NORTHERN CLINICS OF İSTANBUL
VOL 12 • NO 4 • YEAR 2025

P ISSN 2148-4902
E ISSN 2536-4553

Journal Abbreviation: North Clin Istanb

INDEXED IN PUBMED, EUROPE PMC, WEB OF SCIENCE, DOAJ, TRDİZİN, THE TURKIYE SCIENCE CITATION INDEX, TÜRKMEDLINE, EBSCO, CEEAS, SCOPUS, CLIO, PROQUEST, OUCI, SCILIT AND HINARI/ RESEARCH4LIFE.

NORTHERN CLINICS OF ISTANBUL
İSTANBUL KUZEY KLİNİKLERİ

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İSTANBUL KUZEY KLİNİKLERİ

YEAR 2025
VOLUME 12
NUMBER 4

P ISSN 2148 - 4902
E ISSN 2536 - 4553

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Indexed in PubMed, Europe PMC, Web of Science, DOAJ, TRDIZIN, the Turkiye Science Citation Index, TurkMedline, EBSCO, CEEAS, Scopus, CLIO, ProQuest, OUCI, Scilit and Hinari/Research4Life.

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Tel: +90 216 550 61 11
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YILDIRIM PRINTING HOUSE
Yuzyil Mah., Massit Matbaacilar Sitesi,
1. Cad., No: 101, Bagcilar, Istanbul, Turkiye
Tel: +90 212 629 80 37
Fax: +90 212 629 80 39

Info

Press date: August 2025
Circulation: 50
Type of publication: Periodical

DESIGN

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Northern Clinics of Istanbul (NCI) is a peer-reviewed journal published 6 issues in a year.
This publication is printed on paper that meets the international standard ISO 9706:1994.
National Library of Medicine recommends the use of permanent, acid-free paper in the production of biomedical literature.

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North Clin Istanb 2025;12(4):387–395
doi: 10.14744/nci.2025.43788

ORIGINAL ARTICLE GENERAL SURGERY

Evaluating liver function test alterations in laparoscopic right adrenalectomy with different retractors

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ABSTRACT

OBJECTIVE: Laparoscopic techniques have emerged as the preferred approach over traditional open surgery for the treatment of adrenal gland disorders. Right laparoscopic adrenalectomy (RLA) typically requires liver retraction for exposure, and various retractors can be used for this purpose. While studies have been conducted on liver injury during liver retraction in upper abdominal surgeries, no research has specifically addressed liver damage during laparoscopic adrenalectomy (LA). This study aims to evaluate the impact of two retractors used for liver retraction during RLA on liver function test results (LFTs) and their clinical significance.

METHODS: This retrospective study included 87 LA patients who underwent surgery for adrenal gland pathology at our institution between 01/01/2010 and 04/30/2024. The patients were divided into two groups: RLA (n=42) and left LA (LLA) (n=45). The RLA patients were further categorized into two subgroups based on the retractor used: 5-blade retractor (FB) (n=22) and full ring retractor (GF) (n=20). Clinicopathological findings, operative outcomes, and laboratory test results were compared across groups.

RESULTS: Postoperative levels of aspartate aminotransferase (AST), alanine aminotransferase, and alkaline phosphatase were significantly higher in the RLA groups (FB and GF) compared to the LLA group (p<0.001, p<0.001, p=0.001, respectively). Although no statistically significant difference was observed between groups, the median length of stay (LOS) was slightly shorter in the FB group (2 (2–3), p=0.058). There were no significant differences between FB and GF groups in terms of operation time, LFTs, complications, or mortality. Correlation analysis showed a statistically significant positive correlation between postoperative AST levels and lesion size (rho=0.31, p=0.045). Additionally, patients with functional adrenal pathologies had a significantly longer hospital stay compared to those with nonfunctional pathologies (2 (2–2.25) vs. 3 (2–3.5), p<0.001).

CONCLUSION: In RLA procedures, the LFT values were higher compared to LLA procedures. The effects of FB and GF retractors on surgical outcomes and LFT values were similar, indicating both retractors can be safely used during RLA surgeries. While no clinical impact was detected, caution is advised regarding potential liver injury during RLA procedures.

Keywords: 5-blade fan retractor; golden finger retractor; laparoscopic adrenalectomy; liver function tests; liver retractors.

Cite this article as: *Dincer HA, Alkan I, Dogu D, Cennet O, Akkapulu N, Dogrul AB. Evaluating liver function test alterations in laparoscopic right adrenalectomy with different retractors. North Clin Istanb 2025;12(4):387–395.*

The first laparoscopic adrenalectomy (LA) was performed in 1992 by Gagner et al. [1]. Studies comparing LA to the traditional open approach have demonstrated that LA is associated with less pain, reduced bleeding, shorter hospital stays, faster return to normal activities, and a lower incidence of incisional hernias.

Received: January 20, 2025 *Revised:* April 02, 2025 *Accepted:* May 22, 2025 *Online:* August 19, 2025

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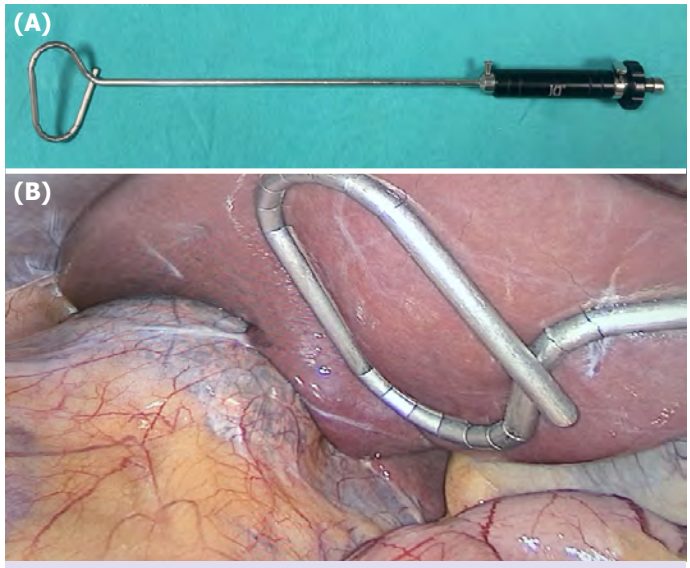

FIGURE 1. **(A)** Golden finger retractor. **(B)** Use of retractor during surgery.

Consequently, the laparoscopic approach has become the standard treatment for the surgical management of adrenal pathologies [2, 3]. Despite its benefits, carbon dioxide (CO_2) pneumoperitoneum has adverse effects on the circulatory and respiratory systems. One such effect is the reduction in portal blood flow, potentially leading to elevated serum transaminase levels [4]. Additionally, elevated liver function tests (LFTs) have been observed after laparoscopic cholecystectomy (LC) procedures, a phenomenon also associated with pneumoperitoneum [5, 6].

Various techniques have been described for LA, but the transabdominal approach is the most commonly preferred [7, 8]. Due to anatomical differences, surgical techniques vary between the right and left adrenal glands. The right adrenal gland is partially retrocaval and drains directly into the inferior vena cava (IVC). Additionally, it is in close proximity to the liver and the second portion of the duodenum. Right adrenal surgery is considered more challenging than left adrenal surgery due to anatomical features [9, 10].

In some upper abdominal minimally invasive surgeries, liver retraction is required to achieve adequate exposure, and various retractors are used for this purpose. Liver retraction is most frequently necessary during laparoscopic and robotic gastric surgeries. However, during these retractions, liver lacerations or ischemia may occur. Retractor-associated liver injury has been reported in laparoscopic gastrectomies performed for gastric cancer [11–14], Nissen fundoplication surgeries [15, 16], and bariatric surgeries for morbid obesity [17, 18]. Additionally, retractor-related liver injury has also been reported during right nephrectomy [19] and right laparoscopic adrenalectomy (RLA) procedures [20].

Highlight key points

- In laparoscopic right adrenalectomy, there is a risk of liver injury.
- Liver function tests are elevated in laparoscopic right adrenalectomy; however, no clinically significant negative effects on patients have been demonstrated.
- Both the 5-blade fan retractor® and the golden finger retractor® can be safely used with similar results for liver retraction.
- There is a correlation between the size of the adrenal lesion and liver function test results. Caution should be exercised in cases with larger lesions to prevent potential liver damage.

During RLA, liver injury or pressure-related localized liver ischemia may occur during exposure. To the best of our knowledge, in the literature, no study has examined the effects of different types of retractors used for liver retraction during RLA on LFTs. This study aims to evaluate the effects and clinical significance of two types of retractors used for liver retraction on LFTs.

MATERIALS AND METHODS

Study Design and Patient Selection

Patients who underwent LA at our institution between January 1, 2010, and April 30, 2024, were retrospectively analyzed. Inclusion criteria included all patients over 18 years old who underwent LA for adrenal gland pathology, with the procedure completed laparoscopically. Exclusion criteria comprised open surgeries, conversion to open surgery, concomitant procedures, preoperative abnormal LFTs, history of upper abdominal surgery, and patients with incomplete follow-up data.

A total of 87 patients were included in the study. The choice of retractor was based on the surgeon's preference. Due to the retrospective nature of the study, no randomization was performed; instead, groups were formed based on the types of retractors used. Patients undergoing RLA (n=42) were grouped based on the type of liver retractor used: full ring Golden Finger retractor® (GF-retractor group) (Kangji Medical Instrument Co., Zhejiang, China) (n=22) (Fig. 1) or five blade fan retractor® (FB-retractor group) (Covidien IIc, Massachusetts, USA) (n=20) (Fig. 2). Left laparoscopic adrenalectomy (LLA) patients (n=45) were included as the control group.

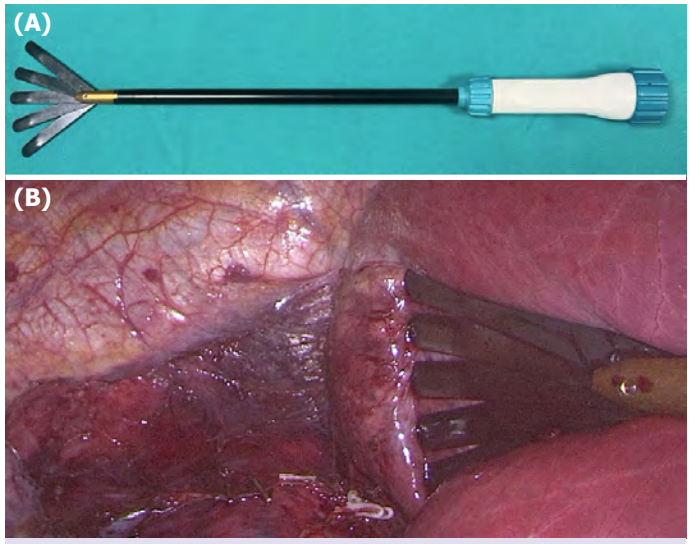

FIGURE 2. **(A)** 5-blade fan retractor. **(B)** Use of retractor during surgery.

Demographic, Clinical, and Laboratory Features

Clinicopathological findings, operative outcomes, and laboratory test results were analyzed between groups. Clinicopathological findings included demographic characteristics, body mass index (BMI), Charlson Comorbidity Index (CCI), lesion location, size, and preoperative diagnosis. The evaluated operative outcomes were operation time, estimated blood loss (EBL), complications classified according to the Clavien-Dindo Classification (C-D), length of hospital stay (LOS), mortality, and re-admission rates.

Laboratory findings included preoperative and postoperative day-1 levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and bilirubin. The reference ranges in our institution's laboratory are as follows: AST 0–50 U/L, ALT 0–50 U/L, ALP 30–120 U/L, GGT 0–55 U/L, and bilirubin 0.3–1.2 mg/dL.

Definitions

The Charlson Comorbidity Index is a reliable, simple, and widely used scoring system for assessing mortality risk associated with comorbid conditions [21]. It predicts 1-year mortality by assigning weighted scores to comorbid diseases, ranging from 1 to 6, with the total score representing the cumulative risk. Higher CCI scores indicate increased mortality risk and more severe of comorbidities.

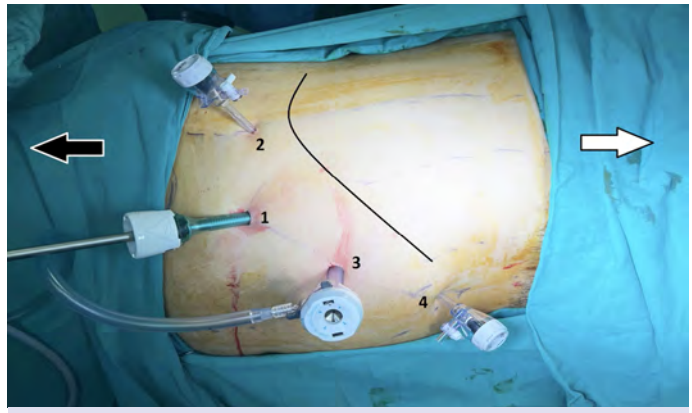

FIGURE 3. Patient position and trocar sites in laparoscopic right adrenalectomy. White arrow, patient's head; Black arrow, patient's feet; black line, subcostal line; 1, camera port; 2 and 3, working ports; 4, liver rectactor port.

