. 2006;35(6):462-4.
36. Trinidad SB, Fryer-Edwards K, Crest A, et al. Educational needs in genetic medicine: primary care perspectives. Community Genet. 2008;11(3):160-5.
37. Friedman L, Cooper HP, Webb JA, Weinberg AD, Plon SE. Primary care physicians' attitudes and practices regarding cancer genetics: a comparison of 2001 with 1996 survey results. J Cancer Educ. 2003;18(2):91-4.
38. Poppelaars FA, Adèr HJ, Cornel MC, et al. Attitudes of potential providers towards preconceptional cystic fibrosis carrier screening. J Genet Couns. 2004;13(1):31-44.
39. Welkenhuysen M, Evers-Kiebooms G. General practitioners and predictive genetic testing for late-onset diseases in Flanders. Community Genet. 2002;5(2):128-37.
40. Lowstuter KJ, Sand S, Blazer KR, et al. Influence of genetic discrimination perceptions and knowledge on cancer genetics referral practice. Genet Med. 2008;10(9):691-8.
41. Pichert G, Dietrich D, Moosmann P, Zwahlen M, Stahel RA, Sappino AP, et al. Swiss primary care physicians' knowledge, attitudes, and perception towards genetic testing for hereditary breast cancer. Fam Cancer. 2003;2(3-4):153-8.
42. Nagle C, Lewis S, Meiser B, Gunn J, Halliday J, Bell R, et al. Exploring general practitioners' experience of informing women about prenatal screening tests for foetal abnormalities: a qualitative focus group study. BMC Health Serv Res. 2008;8:114.
43. Acheson LS, et al. Clinical genetics issues encountered by family physicians. Genet Med. 2005;7(7):501-8.
44. Morgan S, McLeod D, Kidd A, Langford B, et al. Genetic testing in New Zealand: the role of the general practitioner. N Z Med J. 2004;117(1206):U1178.
45. Hindorff LA, Burke W, Laberge AM, Rice KM, Lumley T, Leppig K, et al. Motivating factors for physician ordering of factor V Leiden genetic tests. Arch Intern Med. 2009;169(1):68-74.
46. Welkenhuysen M, Evers-Kiebooms G, et al. General practitioners and predictive genetic testing for late-onset diseases in Flanders: what are their opinions and do they want to be involved? Community Genet. 2002;5(2):128-37.
47. Sabatino SA, McCarthy EP, Phillips RS, Burns RB, et al. Breast cancer risk assessment and management in primary care: provider attitudes, practices, and barriers. Cancer Detect Prev. 2007;31(5):375-83.
48. Mathers J, Greenfield S, Metcalfe A, Cole T, Flanagan S, Wilson S, et al. Family history in primary care: understanding GPs' resistance to clinical genetics—qualitative study. Br J Gen Pract. 2010;60(574):e221-30.
49. Williams JL, Collingridge DS, Williams MS, et al. Primary care physicians' experience with family history: an exploratory qualitative study. Genet Med. 2011;13(1):21-5.
50. Suther S, Goodson P, et al. Barriers to the provision of genetic services by primary care physicians: a systematic review of the literature. Genet Med. 2003;5(2):70-6.