The Clavien-Dindo Classification is a widely utilized tool for evaluating postoperative complications, encompassing both morbidity and mortality [22]. The C-D grades range from Grade I to Grade V as follows:

- Grade I: Any deviation from the normal postoperative recovery not requiring pharmacological or invasive interventions.
- Grade II: Deviations requiring pharmacological treatment.
- Grade III: Deviations necessitating surgical, endoscopic, or radiological interventions (IIIa without general anesthesia; IIIb under general anesthesia).
- Grade IV: Life-threatening complications requiring intensive care management (IVa single organ dysfunction; IVb multiorgan dysfunction).
- Grade V: Death of the patient because of postoperative complications.

Surgical Technique

In our institution, a 4-trocar technique is used for RLA (Fig. 3). Under general anesthesia, the patient is positioned in the left lateral decubitus position. A 10-mm trocar is inserted using the Hasson technique (open technique) at the anterior axillary line, approximately 3–4 cm below the subcostal line. After establishing pneumoperitoneum with an intraabdominal pressure of 12 mmHg, under direct vision, a 5-mm trocar is inserted at the midaxillary line, and a 10-mm trocar is placed at the midclavicular line.

The right triangular ligament of the liver is divided using the laparoscopic LigaSure™ vessel sealing system (Medtronic, Minneapolis, USA) to allow medial retraction of the liver. If a GF retractor is used, an additional 5-mm trocar is inserted at the xiphoid area, while a 10-mm trocar is inserted if an FB retractor is employed. The liver is retracted medially to expose the IVC. The peritoneum overlying the IVC is incised, and the right adrenal gland is visualized. Dissection is carried out in the plane between the gland and the IVC, identifying the central vein draining directly into the IVC. The central vein is looped, clipped with hemoclips, and divided.

The adrenal gland is retracted laterally for traction, dissected from the retroperitoneum using the LigaSure™, and placed in a specimen bag. The adrenal gland is removed through one of the trocar sites, which is widened as necessary.

For LLA, liver retraction is not required. Instead, the spleen and pancreas are medialized to access the adrenal gland, and the procedure is completed following the same principles as RLA.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Categorical variables were presented as frequencies and percentages. The distribution of continuous variables was assessed using the Shapiro–Wilk test. Variables with a normal distribution were reported as mean±standard deviation, whereas non-normally distributed variables were presented as median and interquartile range (Q1–Q3).

Comparisons between categorical variables were conducted using the chi-square test. For continuous variables, one-way analysis of variance (ANOVA) was used when the data were normally distributed, while the Kruskal–Wallis test was applied for non-normally distributed variables. When significant differences were found, post hoc comparisons were conducted using the Mann–Whitney U test. Correlations between variables were analyzed using Spearman's rank correlation coefficient. A two-tailed p-value of <0.05 was considered statistically significant.

Sample size estimation was conducted using G*Power software (version 3.1.9.7, Heinrich Heine University, Düsseldorf, Germany). A power analysis, assuming a medium effect size (Cohen's d = 0.5), an alpha level of 0.05, and a statistical power of 0.80 with equal group allocation (N1/N2=1), determined that a total sample size of 84 patients (42 per group for RLA and LLA) is adequate.

Ethical Approval

The Institutional Review Board of Medicine, Hacettepe University, approved this study (SBA 24/55; 2024/09-19).

RESULTS

Of the 87 patients included in the study, 23 (26.44%) were male. Characteristics such as age, BMI, ASA score, CCI, and past surgical history were similar among the groups (p=0.47, 0.32, 0.13, 0.49, and 0.83, respectively). Hormonal activity was observed in 45% of patients in the FB-retractor group, 54.5% in the GF-retractor group, and 53.3% in the LLA group, with no statistically significant difference between the groups (p=0.79). The median long-axis dimensions of adrenal lesions were also similar among the groups (p=0.99).

In the FB-retractor group, the most common surgical indications were adrenal adenoma (50%), Conn syndrome (25%), and Cushing syndrome (15%). In the GF-retractor group, they were adrenal adenoma (27.3%), Conn syndrome (22.7%), and pheochromocytoma (18.2%). In the LLA group, the leading indications were adrenal adenoma (44.4%), Cushing syndrome (22.2%), and Conn syndrome (20%) (p=0.52) (Table 1).

The median operation times were 101.8±21.3 minutes for the FB-retractor group, 114.3±24.5 minutes for the GF-retractor group, and 116.1±32.7 minutes for the LLA group. Statistical analysis revealed no significant differences among the three groups. (p=0.17). Intraoperative bleeding occurred in five patients overall. No bleeding was observed in the FB-retractor group, while two patients in the GF-retractor group (100 mL and 150 mL) and three patients in the LLA group (30 mL, 50 mL, and 350 mL) experienced intraoperative bleeding.

The median length of hospital stay was slightly higher in the GF-retractor group (3 (2–4)), but it was not statistically significant (p=0.058). Two patients needed a one-day ICU stay, both from the LLA group. No mortality or re-operation occurred in any group. Re-admission occurred in only one patient from the LLA group. The C-D score was two in only one patient; all others had a score of one.

Regarding pathological outcomes, the most common diagnoses were adrenal adenoma (75%) and pheochromocytoma (15%) in the FB-retractor group, adrenal adenoma (54.5%) and pheochromocytoma (18.2%) in the GF-retractor group, and adrenal adenoma (77.8%) and

Table 1. Demographic and clinicopathological characteristics of patients

	Right adrenalectomy (n=42)		Left adrenalectomy (n=45)	p
	5-blade fan retractor (n=20)	Golden finger retractor (n=22)		
Age (years) (mean±SD)	49±9.7	45±12.7	48.4±12.5	0.469[1]
Sex, male (%)	25	36.4	22.2	0.461[3]
Body mass index (median Q1–Q3)	22.9 (22–29.67)	25.5 (22.65–32.93)	24.6 (22.75–31.75)	0.323[2]
ASA score (%)				0.125[3]
1				
2	40	13.6	15.6	
3	45	72.7	60	
Comorbidities, (%)	15	13.6	24.4	
None				0.216[3]
Diabetes mellitus	10	31.8	20	0.775[3]
Hypertension	15	13.6	20	0.114[3]
Cardiovascular disease	80	50	57.8	0.488[3]
Hyporthyroidism	15	4.5	13.3	0.515[3]
Other	20	9.1	11.1	0.370[3]
Charlson comorbidity index (median, Q1–Q3)	10	22.7	11.1	0.487[3]
Past surgical history, (%)	1 (0–1.75)	0 (0–1)	1 (0–1)	0.830[3]
Hormonal status (functionality), (%)	30	36.4	37.8	0.787[3]
Length of longer axis of the lesion (median Q1–Q3)	45	54.5	53.3	0.990[2]
Preoperative diagnosis, (%)	30 (18–48.5)	32 (16.5–45.5)	30 (21.5–41)	
Adrenal adenoma				0.523[3]
Adrenal adenoma	50	27.3	44.4	
Conn syndrome	25	22.7	20	
Adrenal nodular hyperplasia	0	4.5	0	
Adrenal cyst	0	4.5	2.2	
Cushing syndrome	15	13.6	22.2	
Pheochromocytoma	5	18.2	11.1	
Myelolipoma	5	4.5	0	
Metastasis	0	4.5	0	

1: ANOVA; 2: Kruskal-Wallis Test; 3: Chi-Square; SD: Standard deviation; Q: Quartile; ASA: American Society of Anesthesiologist; ANOVA: Analysis of variance.

endothelial cyst (8.9%) in the LLA group. There were no statistically significant differences observed among the groups (p=0.4) (Table 2).

Analysis of LFTs revealed significant differences in postoperative AST, ALT, and ALP levels among the groups (p<0.001, p<0.001, and p=0.001, respectively) (Table 3). Post-hoc analysis using the Mann-Whitney U test showed significant differences between the FB-retractor and LLA groups (postoperative AST, ALT, and ALP; p<0.001, p<0.001, and p=0.001, respectively) and between the GF-retractor and LLA groups (postoperative AST, ALT, and ALP; p<0.001, p<0.001, and p=0.001, respectively). However, no differences were observed between the FB-retractor and GF-retractor groups for postoperative AST, ALT, and ALP levels (p=0.52, p=0.88, and p=0.88, respectively).

Table 2. Operative and pathological examination results of the patients

	Right adrenalectomy (n=42)		Left adrenalectomy (n=45)	p
	5-blade fan retractor (n=20)	Golden finger retractor (n=22)		
Duration of operation (minutes), mean±SD	101.8±21.3	114.3±24.5	116.1±32.7	0.167[1]
Length of hospital stay (days), median (Q1-Q3)	2 (2–3)	3 (2–4)	2 (2–3)	0.058[2]
Pathological report, (%)				0.398[3]
Adrenal adenoma	75	54.5	77.8	
Adrenal nodular hyperplasia	0	4.5	0,0	
Adrenocortical carcinoma	0	9.1	2.2	
Endothelial cyst	5	4.5	8.9	
Pheochromocytoma	15	18.2	6.7	
Myelolipoma	5	4.5	0,0	
Other	0	4.5	4.4	

1: ANOVA; 2: Kruskal-Wallis Test; 3: Chi-Square; SD: Standard deviation; Q: Quartile.

Among RLA patients, a statistically significant positive correlation was found between postoperative AST levels and the lesion's long-axis size (rho=0.31, p=0.045). However, there was no significant relationship between lesion size and operation time (p=0.79) (Table 4).

Patients with functional adrenal lesions had significantly longer LOS in the hospital compared to those with non-functional lesions (3 (2–3.5) vs. 2 (2–2.25), respectively, p<0.001) (Table 5).

DISCUSSION

This study demonstrated that postoperative day-1 AST, ALT, and ALP levels were significantly higher in RLA surgeries compared to LLA surgeries. It also showed that the GF and FB retractors had similar effects on LFTs elevation during RLA, and that these increases in LFTs values did not result in any clinically adverse consequences. Additionally, a statistically significant positive correlation was found between lesion size and AST levels. It was also observed that patients with functional lesions had longer LOS compared to those with non-functional lesions.

Previous studies have discussed that anesthetic agents, the pneumoperitoneum created during laparoscopy, patient positioning, and manipulation of the liver during surgery may lead to occult liver damage, resulting in postoperative elevation of LFTs [23–26]. During pneumoperitoneum, when intra-abdominal pressure is increased from 10 mmHg to 15 mmHg, splanchnic circulation slows, and hepatic blood flow decreases by 39% [4]. While some studies suggest that LFT elevation occurs without clinical significance [5, 27, 28], Meierhenrich et al. [29] demonstrated increased liver blood flow during laparoscopic surgery using transthoracic echocardiography. Furthermore, another study comparing high-pressure and low-pressure laparoscopic surgeries noted that high pressure had a negative effect on LFTs [6]. In our study, both RLA and LLA surgeries were performed with an intra-abdominal pressure set at 12 mmHg, a level considered low pressure, to minimize the impact of pneumoperitoneum on LFTs.

A study on the effect of patient positioning during laparoscopic colectomy surgeries, without liver manipulation, found no significant difference in abnormal LFT elevation between head-up and head-down positions (4.4% vs. 5.5%, respectively) [30]. Although temporary liver dysfunction may occur after general anesthesia, it is generally accepted that anesthesia does not have a clinically significant impact on LFTs [5, 31]. In our study, similar anesthetic agents were used for all patients, which helped homogenize the potential effects of anesthesia on LFTs across both groups. Additionally, the absence of elevated LFTs in the LLA group—despite liver compression in the lower abdominal region—further supports the notion that patient positioning does not significantly affect LFTs.

Table 3. Preoperative and postoperative liver function test results of the patients

	Right adrenalectomy (n=42)		Left adrenalectomy (n=25)	p
	5-blade fan retractor (n=20)	Golden finger retractor (n=22)		
Preoperative				
AST, median (Q1–Q3)	17.5 (15–21.75)	21.5 (16–26.25)	19 (16–23)	0.3192
ALT, median (Q1–Q3)	20.5 (14.25–24.75)	21.5 (18.25–34.25)	20 (14–26)	0.8152
ALP, median (Q1–Q3)	72 (55.75–89.75)	71 (52–98.25)	67 (61–76,5)	0.3182
GGT, median (Q1–Q3)	19 (11.25–30)	23 (18.75–31.5)	19 (15,5–31)	0.3032
Total bilirubine, median (Q1–Q3)	0.48 (0.4–0.72)	0.43 (0.36–0.57)	0.51 (0.39–0.64)	0.8662
Direct bilirubine, median (Q1–Q3)	0.09 (0.04–0.12)	0.09 (0.07–0.12)	0.09 (0.06–0.12)	0.8662
Postoperative day 1				
AST, median (Q1–Q3)	159 (57.75–223.75)	94.5 (75–126.75)	21 (18.5–26.5)	<**0.001**[2]
ALT, median (Q1–Q3)	110 (51–220)	114 (71–177.5)	19 (14.5–26)	<**0.001**[2]
ALP, median (Q1–Q3)	68.5 (53.25–88)	71 (54–82.25)	50 (40.5–63)	**0.001**[2]
GGT, median (Q1–Q3)	27.5 (16–42.25)	22 (16–29.25)	19 (15–25)	0.1052
Total bilirubine, median (Q1–Q3)	0.66 (0.5–1.29)	0.99 (0.46–1.06)	0.61 (0.09–0.2)	0.4572
Direct bilirubine, median (Q1–Q3)	0.22 (0.09–0.47)	0.13 (0.1–0.21)	0.14 (0.09–0.2)	0.3842

1: ANOVA; 2: Kruskal–Wallis Test; 3: Chi–Square; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT, Gamma glutamyl transferase; Q: Quartile.

Table 4. Correlation analysis of lesion long axis length between liver function tests and duration of operation

	Lesion long axis length	
n=42	Rho	p
AST	**0.311**	**0.045**
ALT	0.192	0.223
ALP	0.104	0.511
Duration of operation	0.042	0.793

AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline phosphatase.

Table 5. The relationship between the functionality of lesions and the length of hospital stay

	Nonfunctional (n=42)	Functional (n=45)	p
Length of hospital stay, median (Q1–Q3)	2 (2–2.25)	3 (2–3.5)	<**0.001**

Q: Quartile.

Liver retraction is essential in RLA surgeries because of the anatomical proximity, necessitating the use of various retractors for this purpose. There are reports in the literature, particularly in gastric surgeries, indicating that liver compression caused by retractors can lead to elevated LFTs or liver injury [11, 20]. Mechanically induced liver damage during laparoscopy has been classified into two types [19]. The first type is retraction-associated injury, where the retractor directly causes parenchymal damage. Over-retraction of tissues can lead to tears. The second type is compression-associated liver damage, where prolonged compression causes parenchymal congestion. This results in transient asymptomatic elevation of LFTs, which is often clinically insignificant. Some studies have described this type of damage as retraction transaminitis [16]. However, Tamhankar et al. [32] reported a case of retraction-related liver necrosis. Additionally, cases of retraction-related acute liver failure [19], liver hematoma [15], subcapsular hematoma [20],

and liver atrophy [33] have been reported. It is recommended that the retractor be loosened and its position be adjusted every 30 minutes in order to prevent such injuries [12, 34]. In our study, we observed no instances of direct liver injury in any of the patients, suggesting that the elevated LFTs seen in RLA patients are likely attributable to the secondary mechanism described above. However, the absence of liver injury is also directly related to the experience of the surgeon handling the retractor. Considering the average surgical time of around two hours for our RLA patients, following Hiramatsu et al.'s [12] recommendation of loosening the retractor every 30 minutes may help prevent LFTs elevation.

Different types of retractors have been recommended in various surgeries to reduce retractor-related liver injury [34–36]. These efforts aim to prevent liver damage. In our study, the effects of two different retractor types on LFTs were compared. When comparing operation time, C-D score, LOS, and postoperative LFTs elevation, no significant differences were found between the two retractor types. Based on current data, no definitive conclusion has been reached that would influence retractor selection in clinical practice. Nonetheless, we believe that both GF and FB retractors can be safely used in RLA surgeries.

In right LA surgeries, exposure is more challenging due to factors such as the short length of the adrenal vein, its direct drainage into the IVC, and its location behind the liver [9, 10]. In LA procedures, injuries to the IVC, adrenal vein, or accessory veins may occur in 5–10% of cases [37]. In our study, no major vascular injuries were observed. In the RLA group, two patients experienced bleeding (100 ml and 150 ml), while in the LLA group, three patients experienced bleeding (30 ml, 50 ml, and 350 ml). Although the primary aim of our study was not to investigate adrenalectomy outcomes, the finding that only one patient had a C-D score of two, while the others had a score of one, along with similar operative times, EBL, and LOS durations compared to the literature, suggests that LA surgeries can be safely performed in cases of adrenal gland pathology.

The findings of the present study revealed a statistically significant increase in AST levels as the size of the right adrenal gland increased (rho=0.311, p=0.045). As the size of the adrenal gland increases, longer and more powerful retraction of the liver with retractors is required to dissect the adrenal gland. For this reason, we believe that compression-related liver injury, as previously mentioned, occurs more frequently, leading to an increase in AST levels. Therefore, during the preoperative evaluation, it should be considered that patients with a larger right adrenal gland may be at potential risk for liver damage. Additionally, we observed that patients with functional adrenal pathology had a statistically significantly longer postoperative LOS compared to those with nonfunctional pathology ($p<0.001$). The observed difference can be attributed to the protracted postoperative medical treatments necessary for patients with functional pathology.

Our study had some limitations. The major limitations included its retrospective design, being a single-center study, and the small sample size. Additionally, the lack of preoperative assessment of hepatosteatosis was another limitation of our study.

Conclusion

This study is the first to explore the impact of different retractor types on LFTs during various LA procedures. Although postoperative LFT levels are higher in patients undergoing RLA than in those undergoing LLA, the type of retractor used during RLA appears to have a comparable impact on the degree of LFT elevation. Therefore, the available data are insufficient to warrant a change in clinical practice regarding retractor selection in RLA procedures, and both retractors can be considered safe for use. In addition, since a significant correlation was observed between lesion size and high LFT levels, liver retractors should be used more carefully in large-scale adrenal gland surgeries. While no major clinical effects were noted, it remains crucial to monitor for potential liver injury during RLA surgeries.

Ethics Committee Approval: The Hacettepe University Health Sciences Research Ethics Committee granted approval for this study (date: 21.05.2024, number: 2024/09-19).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used in the study.

Authorship Contributions: Concept – HAD; Design – HAD, IA; Data collection and/or processing – HAD, IA; Analysis and/or interpretation – DD, NA; Literature review – HAD, IA; Writing – HAD, OC; Critical Review – ABD.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):396–403
doi: 10.14744/nci.2024.93342

Original Article PEDIATRICS

Pediatric inflammatory bowel diseases: Effects of disease and treatment regimens on growth and puberty

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ABSTRACT

OBJECTIVE: Inflammatory bowel diseases (IBD) are lifelong conditions that exhibit periods of remission and exacerbation. In addition to gastrointestinal manifestations, they can also cause growth retardation and disorders of puberty in children. The objective of this study was to evaluate the effects of the disease and the treatment regimens on growth and puberty in children and adolescents with IBD.

METHODS: A retrospective screening of patients aged 2 to 18 years with a minimum of six months of follow-up due to inflammatory bowel disease (IBD) between January 2016 and April 2022 was conducted. The growth parameters were compared between disease groups, gender groups, disease activity and level of inflammation groups, and treatment regimen groups. The effects of treatment protocols on growth were evaluated by comparing the data before and after treatment, and the pubertal status of patients was evaluated by comparing them with healthy children.

RESULTS: A total of 58 patients were evaluated, comprising 29 individuals with Crohn's Disease (CD) and 29 with ulcerative colitis (UC). The growth and pubertal development of patients at the time of diagnosis did not differ based on gender or the specific disease type. A negative deviation from the target height was observed to be more prevalent in patients with Crohn's disease. Following treatment, patients exhibited a significant improvement in weight and BMI SDS, although no significant change in height SDS was observed. In comparison to healthy Turkish children, the patients exhibited a delayed pubertal progression, despite the normal onset of puberty.

CONCLUSION: Children and adolescents with IBD exhibited no significant adverse effects on linear growth at diagnosis or during the follow-up period, regardless of the primary disease and the treatment protocols. This was likely due to their timely diagnosis and successful treatment. It is important to monitor puberty, as it may progress more slowly or even cease in these patients compared to healthy children.

Keywords: Growth; inflammatory bowel disease; puberty

Cite this article as: *Gurler E, Gerenli N, Celtik C, Dursun F, Kirmizibekmez H. Pediatric inflammatory bowel diseases: Effects of disease and treatment regimens on growth and puberty. North Clin Istanb 2025;12(4):396–403.*

Inflammatory bowel diseases (IBD) are chronic, recurrent, and progressive immune-mediated gastrointestinal system diseases of children and adults. Ulcerative colitis (UC) and Crohn's disease (CD) are the two main types, characterized by attacks and periods of remission [1]. Growth retardation and malnutrition are common extraintestinal manifestations, often presenting before diagnosis and persisting during follow-up. Retardation

Received: February 08, 2024 *Revised:* April 28, 2024 *Accepted:* July 25, 2024 *Online:* August 19, 2025

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in growth and puberty not only indicates disease activity but also reflects the effectiveness of treatment. Growth retardation in CD can vary in severity and may precede diagnosis or occur as the sole presenting symptom [2].

Children often experience rapid weight loss at diagnosis and during active disease phases, with a frequency of 85% in CD and 65% in UC at diagnosis [3]. CD typically causes more significant linear growth impairment compared to UC [4]. The rate of growth retardation is about 10% in UC, while up to 40% in CD, which iseven more in younger patients [5, 6].

Bone maturation and development of puberty may also be delayed due to malnutrition in the long term. The recurrent and active courses of the disease may be responsible for pubertal arrest by suppressing the hypothalamic-pituitary-gonad axis. Secondary amenorrhea is seen frequently in girls following rapid and severe weight loss [7]. The hypothesis was that malnutrition and increased levels of proinflammatory cytokines may impair the onset and progression of puberty during the active phases of the disease [8]. However, evidence regarding puberty disorders in children with IBD remains limited.

As the number of children and adolescents diagnosed with IBD increases, the diagnosis and follow-up of endocrine problems such as growth retardation and puberty disorders are becoming increasingly important. A multidisciplinary approach is essential for the management of long-term problems in children with IBD. The objective of this study was to evaluate the growth and pubertal status of children and adolescents with IBD, including the frequency of growth and pubertal disorders, the clinical features and factors affecting them, and the differences between treatment regimens.

Highlight key points

- Inflammatory bowel diseases can result in growth retardation and disorders of puberty, in addition to gastrointestinal manifestations, in children.
- The use of pharmacological doses of steroids in induction, the total duration of steroids, and the use of biological agents did not result in a significant change in linear growth.
- The results suggest that the management of the inflammatory process may overcome the growth suppression of steroids.
- The pubertal tempo may be slowed in children with inflammatory bowel disease.

MATERIALS AND METHODS

The study group consisted of patients aged 2-18 years who were followed up with a diagnosis of IBD in the Pediatric Gastroenterology Clinic between January 2016 and April 2022. The study was approved by the Umraniye Training and Research Hospital Ethics Committee (date/number: 31.03.2022 / B.10.1TKH.4.34.H.GP.0.01/13) and is in accordance with the Declaration of Helsinki. Data on symptoms, laboratory values, and endoscopic findings at admission were collected from the hospital information system.

The measurements of the patients included in the study were conducted with the subjects wearing indoor clothes and without having eaten for at least eight hours. The height of each subject was measured without shoes, with the heel, hip, and scapula in contact with the measuring board, and with the head and face in a straight position. The measurements were determined using a height meter with a 1-mm sensitivity and a digital scale with a 100-gram sensitivity. Body weight, height, and body mass index SDS values were calculated using the Child Metrics application, created with references for Turkish children [9, 10].

The nutritional status of the patients was evaluated according to body mass index (BMI). Those with BMI SDS values below -3 SDS were classified as severely malnourished, those between -3 and -2 were classified as moderately malnourished, and those between -2 and -1 were classified as mildly malnourished [11].

The target height is the sex-adjusted midparental height, which is calculated by subtracting 13 cm from the father's height and averaging it with the mother's height for females, and by adding 13 cm to the mother's height and averaging it with the father's height for males. The difference between the patients' height SDS and target height SDS (height SDS - target height SDS) at diagnosis and at the last visit were recorded.

Short stature was defined as a height that was two or more standard deviations below the mean for Turkish children of that sex and chronological age. Patients who were below -2 SDS and who had insufficient growth velocity (less than 7 cm per year between the ages of 2–4, and less than 5 cm per year between the ages of 4 and puberty) were defined as those with growth retardation [12].

Puberty was assessed using the Tanner–Marshall method [13, 14]. Stage-1 is classified as prepubertal (no breast development in girls, and testis volumes of less than 4 ml for boys), Stage-2 (breast buds in girls, and tes-

tis volumes of 4-10 ml), Stage-3 (elevated breasts in girls, and testis volumes of 10-15 ml in boys), Stage-4 (areolar mound in girls, and testis volumes of 15-20 ml in boys) were classified as pubertal process, and Stage-5 (adult contours of breasts and mature areolae in girls, and testis volumes of more than 20 ml in boys) was classified as post-pubertal process. Pubertal progression was considered to be slow if there was minimal or no change in the stage of breast, pubic hair, or genital development within six or more months. The comparison of the mean age at pubertal stages of the male and female patients in the study group with the mean age of healthy Turkish children was made using the reference by Semiz et al. [15].

Fecal calprotectin levels of <50 μg/g were considered no inflammation, 5–100 μg/g as moderate inflammation, and >200 μg/g as severe inflammation [16]. PUCAI was used for the evaluation of disease activity for those with UC [17]. PCDAI was used for those with CD [18]. Those with a PUCAI score of <10 for UC were classified as remission/inactive disease, 10–34 as mild, 35–64 as moderate, and ≥65 as severe disease activity. Those with a PCDAI score of <10 for CD were classified as remission/inactive disease, 10–27.5 as mild, 30–37.5 as moderate, and >40 as severe disease activity.

Paris classification was used for the classification of endoscopic findings [19]. Among the treatment parameters, number of relapses, whether steroids were taken in induction, the total duration of steroid use, and whether biological agents were used were investigated.

Statistical Analysis

The data were analyzed using the SPSS Statistics version 25 (USA: IBM Corp.) program. The distribution of the data was evaluated with the Shapiro Wilk test. The results of the descriptive analyses were shown as mean±SD for normally distributed, and as the median and interquartile range for non-normally distributed data. Chi-square test was used to compare categorical data in independent groups, and Fisher's exact test was used for tables with insufficient sample size. Independent groups were compared by using an Independent-samples T test for parameters with normal distribution, and a Mann-Whitney U test for those with non-normal distribution. One-way Anova for normally and Kruskal Wallis test for non-normally distributed parameters were applied for more than two group comparisons. Related groups were compared using Paired-samples T test for normal distributed and Wilcoxon signed ranks test for non-normally distributed variables. Pearson correlation tests were used for normally, and Spearman correlation tests for non-normally distributed variables. The level of significance was accepted as $p<0.05$.

RESULTS

A total of 58 patients were included in the study group, comprising 29 individuals with UC and 29 with CD. The mean age at diagnosis was 12.2±3.4 years (range: 2.2-17 years). The duration between the onset of symptoms and diagnosis (delta diagnosis time) was 7.58±6.88 months, with no significant difference between disease groups. The male-to-female ratio (M/F) was 0.81 in patients with UC and 1.07 in those with CD.

The most common complaints were diarrhea (96.5%), abdominal pain (93.2%), blood in the stool (75.8%) and mucus (72.5%) in patients with UC and abdominal pain (94.8%) and diarrhea (93.1%) in CD patients. The most frequent physical examination finding was abdominal tenderness, with a prevalence of 46.5% among all patients. Perianal disease was observed in patients with CD patients. The most common extraintestinal findings were anemia in 62%, arthritis/arthralgia in 31%, and growth retardation in 20.7% of all patients.

Among the 29 patients with CD, 12 (41.4%) had severe disease activity, 4 (13.8%) had moderate disease activity, and 13 (44.8%) had mild disease activity at diagnosis. Among 29 patients with UC, one (3.5%) had severe disease activity, 11 (37.9%) had moderate disease activity, and 17 had (58.6%) mild disease activity. Regarding the degree of inflammation based on fecal calprotectin levels, 19 (57.5%) patients with CD and 14 (42.5%) with UC had severe inflammation. According to the Paris classification, 37.9% of UC patients had pancolitis, 27.6% had left colon involvement, 13.8% had extensive involvement (involvement beyond the splenic flexure but not reaching hepatic flexure), and 17.2% had ulcerative proctitis. Among CD patients, 62.1% had ileocolonic involvement, 17.2% had colonic involvement and 17.2% had ileal/limited cecum involvement.

The weight, height, BMI, and growth velocity SDS values were compared between UC and CD patients, and no significant differences were observed. More retardation from the target height was observed in patients with CD (Table 1). The follow-up period spanned a range of 6 months to 72 months, with a mean duration of 23.62±19.8 months. There was no significant differ-

Table 1. Anthropometric measurements and growth velocity of the patients at diagnosis

Growth parameter	Ulcerative colitis (n=29)	Crohn's disease (n=29)	p
Age at diagnosis (year)	12.0 (4.96)	14.0 (3.75)	0.159[b]
Weight SDS	-1.20±1.4	-0.85±1.8	0.112[a]
Height SDS	-0.54±1.7	-0.23±1.2	0.317[a]
Body mass index SDS	-1.16±2.0	-1.04±1.9	0.111[a]
Growth velocity SDS	-0.18±1.99	0.10±2.09	0.614[a]
Height SDS - target height SDS	0.29 (1.64)	-0.27 (1.25)	0.046[b]*

SDS: Standard deviation score; a: Independent-Samples T test; results are shown as mean±standard deviation; *: P<0.05; b: Mann-Whitney U test; results shown as median (interquartile range).

Table 2. Tanner Stages of the patients with ulcerative colitis and Crohn's disease

Tanner stage	Ulcerative colitis 29 (100%)	Crohn's disease 29 (100%)
Stage-1	4 (13.7)	2 (6.80)
Stage-2	3 (10.3)	6 (20.6)
Stage-3	1 (3.40)	0 (0.00)
Stage-4	6 (20.6)	9 (31.0)
Stage-5	15 (51.7)	12 (41.3)

Table 3. Comparison of mean ages of the patients according to pubertal stage with healthy Turkish children

Gender/Tanner stage	Study group age (Mean±SD)	Reference age (Mean±SD)	p
Girl/Tanner-2	11.79±0.41	10.16±0.97	0.113
Girl/Tanner-4	15.31±2.11	12.97±1.17	0.042*
Menarche	12.54±1.02	12.41±0.92	0.534
Male/Tanner-2	12.20±1.57	11.76±1.76	0.431
Male/Tanner-4	14.70±1.71	13.17±0.87	0.022*

SDS: Standard deviation; *: P<0.05. One-sample T-test.

ence between UC and CD in terms of anthropometric measurements at the end of the follow-up period.

There were no statistically significant differences in weight, height, BMI, growth velocity, or retardation from target height SDS parameters between boys and girls with IBD. The growth velocity, adjusted for pubertal stage and excluding patients who had completed linear growth, was found to be within the normal range in 19 patients (11 UC, 8 CD) and reduced in 12 patients (5 UC, 7 CD). The frequency of low growth velocity did not demonstrate a statistically significant difference between the disease groups (p=0.379).

The pubertal stages of the entire study group (30 female, 28 male) were evaluated. The girls were divided into four groups: four were prepubertal, two were at Tanner Stage 2, six were at Stage 4, and 18 were at Stage 5. Of the 28 boys, two were prepubertal, seven were at Stage 2, one was at Stage 3, nine were at Stage 4, and nine were at Stage 5. Table 2 presents the Tanner stages of patients with UC and CD. A comparison of the mean age at pubertal stages of male and female patients in the study group with the mean age of healthy Turkish children is presented in Table 3. In terms of mean age, there was no significant difference between the entire study group and healthy children in the early pubertal stage. However, the mean age in Stage-4 was significantly higher in the study group, suggesting that while there was no delay in the onset of puberty, there was a delay in the progression. Upon exclusion of prepubertal patients, 40% of pubertal patients with UC were in different stages of puberty, while 60% had completed puberty (Tanner Stage 5). In contrast, 55% of patients with CD were in the pubertal period, while 45% had completed puberty. The chi-square test indicated that there was no statistically significant difference between the disease groups (p=0.262).

Extraintestinal findings were observed in three (50%) patients in the prepubertal period, in seven (25%) patients in the pubertal period, and in five (18.5%) patients in the postpubertal period. The frequency of malnutrition in patients presenting with extraintestinal findings or an accompanying disease was significantly lower compared to other patients (p=0.040, p=0.011, respectively).

According to the Paris classification, 37.4% of the patients with UC had pancolitis, and 62.1% of the patients with CD had ileocolonic involvement. Height and retardation from target height were significantly lower in UC patients with pancolitis than in others (p=0.040,

Table 4. Growth parameters according to the disease activity and the degree of inflammation

Growth parameter	Disease severity mild (n=30)	Disease severity moderate/severe (n=28)	p	Inflammation mild/moderate (n=3)	Inflammation severe (n=33)	p
Weight SDS	-0.81±1.64	-1.01±1.78	0.717[a]	0.08±2.4	-1.05±1.65	0.615[a]
Height SDS	0.18±0.65	-0.55±1.55	0.190[a]	-0.06±1.03	-0.34±1.4	0.727[a]
BMI SDS	-1.52±2.34	-0.87±1.72	0.181[a]	0.03±2.05	-1.19±1.91	0.643[a]
Growth velocity SDS	-0.44±1.88	-0.26±2.11	0.212[a]	0.42±3.3	0.09±2.09	0.809[a]
Height – target height (SDS)	0.26 (0.22)	-0.04 (1.37)	0.332[b]	0.12 (0.28)	0.18 (1.3)	0.977[b]

SDS: Standard deviation score; a: Independent-Samples T test; results are shown as mean±standard deviation; b: Mann-Whitney U test; results shown as median (interquartile range).

Table 5. Comparison of growth and disease activity parameters before and after treatment

Growth and disease activity parameters	Before treatment	After treatment	p
Weight SDS (mean±standard deviation)	-0.63±1.53	-0.37±1.20	0.048[a]*
Height SDS (mean±standard deviation)	-0.25±1.19	-0.10±0.94	0.224[a]
BMI SDS (mean±standard deviation)	-0.68±1.62	-0.37±1.19	0.045[a]*
Delta-target height SDS, median (quartile difference)	0.02 (1.56)	0.32 (1.59)	0.108[b]
PCDAI for CD, median (min–max/IQR)	30.0 (15–70/22.5)	5.00 (0–15/10.0)	0.000[b]*
PUCAI for UC, median (min–max/IQR)	30.0 (12.5–60/22.5)	0.00 (0–17.5/0.00)	0.000[b]*

a: Paired Samples T Test; b: Wilcoxon Signed Ranks Test; SDS: Standard deviation score; BMI: Body Mass Index; CD:Crohn's disease; UC: Ulcerative colitis; PCDAI: Pediatric Crohn's Disease Activity Index; PUCAI: Pediatric Ulcerative Colitis Activity Index. Since the median and interquartile range (IQR) values of PUCAI and PCDAI scores at diagnosis in UC and CD patients were found to be the same by chance, they were also shown in the table as "median (minimum–maximum/IQR)".

p=0.022, respectively). However, in CD patients, groups according to the sites of involvement (ileocolonic and others) showed no significant difference in terms of growth parameters. There were no significant differences in growth parameters according to the disease activity scores or according to the level of inflammation (Table 4).

Steroid use was employed in induction therapy in 41 of the patients. In 30 of these patients, the steroid treatment could be reduced within three months. However, 19 patients required steroid treatment again within one year. In the induction treatment phase, steroid plus mesalazine was initiated in one patient, steroid plus azathioprine in one patient, azathioprine plus mesalazine in seven patients, and all three drugs were initiated simultaneously in 39 patients. As maintenance treatment, 56 patients received mesalazine, three patients received salazoprine, 44 patients received azathioprine, 11 patients received infliximab, five patients received adalimumab, one patient received vedolizumab, and five patients received methotrexate. There was no significant difference in the weight SDS, height SDS, BMI SDS, growth velocity SDS and deviation from target height parameters between patients who had received glucocorticoids during induction and who did not (p=0.444. p=0.122. p=0.695. p=0.901. p=0.953, respectively). The comparison of these growth parameters at last visit between patients who received biological agents and who did not show any significant difference (p=0.333. p=0.254. p=0.066. p=0.244. p=0.743, respectively).

A comparison of the growth parameters and disease activity parameters of our patients at diagnosis and after treatment revealed a significant improvement in the disease activity scores, as well as a significant improvement in the weight and BMI SDS values (Table 5).

Analyses revealed no correlation between growth parameters and disease scores at diagnosis or after treatment, time to diagnosis, number of stools per day, fecal calprotectin, number of relapses, or duration of steroid administration. There was no correlation between the stage of puberty and the number of relapses, the duration of steroid use, or disease severity scores. The post-treatment PUCAI/PCDAI scores were significantly lower in 57 patients and equal in only one patient (p=0.000). There was no correlation between the PUCAI/PCDAI scores and the anthropometric data, biochemical parameters, and pubertal stages at diagnosis. Similarly, no correlation was observed between fecal calprotectin levels and anthropometric data, biochemical parameters, and pubertal stage.

DISCUSSION

The results of this study demonstrated a notable improvement in weight and BMI SDS following treatment. Children and adolescents with IBD had no significant adverse effects on linear growth at diagnosis or during follow-up, regardless of the primary disease or treatment protocol. The treatment protocols, including the use of pharmacologic doses of steroids in induction, the total duration of steroids, and the use of biological agents, did not result in a significant change in growth in accordance with the literature. This suggests that the management of the inflammatory process and maintenance of disease control overcomes the growth suppression effect of steroids.

Anthropometric parameters were not different between boys and girls in our study, contrary to the report of the Pediatric CD Study Group which showed lower height gains in boys than in girls [20]. Systemic diseases with a rapid onset May 19, 2024lead to negative energy balance. Weight loss is evident at first, and linear growth is affected after the process is prolonged and diagnosis is delayed. Our patients with UC may have had height SDS values above the target height line at presentation due to positive environmental conditions before the onset of the disease. Possibly the diagnostic process did not take long enough to affect height. Patients with CD exhibited greater negative deviations from the target height at diagnosis. Since CD manifests with more insidious and non-specific findings, the diagnosis may be relatively late. When the negative effects on the nutritional status may develop over a longer period linear growth may also decrease. Another very important effect on growth is the process of chronic systemic inflammatory condition, which is more pronounced in CD. In the study of Jin et al. [21], patients with moderate and severe disease according to the PCDAI had lower IGF-1-SDS values. Song et al. [22], also found a statistically significant decrease in weight and BMI SDS in patients with higher PCDAI for CD patients.

Weight and BMI improved significantly with treatment while height was not significantly different after treatment in the follow-up period of up to 72 months (with a mean of 23 months). The disease activity scores were significantly improved after, confirming a successful suppression of disease, while height values were similar. This suggested that the height was already not affected at diagnosis. It can also be claimed that the negative effects of systemic inflammation and both positive and negative effects of the treatment agents provided a balanced outcome during treatment. In the study of Jin et al. [21], it was also reported that height did not change significantly, while weight increased significantly with treatment in 70 patients. Pfefferkorn et al. [23] and Vasseur et al. [24] also found no difference in height during a 2-year period.

Corticosteroids are still the most widely used agents to achieve remission in acute relapses. There are many studies investigating the effect of steroids on growth parameters. The fact that the growth parameters of the patients who received and did not receive steroid in induction treatment were not significantly different, suggesting that steroids may have less of an effect on growth than disease inflammation. In a study by Motil et al. [25], a negative relationship was found between linear growth and disease activity, but there was no relationship between steroid treatment and linear growth. In another study by Malik et al. [26], there was no significant difference between the groups that received and did not receive steroids.

In a study evaluating the adult height of patients, it was reported that patients with childhood-onset IBD reached a lower adult height than in the general population and healthy siblings. In patients with severe inflammation the final adult height was found to be lower than the others [27]. Malik et al. [26] concluded that even if disease control is good, growth is affected because of several interrelated factors.

When we analyzed our patients according to their nutritional status, patients with malnutrition had more insidious findings while patients with better nutritional status at diagnosis had more additional diseases or extraintestinal findings, suggesting that findings due to extraintestinal comorbidities may lead to earlier diagnosis.

The patients with pancolitis due to UC showed significantly lower height and more negative deviations from target height, compared to patients with less regional involvement, suggesting a reduced growth in this subgroup. However, the small number of our patients in the subgroups makes it difficult to conclude. In the study by Kim et al. [28] in which 594 patients were evaluated, pancolitis was the most common type in UC, and the height SDS of the patients was found to be significantly lower than in other types of involvement. Growth parameters were not different among the groups of involvement, as well as the groups of inflammation and groups of disease severity in patients with CD. There are studies showing the effects of ileal disease involvement on disease activation and severity of inflammation [21]. In the study of Song et al. [22], no significant correlation was found between weight, height, BMI, and disease region, while Timmer et al. [29] reported that the delay in diagnosis and growth retardation were higher in patients with ileal involvement in CD.

A comparison of the mean age at pubertal stages of male and female patients in the study group with data from healthy Turkish children revealed that the ages of girls and boys with Tanner Stage-4 were significantly higher than those of healthy Turkish children [15]. These data indicate that although there is no delay in the onset of puberty in children with IBD, there may be a slowdown in the rate of pubertal progression. This may be due to the disease itself, treatments, and nutritional deficiencies. Considering the diseases, the fact that those with UC are more in the post-pubertal period may suggest that those with CD are at higher risk for delayed puberty. In the study of Jin et al. [21], 11 of 109 patients (10.1%) presented with delayed puberty and had a significantly lower BMI. In the same study, 8 out of 31 patients who experienced menarche before the diagnosis developed secondary amenorrhea. In our study, no significant difference was observed in the age of menarche between healthy Turkish children. However, secondary amenorrhea was identified in two patients with Crohn's disease (CD). The relationship between inflammatory bowel disease (IBD) and menstrual changes has not yet been fully elucidated.

The main limitation of this study was the relatively small sample size. Nevertheless, the patients in the study group were monitored on a regular basis, providing valuable insights into the growth velocity and pubertal progression in this specific chronic disease of childhood. The effects of IBD on growth have been investigated in some larger cohorts, but there is a clear need for further studies on the onset and progression of puberty.

Conclusion

While weight and BMI were affected at diagnosis and improved with treatment, linear growth markers were not affected at diagnosis and did not show a significant difference under treatment. Although the age at which puberty begins remains unchanged in individuals with IBD, the pubertal tempo may slow down, or the patients may have been diagnosed at relatively advanced stages of puberty. The potential adverse effects of therapeutic agents on linear growth are offset by successful control of the disease.

Ethics Committee Approval: The Umraniye Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 31.03.2022, number: B.10.1TKH.4.34.H.GP.0.01/13).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The authors declared that artificial intelligence (AI) supported technologies were not used in our study.

Authorship Contributions: Concept – HK, NG; Design – HK, NG; Supervision – HK, NG; Fundings –HK; Materials – EG, CC, FD; Data collection and/or processing – EG, CC; Analysis and/or interpretation – HK; Literature review – EG, NG, FD; Writing – HK, EG; Critical review – HG, NG.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):404–412
doi: 10.14744/nci.2024.78545

ORIGINAL ARTICLE PEDIATRICS

The effect of Rituximab on B cells in pediatric autoimmune rheumatic diseases

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ABSTRACT

OBJECTIVE: This study examines how demographic factors and disease conditions affect B-cell depletion and regeneration after Rituximab (RTX) infusion in pediatric patients with rheumatic conditions.

METHODS: We retrospectively reviewed 27 patients approved by the Institutional Review Board, all of whom received at least one RTX infusion, analyzing 99 lymphocyte subunits. Inclusion: patients under 18 at their first RTX infusion, diagnosed with pediatric rheumatologist-confirmed autoimmune diseases. B-cell depletion was defined as a CD19 positive B Cells (CD19+) count below 10 cells/μL, assessed at 6- and 12-months post-RTX infusion. Complete regeneration was defined as CD19+ ≥170 cells/μL using adolescent norms.

RESULTS: Most patients had connective tissue disorders (CTD); Systemic Lupus Erythematosus, Sjögren's Disease, Systemic Sclerosis; n=17; 63%), followed by vasculitis (n=5; 18.5%), juvenile dermatomyositis (n=4; 14.8%), and miscellaneous conditions (n=1; 3.7%). Among patients with CTD, 4 out of 12 (33%) had B-cell depletion at 6 months. At 12 months, 3 out of 6 (50%) achieved CD19+ counts ≥10 cells/μL, while 5 out of 6 (83%) did not reach normal levels of CD19+ (≥170 cells/μL). No significant correlation existed between immunosuppressants (mycophenolate mofetil, methotrexate, azathioprine, cyclosporine, cyclophosphamide) and CD19+≥10 cells/μL at 6 or 12 months. However, hydroxychloroquine significantly differed for persistent depletion at 12 months.

CONCLUSION: This study demonstrates that demographic factors and disease conditions influence B-cell depletion and regeneration in pediatric patients treated with RTX for rheumatic conditions. The findings highlight the variability in response to RTX and suggest that factors such as hydroxychloroquine use may impact long-term B-cell levels.

Keywords: Autoimmune diseases in children; B cell depletion; pediatric rheumatology; rituximab treatment.

Cite this article as: *Ozomay Baykal G, Sozeri B. The effect of Rituximab on B cells in pediatric autoimmune rheumatic diseases. North Clin Istanb 2025;12(4):404–412.*

Rituximab (RTX) is a human chimeric anti-CD20 monoclonal antibody, a licensed B-cell depleting agent. CD 20 is a B lymphocyte transmembrane protein, is expressed on peripheral and malignant B cells [1]. RTX, widely used for lymphomas and posttransplant lymphoproliferative disorders in adults and children, it is approved in the United States for B cell malignancies, rheumatoid arthritis, granulomatosis with polyangiitis, microscopic polyangiitis, and pemphigus vulgaris [2–4]. In off-label use, it benefits children with the mentioned conditions and antibody-mediated autoimmune diseases such as systemic lupus erythematosus (SLE), multiple sclerosis, immune thrombocytopenic purpura, and IgG4-related disease [5, 6]. Autoimmune diseases exhibit diverse clinical presentations, often involving intricate pathophysiology. Recent immunology insights identi-

Received: August 09, 2024 *Revised:* September 03, 2024 *Accepted:* October 07, 2024 *Online:* August 26, 2025

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fy cellular and molecular targets with potential impact on the pathogenesis of various autoimmune diseases. Notably, recognizing the central role of B lymphocytes in pathogenesis suggests their elimination as a valuable therapeutic goal [7]. Following the RTX treatment, there is an immediate rapid decrease of circulating CD20+ B cells [8]. Most patients experience this dose-dependent effect for 2–3 months; however, in some cases, it lasts for 6 months before slowly reversing [9].

Some disorders like SLE show less pronounced depletion due to differences in drug effects [10, 11]. Detectable RTX in circulation for months suggests a potential lasting effect roll p [12]. Long-lived plasma cells, responsible for antibody production, are minimally affected by RTX [13–15]. RTX-induced B cell depletion is variable in the many autoimmune and lymphoproliferative disorders, reflecting the various phenotypic and functional variability of human B cell populations involved in diverse pathogenetic functions in inflammatory and malignant disorders [16, 17].

Although several methods have been used to investigate the impact of RTX therapy on biochemical biomarkers, no comprehensive, precise cytofluorimetry regimen to use before and after RTX treatment has yet to be devised [18]. There are conflicting reports in the literature on whether RTX-induced B cell depletion assessment could be a prognostic marker of disease relapses [11, 19–21]. Antibody levels might decrease, but clinical improvement in many patients correlates with lower autoantibody levels [10, 11, 19, 22].

The aim of this investigation is to examine the factors impacting the timing of B cell depletion and regeneration after RTX infusion in pediatric rheumatologic patients.

MATERIALS AND METHODS

Study Design

This study was designed to examine the CD-19 levels in patients treated with RTX.

Participants

Inclusion Criteria

In our study, a total of 27 patients have been examined for their CD-19 levels. Individuals aged 18 or below at their initial RTX infusion, diagnosed and being followed with an autoimmune disorder at Umranıye Research and Training Hospital between December 2016 and July 2023, were enrolled.

Highlight key points

- Demographic factors and specific disease conditions significantly affect B-cell depletion and regeneration in pediatric patients treated with Rituximab for rheumatic diseases.
- A considerable number of patients with connective tissue disorders show incomplete B-cell regeneration within 12 months post-Rituximab treatment.
- Hydroxychloroquine use is linked to sustained B-cell depletion at 12 months, suggesting its influence on long-term B-cell recovery.
- Rituximab treatment in pediatric rheumatic diseases is associated with a low incidence of infection-related hospitalizations, indicating its safety.
- Other immunosuppressive medications, except hydroxychloroquine, do not show a significant correlation with B-cell regeneration, highlighting the need for individualized treatment strategies.

Exclusion Criteria

Patients who did not receive at least 1 round and were diagnosed over the age of 18 were not included in this study.

Limits of the Study

The relatively small sample size of 27 patients, and even smaller subgroups based on disease diagnosis, may limit the generalizability of the findings. CD-19 values of each patient, which were analyzed at certain periods, were not available.

Ethics Approval

Ethical considerations were strictly adhered to in accordance with the principles of the Declaration of Helsinki (2013 revision).

The study has the ethics committee approval from the Umranıye Training and Research Hospital with the number of: B.10.1.TKH.4.34.H.GP.0.01/369 on 28/11/2022.

Interventions

The administration of RTX adhered to institutional protocols: two infusions separated by two weeks, 750 mg/m² (max 1 g) each. Premedication included iv methylprednisolone (2 mg/kg; max 100mg) to reduce RTX infusion reaction risk and as disease treatment. RTX was administered to inpatients at our clinic. Repeat RTX dosing depended on clinical disease activity, often redosed if B cell repletion occurred during a flare.

Outcome Measures

Depletion of B cells was defined as a CD19+ count that is lower than 10 cells/μL in the study of Mitchell et al [23]. Levels were evaluated at 6- and 12-months post-RTX infusion. Complete regeneration of B cells to normal levels was characterized by a CD19+ level of ≥170 cells/μL using adolescent norms [24]. Patients exhibiting <170 cells/μL at 12 months or beyond after the last RTX infusion fulfilled the criteria for persistent B cell depletion. CD19+ levels were determined at the discretion of the provider, with data missing for 11 patients at 6 months and 17 patients at 12 months. Eight patients underwent CD19+ level assessments just before the culmination of 12 months, with counts exceeding 10 but falling short of 170 cells/μL, designated as repleted at ≥10 cells/μL but lacking complete regeneration at ≥170 cells/μL.

Statistical Analysis

Data was extracted, compiled, and analyzed using SPSS v22.0 statistical software. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) statistical software. Continuous variables were presented with observation count, mean and standard deviation, or median and range. Qualitative values were expressed as patient count and percentage for relevant variables. Analysis of variance (ANOVA), Kruskal-Wallis and chi-squared tests were applied to continuous (age, rounds) and nominal variables (gender, B cell response, concurrent immunosuppression), respectively, utilizing the significance of $p<0.05$. For data following a normal distribution, the mean (standard deviation) is reported, while for non-normally distributed data, the median (range) is presented.

Throughout this study, there have not been any sections partially or entirely supported, sourced or scripted by the use of a Large Language Model (LLM).

RESULTS

We conducted a retrospective review approved by the Institutional Review Board involving 27 patients who underwent at least one RTX infusion. The analysis covered 99 lymphocyte subunits. Among the 27 eligible patients, the majority were females (n=22; 81%) and males (n=5; 19%). The median age at diagnosis was 13.00 (min-max=1-17.8 years), while the median age at initial RTX infusion was 17 years (min-max=5-17.9 years). The median number of RTX rounds was 1.5 (min-max=1-5.5).

Patients were primarily diagnosed with connective tissue disorders (CTD; SLE, Sjögren diseases, Systemic sclerosis; n=17; 63%), followed by vasculitis (n=5; 18.5%), juvenile dermatomyositis (n=4; 14.8%), and miscellaneous conditions (n=1; 3.7%). A significant difference for age at diagnosis versus diagnostic categories have been observed (p=0.026) (Table 1). The median age of vasculitis patients was 17.8, while for JDM patients it was 6.2. Mean CD19+ levels before RTX were 271 cells/μL (min–max=57-359 cells/μL). The number of RTX rounds used in each group did not differ significantly (p=0.376) (Table 1).

Following the administration of RTX, we observed distinct patterns in B cell repletion among our patient cohort. At the 6-month mark post-RTX infusion, exactly 50% of the patients (8 out of 16) exhibited sustained depletion, characterized by CD19+ levels below 10 cells/μL. Conversely, the remaining half of the cohort (n=8, 50%) demonstrated CD19+ levels of 10 cells/μL or higher. It is noteworthy that a significant proportion of those with persistent depletion at the 6-month milestone presented with connective tissue diseases (CTD), constituting 50% of the affected subgroup (n=4 out of 8).

As we extended our observation to the 12-month interval post-infusion, we noted a notable shift in the dynamics of CD19+ levels. At this juncture, 50% of the patients (5 out of 10) had successfully replenished their CD19+ levels to 10 cells/μL or more. However, most patients, precisely 80% (8 out of 10), still grappled with depletion below the threshold of 170 cells/μL. These findings are succinctly summarized in Table 1.

Within the CTD group, 4 out of 12 (33%) patients continued to have low CD19+ levels at 6 months. By the 12-month mark, 3 out of 6 (50%) patients showed CD19+ regeneration to 10 cells/μL or more, while 5 out of 6 (83%) did not achieve normal levels (170 cells/μL) of CD19+. While both vasculitis patients (n=2) exhibited depletion at 6 months, they regained normal CD19+ levels by 12 months, highlighting a faster recovery compared to other disease categories. Despite this observation, no significant differences in depletion or regeneration rates were detected between diagnostic groups at 6 or 12 months. No statistical significance was observed for depletion at 6 months (p=0.082), 12 months (p=0.549), or failure to regenerate by 12 months (p=0.116) in relation to diagnosis.

Table 1. Demographic data by diagnosis

Variable	Diagnostic Category, n (%)					p
	Total (n=27)	CTD (n=17)	JDM (n=4)	Vasculitis (n=5)	Miscellaneous (n=1)	
Sex						0.497
Female	22	15	3	3	1	
Male	5	2	1	2	0	
Age at initial Rituximab infusion (years)						**0.026**
Mean±SD	14.66±1.25	15.2±3.33	8.1±4.73	17.6±0.67	17±0	
Median	17.00	17.20	6.20	17.80	17.00	
CD19+<10 cells at 6 months following RTX	8 (50)	4 (33.3)	2 (100)	2 (100)	0	0.069
CD19+<10 cells at 12 months following RTX	5 (50)	3 (50)	2 (66.7)	0 (0)	0	0.513
CD19+<170 cells at 12 months following RTX	8 (80)	5 (83.3)	3 (100)	0 (0)	0	0.091
Rounds (n)						0.376
Mean±SD	1.93±0.43	2.17±1.48	1.875±0.63	1.3±0.45	1±0	
Median	1.50	1.5	2	1	1	

SD: Standard deviation; CTD: Connective tissue disorders; JDM: Juvenile dermatomyositis.

Regarding additional factors, there were no significant correlations detected between age (p=0.478), gender (p=0.209), or the quantity of rounds of RTX treatment (p=0.359) and CD19+ levels falling below the established threshold of 170 cells/μL at 12 months post-administration of RTX. Similarly, no associations were noted between age, gender, or the frequency of RTX treatments and B cell depletion below 10 cells/μL either at 6 months or 12 months following the treatment.

The frequency of administrations of RTX exhibited similarity in both genders. A paired comparison of patients with available CD19+ levels at both 6 and 12 months demonstrated no statistically significant concordance (p>0.05), particularly concerning the absence of replenishment at 6 months and enduring depletion below standard levels at 12 months. Patients who sustained low CD19+ levels at 6 months (CD19+ levels less than 10 cells/μL) typically did not reach CD19+ counts surpassing 170 cells/μL by 12 months post-RTX, with four out of five falling into this category. Conversely, among the patients who achieved repletion (10 cells/μL or higher) at 6 months, three did not revert to standard levels by 12 months following the administration of RTX.

Among the 27 patients, 20 (74%) received concurrent immunosuppression (IS). Specifically, 18 (67%) were treated with mycophenolate mofetil (MMF), 2 (35%) with methotrexate (MTX), and 1 with cyclophosphamide (CYC). Out of the patients administered CYC, 2 out of 8 (25%) remained depleted at 6 months (p=0.131). Similarly, among the five patients who did not replenish (CD19+ count <10 cells/μL) at 12 months, two had been treated with CYC (p=0.114). No significance was found between simultaneous CYC use and restoration to standard levels by 12 months post-RTX (p=0.429). Additionally, there was no substantial correlation between simultaneous use of MMF, MTX, CYC, cyclosporin, azathioprine or tacrolimus and CD19+ levels ≥10 cells/μL at 6 or 12 months. However, a statistically significant difference was observed with hydroxychloroquine and enduring depletion at 12 months (Table 2).

A statistically significant difference was observed in the reduction of CD19+ levels during the 1st and 2nd months following RTX between autoimmune diseases and vasculitis (Polyarteritis Nodosa, Granulomatosis with polyangiitis, Henoch Schoenlein Purpura). The decline during these months was more pronounced in autoimmune diseases (Table 3).

Safety Assessment

Out of the 27 patients, 14 (52%) were admitted to the hospital due to non-life-threatening infections. Among these, three patients were hospitalized twice, and one pa-

Table 2. Concurrent treatment and CD19+ levels

	CD19+<10 cells/μL n (%) at 6th month			CD19+<10 cells/μL n (%) at 12th month			CD19+<170 cells/μL n (%) at 12th month		
	Yes	No	p	Yes	No	p	Yes	No	p
Mycophenolate mofetil			0.248			1			0.429
No (n=9)	1 (25)	3 (75)		1 (50)	1 (50)		2 (100)	0 (0)	
Yes (n=18)	7 (58.3)	5 (41.7)		4 (50)	4 (50)		6 (75)	2 (25)	
Methotrexate			1			0.292			0.598
No (n=25)	7 (50)	7 (50)		4 (44.4)	5 (55.6)		7 (77.8)	2 (22.2)	
Yes (n=2)	1 (50)	1 (50)		1 (100)	0 (0)		1 (100)	0 (0)	
Azathioprine			0.055			0.114			0.429
No (n=22)	5 (38.5)	8 (61.5)		3 (37.5)	5 (62.5)		6 (75)	2 (25)	
Yes (n=5)	3 (100)	0 (0)		2 (100)	0 (0)		2 (100)	0 (0)	
Cyclosporine			0.131			0.114			0.429
No (n=25)	6 (42.9)	8 (57.1)		3 (37.5)	5 (62.5)		6 (75)	2 (25)	
Yes (n=2)	2 (100)	0 (0)		2 (100)	0 (0)		2 (100)	0(0)	
Methylprednisolone			0.522			0.114			0.236
No (n=7)	2 (66.7)	1 (33.3)		0 (0)	2 (100)		1 (50)	1 (50)	
Yes (n=20)	6 (46.2)	7 (53.8)		5 (62.5)	3 (37.5)		7 (87.5)	1 (12.5)	
Hydroxychloroquine			0.248			0.292			**0.035**
No (n=7)	3 (75)	1 (25)		0 (0)	1 (100)		0 (0)	1 (100)	
Yes (n=20)	5 (41.7)	7 (58.3)		5 (55.6)	4 (44.4)		8 (88.9)	1 (11.1)	
Cyclophosphamide			–			–			–
No (n=26)	8 (50)	8 (50)		5 (50)	5 (50)		8 (80)	2 (20)	
Yes (n=1)	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Tacrolimus			0.302			–			–
No (n=25)	7 (46.7)	8 (53.3)		5 (50)	5 (50)		8 (80)	2 (20)	
Yes (n=2)	1 (100)	0 (0)		0 (0)	0(0)		0 (0)	0 (0)	

Table 3. CD 19 levels for disease categories

Diseases	CD19+ levels at 1–2 months after RTX treatment (Mean)	SD	p
Autoimmune diseases (n=18)	6.46	20.71	**0.014**
Vasculitis (n=3)	153.0	261.5	

SD: Standard deviation; RTX: Rituximab.

tient experienced three hospitalizations. Among the 13 infection-related hospitalizations, one (44%) was due to syncope. An allergic reaction to RTX infusion was reported in one patient. No other adverse events linked to RTX infusion or CD19+ levels were noted. Independent of CD19+ counts, 20 patients out of 27 (74%) received intravenous immunoglobulin (IVIg) for reasons unrelated to disease management. In five patients, hypogammaglobulinemia was confirmed. Among the remaining 22 patients, IgG levels were checked and found to be within the normal range. Prior to RTX infusion, the mean B cell count was 271 cells/μL (range=57–359). Following RTX, at the six-month mark, B cell counts below 10 cells/μL were observed in 46.6% of patients. Within our

Table 4. CD19+ levels after RTX treatment by changes the months

Months (after RTX treatment)	CD19+ (mean diff. µl)	p
CD19+ levels at 1-2 months after RTX treatment	**236.8**	**0.003**
CD19+ levels at 3-4-5 months after RTX treatment	**275.3**	**0.009**
CD19+ levels at 6-7-8 months after RTX treatment	**223.7**	**0.004**
CD19+ levels at9-10-11 months after RTX treatment	-65.9	0.970

RTX: Rituximab.

study, we conducted a thorough examination of CD19+ levels depletion over distinct monthly intervals. This analysis resulted in the categorization of our observations into four distinct groups: the initial 2 months, the subsequent 3 months, the subsequent 3 months, and the final 3 months following the administration of RTX infusion. Our rigorous evaluation of these categories revealed a statistically significant difference in CD19+ depletion between the first three categories, as indicated in Table 4.

DISCUSSION

This study represents a comprehensive investigation into the dynamics of B cell repletion following RTX treatment among pediatric rheumatologic patients, shedding light on the influence of demographic factors on this process. While existing literature documents variable timelines for B cell repletion post-RTX treatment, our study explores the effect of the demographic factors on the frequency of B cell depletion and full regeneration in this patient cohort [5–7, 20, 22].

Previous knowledge regarding RTX for the depletion of B cells primarily stems from studies involving adult patients, which predominantly focus on effects in closer periods. For instance, Popa et al. [24] reported persistent B cell depletion 12 months post-RTX treatment in adults with rheumatoid arthritis (RA). Similarly, Thiel et al. [25] reported prolonged B cell depletion in adult cohorts encompassing connective tissue diseases (CTD), RA, and vasculitis, spanning a duration of more than 12 months. They defined full regeneration of B cells, which appear to be in absolute form, surpassing 70 cells/µL. Notably, patients with vasculitis exhibited a significantly higher failure rate in achieving total regeneration, exceeding 90%, compared to 7% in RA. Additionally, CTD levels which tend to be at 12% are in this scope. When the exact time of depletion after RTX infusion was examined, it was observed that this condition occurred most often in the ninth month in patients diagnosed with CTD and RA, and this period extended to the 26th month in patients diagnosed with vasculitis [24–26].

In our study, we present data from a clinically noteworthy group of 27 pediatric individuals subjected to RTX such as, vasculitis, juvenile dermatomyositis, SLE, and juvenile idiopathic arthritis (JIA). This investigation primarily delves into the enduring impacts of RTX treatment on B cell depletion and regeneration. About half sustained depletion with counts below 10 cells/µL at 6 months, while roughly 50% achieved regeneration to levels surpassing 10 cells/µL at 12 months. Eighty percent of the patients did not reach standard CD19+ levels (above 170 cells/µL) within one year following the final dose of RTX treatment. Despite the majority of the patients in our study being diagnosed with SLE, no statistically significant difference was observed between diagnostic groups in terms of sustained depletion from 6 to 12 months. Interestingly, our data indicates that patients who fail to achieve CD19+ levels of 10 cells/µL or higher within 6 months have an 80% likelihood of persistent depletion at 12 months. This finding closely aligns with the results of Mitchell et al. [23].

Age and gender were examined as demographic factors influencing the variability in B-cell depletion and regeneration. In according to our data did not uncover any statistically significant correlation between age and B cell regeneration, nor did it detect variances in the frequency of RTX treatments administered across distinct disease categories. Moreover, no statistically significant difference was found between the mean number of treatments and CD19+ depletion at 6 and 12 months or remaining below normal levels at 12 months. These findings suggest that B cell normalization may be influenced by patient-specific factors, potentially involving genetic predispositions or unique disease manifestations that regulate CD19+ levels post-RTX treatment. This suggests that

additional therapeutic interventions may have an impact on B cell regeneration.

Despite the well-established B cell reducing effects of cyclophosphamide (CYC), Thiel et al. [25] demonstrated an independent relationship between CD19+ proliferation and cumulative CYC dose [21–24, 26]. They showed that in our pediatric cohort, patients mostly increased their CD19+ levels to 10 cells/μL or more within the first 12 months, regardless of their primary disease. Notably, approximately 55% of Mitchell et al.'s [23] study underwent combined RTX and CYC treatments. Although we observed a trend hinting at potential statistical significance in persistent B cell depletion at both 6 and 12 months when CYC was involved, there was no correlation between CYC usage and the incidence of complete regeneration to normal CD19+ levels. These findings strongly suggest a potential synergistic effect of RTX and CYC, suppressing B cell numbers and extending the duration of B cell depletion in tandem.

In our study, we identified a significant difference between age groups and disease categories. Half of the patients (50%) continued to experience depletion at both 6 and 12 months. By the 12-month mark, 80% of patients achieved CD19+ regeneration levels of 170 cells/μL or lower but did not reach normal CD19+ levels. Notably, we did not observe any severe adverse events linked to RTX infusion or CD19+ levels. Irrespective of CD19+ counts, 74% of our patients received intravenous immunoglobulin (IVIG) for reasons unrelated to disease management. Out of the 27 patients, 52% were admitted to the hospital due to non-life-threatening infections, including acute gastroenteritis and upper respiratory infections.

The cohort of pediatric patients in Mitchell et al.'s investigation exhibited a remarkable resilience to RTX treatment, with a mere 14% necessitating hospitalization due to non-life-threatening infections while undergoing RTX. However, there are also studies showing a 30% infection rate during the treatment process in patients using only RTX. [23, 27]. Importantly, about 44% of those hospitalized had CD19+ levels below 10 cells/μL. Studies have shown that different CD19+ levels affect the hospitalization rate. Nevertheless, these findings are limited by their concentration on a single time point, as CD19+ levels were not consistently monitored in their cohort. The timing of CD19+ level assessments concerning infection episodes remained unclear, and prolonged B cell depletion might indeed increase the vulnerability to infections. Notably, Mitchell et al. [23] study experienced infrequent hypogammaglobulinemia necessitating IVIG replacement therapy, although immunoglobulin levels were not consistently measured, and prophylactic IVIG was administered in some cases prior to confirmed hypogammaglobulinemia. Patients with prolonged B cell depletion were at very high risk of severe infections, emphasizing the importance of monitoring immunoglobulin levels and B cell in RTX-treated patients. Some individuals might require IVIg replacement therapy, especially when confronted with B cell depletion.

Mitchell et al. [23] similarly emphasize the variability in the timing of proliferation and B-cell regeneration, aligning with our study's findings. Considering the cytotoxic effects of CYC on lymphocytes, normalization of B cell numbers is delayed when RTX and CYC are administered together. Our study makes an important contribution to the literature by examining the relationship between demographic factors and B cell regeneration responses. Our results show that other demographic variables, including primary disease diagnosis, do not significantly influence B cell depletion and full regeneration. However, larger studies are needed to further investigate the complex interplay between demographic factors and B cell regeneration.

It's crucial to acknowledge the limitations of our retrospective study, marked by the absence of a universally agreed-upon definition for depletion and regeneration in the existing literature. In our investigation, the cutoff definitions for CD19+ were established based on specific literature [8, 23, 28, 29]. Thiel et al. [25] adopted a lower regeneration threshold, set at CD19+ counts of 70 cells/μL or higher, and defined depletion as fewer than 1 cell/μL [23]. While reducing the CD19+ threshold could potentially heighten sensitivity, the clinical significance of such adjustments remains uncertain. Moreover, CD19+ were not consistently monitored in our cohort, usually occurring before subsequent RTX treatments. Consequently, the absence of B cells might refer to disease remission. Importantly, Charles et al. [30] found no difference in disease relapse rates among adult ANCA-associated vasculitis patients, regardless of whether they received RTX treatment on a fixed schedule or based on CD19+ levels [23]. Although regular monitoring of B cells may hold potential benefits, the clinical relevance concerning absolute values and disease activity remains unclear. The response of B cells to RTX is multifaceted, with the underlying pathophysiology of B cell elimination and reconstitution yet to be fully elucidated. Consequently, a larger study population is needed to fully assess the potential risk factors contributing to persistent B cell depletion.

Conclusion

Extensive investigation is needed to unravel the intricate interplay between demographic variables and the regeneration of B cells. Our study sheds light on the tolerability of RTX therapy among pediatric rheumatic disease patients and accentuates the variations in B cell repletion and normalization at 6 and 12 months. The resurgence of B cells appears predominantly unaffected by the number of RTX treatments and the underlying disease, while the utilization of other immunosuppressive therapies, particularly CYC, might extend the duration of B cell depletion. Our study adds significant insights by exploring the influence of demographic variables on responses to B cell regeneration. Notably, we observed no substantial correlation between demographic factors and the decline and subsequent full recovery of B cells. Nonetheless, a more comprehensive examination is imperative to unravel the intricate dynamics between demographic factors and the regeneration of B cells.

Ethics Committee Approval: The Umraniye Research and Training Hospital Ethics Committee granted approval for this study (date: 28.11.2022, number: B.10.1.TKH.4.34.H.GP.0.01/369).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The authors declared that no artificial intelligence was used to produce this article.

Authorship Contributions: Concept – GOB, BS; Design – GOB, BS; Supervision – GOB, BS; Fundings – GOB, BS; Materials – GOB; Data collection and/or processing – GOB, BS; Analysis and/or interpretation GOB, BS; Literature review – GOB, BS; Writing – GOB; Critical review – GOB, BS.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):413–418
doi: 10.14744/nci.2025.24603

Original Article PEDIATRICS

The optimal number of sessions for biofeedback therapy in children: A retrospective study

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ABSTRACT

OBJECTIVE: Biofeedback electromyography (EMG) involves the transmission of pelvic and abdominal muscle activity to the patient via visual and sometimes auditory feedback, with the ultimate goal of learning to contract and relax the pelvic muscles at the appropriate times through real-time analysis and feedback. To determine the optimal number of biofeedback therapy sessions required for a therapeutic response in the treatment functional voiding dysfunction.

METHODS: The retrospective data of 779 patients who underwent biofeedback therapy at a tertiary pediatric hospital between 2017 and 2023 were analyzed. The study included patients referred for urinary symptoms and uroflow/EMG findings who did not respond to standard urotherapy and behavioral therapy and completed at least 8 biofeedback sessions. During treatment, methods such as EMG biofeedback, pelvic muscle training, and keeping symptom diaries were utilized. Statistical analyses were performed using the Mann-Whitney U test and Chi-Square test.

RESULTS: Of the patients, 62.4% were female, 37.6% were male, and the mean age was 9.05±3.05 years. The most common urinary symptoms were daytime urinary incontinence (59.4%) and nocturnal enuresis (54%). The average number of sessions required for a therapeutic response was 6±1.3. Female patients showed an earlier response to treatment compared to males ($p<0.01$). Younger patients demonstrated faster recovery and better response to therapy ($p<0.05$). Patients who did not respond to therapy had a higher mean age and required more sessions ($p<0.05$). The higher mean number of sessions in non-responders compared to responders was found to be statistically significant ($p=0.001$; $p<0.05$).

CONCLUSION: Biofeedback is an effective and non-invasive treatment method for children with functional voiding dysfunction. Most patients show symptomatic improvement within 1.5–2 months (2–8 sessions - average 6). Male patients may require longer treatment durations, while younger children respond better to therapy. Future studies focusing on factors influencing biofeedback success may contribute to optimizing this treatment.

Keywords: Biofeedback; children; enuresis; incontinence; session; urotherapy.

Cite this article as: *Yener S. The optimal number of sessions for biofeedback therapy in children: A retrospective study. North Clin Istanb 2025;12(4):413–418.*

Urinary incontinence significantly impacts children and their families, causing both physical and psychosocial challenges. While its exact prevalence is difficult to determine, it is estimated to account for approximately 40% of all pediatric urology consultations [1]. In recent years, we have seen in our own practice that this rate has increased up to 60%. In a similar study, the prevalence of stress, urgency, and nocturnal enuresis was reported as 22.95%, 19.34%, and 93.93%, respectively [1]. Gender shows a significant association with stress and urgency-type incontinence, while age is significantly associated with nocturnal enuresis [2].

Received: April 15, 2025 *Revised:* June 11, 2025 *Accepted:* July 15, 2025 *Online:* August 27, 2025

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The first-line treatment for bladder dysfunction is urotherapy, which includes specific behavioral modifications such as scheduled toilet visits, limiting fluid intake in the evening, adopting proper voiding and defecation postures, and managing constipation [3]. When urotherapy alone is insufficient, clinicians explore other treatment options. Biofeedback (BF) therapy is a second-line treatment in selected patients with functional voiding dysfunction [4]. The goal of biofeedback electromyography (EMG) is to retrain pelvic muscles and the bladder-brain connection, teaching proper voiding and defecation habits.

The ultimate aim is for the patient to learn to contract and relax pelvic muscles at appropriate times through real-time analysis and feedback. While biofeedback's effectiveness has been demonstrated in many studies, the optimal duration of therapy required for maximum benefit remains unclear. This treatment is a time- and education-intensive process for patients, families, and healthcare systems.

This study aims to determine the optimal number of biofeedback therapy sessions required for a therapeutic response in the treatment of functional voiding dysfunction.

MATERIALS AND METHODS

Data from all patients undergoing biofeedback therapy at a tertiary pediatric hospital between 2017 and 2023 were retrospectively collected. A total of 779 patients who completed at least 8 sessions of biofeedback therapy were included in the study. Patients with urinary incontinence complaints and voiding symptoms were included in the study. Patients with neurogenic and/or anatomical issues, as well as those who underwent BF therapy solely for constipation/encopresis complaints, were excluded from the study. Patients with urinary symptoms unresponsive to standard urotherapy [3](information, clarification, lifestyle advice, recommendations, behavior modification, recording, and support)were referred for BF sessions. Standard urotherapy was applied for a duration of 6–8 weeks. An initial plan of 8 sessions was outlined for all patients. All sessions were conducted by a single urotherapy nurse. The therapist was a urotherapy and urodynamics unit nurse, trained and experienced in BF therapy, uroflowmetry, and standard urotherapy. Sessions were scheduled weekly, and the decision to extend the number of sessions was made with the consent of the physician, patient, and family.

Highlight key points

- Biofeedback therapy is an effective treatment for functional voiding dysfunction in children.
- It has been shown that results in Biofeedback therapy treatment can be achieved in optimally 6 sessions.
- Factors related to biofeedback success depend on the patient, the family, and the education nurse, and cooperation between them is very important.

The first session consisted of a 10-minute evaluation and a 20-minute biofeedback training segment. Evaluation was performed using the DVISS (Dysfunctional Voiding and Incontinence Symptoms Score) [5]. Subsequent sessions focused on the 20-minute BF training segment. Before starting the BF sessions, anatomical and functional principles related to voiding were explained to the children and their parents with illustrated and figured explanations. Medical Measurement System (MMS) was used in the BF sessions. In the second part, two electromyography (EMG) electrodes were placed at 3 and 9 o'clock on the pelvic floor, and the reference electrode was placed on the anterior aspect of the thigh. Patients were made aware of the function of their pelvic floor muscles using animated figures and were taught how to contract their external urethral sphincter during the sessions. Patients were asked to do these exercises at home. It is recommended to apply it once a day at home until the next session. During the first part of each session, patients were assessed, home progress and biofeedback diaries were reviewed, and education on elimination programs was reinforced. Family-provided BFdiaries documented urinary frequency, incontinence, constipation-encopresis, and adherence to therapy. Symptom improvement was documented in a written summary by the BF nurse based on the diary and patient-family interviews. The number of sessions in which improvement was detected was recorded. Improvement was noted as either 'response present' or 'no response.' According to ICCS (International Children's Continence Society: ICCS) recommendations, the results were classified into two categories:

1. No response: Less than 50% reduction in symptoms.
2. Response: Partial response (50–99% reduction in symptoms) and complete response (100% resolution of symptoms) [6].

Urinary symptoms were categorized into seven thematic groups: Incontinence (daytime urinary leakage), enuresis (nocturnal enuresis), urgency, frequency, uri-

Table 1. Comparison of age and session count according to response status

	n	Mean±SD	Min–Max (median)	p
Age				
No response	227	9.26±2.8	5–17 (9)	0.044*
Response	552	8.96±3.1	5–17 (8)	
Improvement bession				
No response	227	6.79±0.98	3–8 (7)	0.001**
Response	552	6±1.3	2–8 (6)	

SD: Standard deviation; Min: Minimum; Max: Maximum; Mann-Whitney U Test; *: P<0.05; **: P<0.01.

nary retention, giggle incontinence, constipation-encopresis. Bowel dysfunction was recorded in BF diaries based on reports of Bristol Type 1 stool or encopresis (fecal leakage, staining, or soiling). Treatment outcomes were evaluated based on the BF session at which urinary symptoms improved. Improvement was defined as the regression of the patient's initial complaints. Nurse observations were conducted during each session. Physician assessments were performed during the initial examination and after the BF therapy. The final physician assessment was conducted after the last session, incorporating the nurse's report and a face-to-face interview with the family. Health Sciences University Umraniye Training and Research Hospital Ethics Committee reviewed and approved the study design (date: 23.11.2023 decision no: B.10.1.TKH.4.34.H.GP.0.01/452). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

For statistical tests, data were evaluated as response/no response to treatment. Statistical analyses were performed using the NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) software. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, and maximum) were used to evaluate study data. Data distribution was assessed using the Shapiro-Wilk Test. The Mann-Whitney U Test was used for comparisons of quantitative data between two groups, while the Chi-square analysis assessed relationships between qualitative data. Significance was evaluated at $p<0.01$ and $p<0.05$ levels.

RESULTS

Of the patients, 62.4% (n=486) were female, and 37.6% (n=293) were male. The mean age of all patients was 9.05±3.05 years.

The mean age of patients responding to BF treatment was 8.96±3.1, which was younger than those who did not respond. Age and session outcomes are summarized in Table 1. Daytime incontinence (n=463) and nocturnal enuresis (n=421) were the most prevalent urinary symptoms.

The response of the patients to BF treatment was found to be higher in girls with 360 (65.2%) girls and 192 (34.8%) boys. The highest response rate to treatment was observed in the patient group with incontinence (daytime urinary incontinence) with 58.9% (n=325). Findings on urinary symptoms and biofeedback therapy responses are detailed in Table 2.

Patients who responded to treatment were younger on average than non-responders ($p=0.043$; $p<0.05$). Additionally, responders required fewer sessions to achieve improvement than non-responders ($p=0.001$; $p<0.01$).

Given the stable use of anticholinergics and desmopressin during biofeedback therapy (47.8% of patients [451/799] initiated or discontinued usage), medication status was also included in the analysis. It was found that the response to treatment was more effective (56.2%) in patients receiving medication (Table 3).

DISCUSSION

Functional voiding dysfunction has significant psychosocial impacts on children and their families. Biofeedback is a crucial tool for treating this condition; however, retraining voiding mechanics takes time. Understanding the timeframe for clinical improvement can guide clinicians, patients, and families in setting realistic expectations.

Our aim in this study was to determine the optimally number of biofeedback sessions that patients should receive before considering other treatments. We reported symptom improvement by session.

The regression of the patient's complaint after the first session was evaluated as a response to treatment and the average number of sessions was determined as 6±1.3. In addition, the fact that the average age of those who did not respond to treatment was higher than those who did was found to be statistically significant ($p=0.043$; $p<0.05$). As we observed in the clinic, we saw that in rela-

Table 2. Relationship between urinary symptoms and response to biofeedback therapy

	No response	Response	Total	p
Gender				0.011*
Female	126 (55.5%)	360 (65.2%)	486 (62.4%)	
Male	101 (44.5%)	192 (34.8%)	293 (37.6%)	
Incontinence				0.501
Yes	138 (56.3%)	325 (58.9%)	463 (58.1%)	
Enuresis				0.497
Yes	125 (51%)	296 (53.6%)	421 (52.8%)	
Urgency				0.210
Yes	9 (3.7%)	32 (5.8%)	41 (5.1%)	
Frequency				0.144
Yes	11 (4.5%)	14 (2.5%)	25 (3.1%)	
Urinary retention				0.942
Yes	6 (2.4%)	14 (2.5%)	20 (2.5%)	
Giggle				0.459
Yes	2 (0.8%)	8 (1.4%)	10 (1.3%)	
Constipation-encopresis				0.403
Yes	2 (0.8%)	2 (0.4%)	4 (0.5%)	

Chi-Square Test; *: P<0.05.

Table 3. Relationship between medication use and response to biofeedback therapy

	No response	Response	Total	p
Medication use				0.715
No	104 (42.4%)	242 (43.8%)	346 (43.4%)	
Yes	141 (57.6%)	310 (56.2%)	451 (56.6%)	

tively younger patients, education and information about urination in the family and child contributed quickly to the treatment. We can explain the positive effect of the relatively younger age group in the BF treatment as the fact that the game was with visual animations and their motivation was higher. This situation also ensures that they come to therapy sessions willingly.

In one study, it was stated that in most cases, improvement was evident in approximately 3 months [7]. They stated that more than one third of the patients in their series continued with a pause and progress after 9 months. Possible reasons for this lack of progress were selection of patients with severe voiding disorders, patient non-compliance, or decreased clinical benefit due to the increase in session intervals to 3-4 months after the 8th session. Our session duration was determined as 8 weeks. The duration can be extended to once a week depending on the clinical condition and the motivation of the child and the family. However, we believe that the response to treatment may be delayed and delayed in patients who have a very long duration and long session intervals. A statistically significant relationship was found between gender and treatment response ($p=0.011$; $p<0.05$). The higher proportion of females compared to males among those who responded to treatment was also found to be statistically significant ($p=0.001$; $p<0.01$)."

Combs et al [8] found that the average number of biofeedback sessions required to achieve a consistent urodynamic response in a series of 21 patients was approximately 3.7 (approximately 1–3 months after the initial visit). The authors also noted that clinical response required more sessions. In our study, we reported an average of 6 sessions. The earliest response was 2 and the latest was 8 sessions. Another study showed that patients who achieved success with biofeedback underwent an average of 4.1 sessions (3–6 months after the initial visit) [9]. Our results are consistent with these studies, but we suggest that symptomatic improvement is best achieved in 1.5 months.

When we evaluate the response to biofeedback treatment according to the urinary symptoms at presentation, we see that the only significant data is the gender difference. No statistical significance was found in terms of other symptoms and response status. Our data show that boys may have a slower recovery rate than girls and may need biofeedback therapy for a longer period. In another study that reached similar results to ours, the authors found that the maximum recovery time in urinary symptoms was 10 sessions in girls and 22 sessions in boys [7]. It was observed that male patients were less willing to report the latest status of their complaints and their views on the treatment in the feedback interview conducted in the last biofeedback sessions. This finding suggests that we need to do more work on this subject. While gender-based responses to biofeedback have not been previously defined, a similar finding was mentioned in a study conducted in recent years [7]. However, it is known that female patients are included in a higher rate in biofeedback studies. In our series, the rate of female patients was 62.4% of the patients.

Future research will be aimed at understanding patients who do not respond to BF and maximizing BF success. Factors related to biofeedback success affect both the patient, the family, and the education nurse, and cooperation between them is very important. A study on behavioral therapy has shown that behavioral therapy provides 60–80% improvement in children with voiding disorders [10, 11]. Another study reported a 59% success rate with behavioral therapy in patients 4 years of age and older diagnosed with lower urinary tract dysfunction and urinary tract infection [12]. Similarly, in another series, 54% of patients had a voiding disorder symptom score of ≤8.5 after an average of 6 months of behavioral therapy. It was observed that there was a 68% improvement in diurnal enuresis (partial response), 58% in nocturnal enuresis (partial response), 84% in intermittent urination (partial response), and 91% improvement in the complaint of needing to urinate again shortly after urinating (complete response) [13]. In addition to these studies, a systematic review of standard urotherapy indicated that a possible explanation for the low efficacy rates of urotherapy in nocturnal enuresis is the large heterogeneity of the study populations and interventions. They also believe that the duration of intervention and the intensity of the intervention may have an effect on the outcome [14]. We would like to emphasize by mentioning all these studies the importance of family, child and nurse harmony and togetherness. The observation of the nurse applying biofeedback treatment is one of the most effective conditions in the physician's taking the right path for the patient in this process. The attitude of the family and the child's willingness are other factors that support success. Our observational data is that effective success is achieved in a short time in children who regularly apply the nurse's recommendations for voiding training at home. Some of the technical difficulties during the application are that it is difficult to stick the electrode in children with high body weight and the adhesive constantly separates due to sweating. There have been patients who have described leg pain at the end of the session due to incorrect application of biofeedback treatment in relatively older children. This is also a situation that should be kept in mind by the practitioner.

A recent meta-analysis reported no benefit from biofeedback therapy in children with non-neuropathic voiding disorders [15], but a prospective study found that biofeedback therapy helped patients gain control of their voiding function [16]. It has also been shown that children with dysfunctional voiding can be successfully treated with biofeedback therapy with or without animation. Our procedure is animated. It has been observed that presenting several animated visual options to the child allows the child to perceive the treatment as a game and has a positive motivational effect.

Our study has some limitations. It is a single-center, retrospective study, which may introduce observer bias, patient selection bias and limit the validity of our results to other centers. Another limitation is how urinary symptoms were measured. Patients' symptoms were recorded as dichotomous (present/absent), but symptoms are a continuous variable, especially in cases of functional voiding dysfunction. The use of dichotomous outcomes may limit closer analyses of symptom improvement. Adherence to biofeedback treatment is an important aspect of biofeedback and requires compliance from both the patient and the family. This is difficult to measure and therefore cannot be reported.

In conclusion, biofeedback is a simple, effective and noninvasive treatment method for children with functional voiding dysfunction. It improves voiding disorders, voiding patterns and also has a significant impact on the quality of life of both children and their families. This study is one of the large patient sample studies evaluating the treatment process of biofeedback in the management of voiding dysfunction. As we mentioned at the beginning of the discussion in our study, we reported an average of 6 sessions. The earliest response was 2 and the latest was 8 sessions. Our data suggest that most patients who will benefit from biofeedback will improve within the first 1.5-2 months and that other patients may continue to improve over the following periods. Clinical improvement may be slow or ineffective for some patients. In this case, re-evaluation or other treatment options should be considered. Female patients may benefit from biofeedback earlier than male patients and are more likely to receive feedback on progress. The success rate of biofeedback on quality of life may be more effective in children and their families who suffer less from functional voiding dysfunction. This can help physicians guide patients and families about what to expect from treatment biofeedback therapy. We would like to emphasize that the 70.8% treatment response rate observed in our study should be interpreted with caution to avoid overgeneralization and to strengthen directions for future research.

Conclusion

Our study demonstrates that biofeedback therapy is an effective treatment for functional voiding dysfunction in children, with optimal results achieved within 6 sessions. Further research is needed to understand the factors influencing therapy outcomes and to maximize the effectiveness of biofeedback.

Ethics Committee Approval: The Health Sciences University Umraniye Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 23.11.2023, number: B.10.1.TKH.4.34.H.GP.0.01/452).

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: Not declared.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):419–424
doi: 10.14744/nci.2024.79577

Original Article UROLOGY

Comparison of two analgesia applied to periprostatic nerve blockage during transrectal ultrasound guided prostate biopsy

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ABSTRACT

OBJECTIVE: A combination of local anesthetic treatments provides better pain alleviation than periprostatic nerve block (PPNB) alone during a prostate biopsy procedure. The primary objective of this study was to compare Visual Analog Scale (VAS) pain levels during transrectal ultrasound (TRUS)- guided prostate biopsy whilst the use of prilocaine-lidocaine cream, diclofenac suppository, or PPNB only in a prospective, randomized study.

METHODS: This study included 162 patients who had TRUS-guided prostate biopsies performed at the Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Center within a 6 month period, from April to October 2017. Three groups of patients were randomly assigned: group 1 underwent PPNB plus prilocaine-lidocaine cream, group 2 received diclofenac suppository along with PPNB, and group 3 underwent PPNB alone. The VAS was used to measure the degree of pain: VAS 1 was used to record the pain at the time the ultrasound probe was inserted, VAS 2 was used to document the pain during PPNB, and VAS 3 was used to record the pain during needle biopsy. Following the biopsy, any complications or negative consequences were recorded.

RESULTS: Mean age or serum prostate specific antigen (PSA) levels were similar between the three groups. The VAS 1, VAS 2, and VAS 3 pain scores showed statistically significant difference among the three groups (p=0.001). Between groups 1 and 2, there was a statistically significant difference in VAS 1 pain scores (p=0.01). There was no statistically difference in VAS 2 and VAS 3 pain scores between the groups 1 and 2 (p=0.08 and p=0.23, respectively). Patients between the groups 3 and other groups had significantly difference in VAS pain scores (p<0.05).

CONCLUSION: In this study, we highlight that when applied as an adjuvant to PPNB, either 5% prilocaine-lidocaine cream or a 100 mg diclofenac suppository reduced pain levels relative to PPNB alone. When compared to a 100 mg diclofenac suppository, prilocaine-lidocaine cream significantly reduces pain during the insertion and manipulation of the ultrasound probe.

Keywords: Analgesia; anesthesia; biopsy; prostate.

Cite this article as: *Aybal HC, Yikilmaz TN, Basar H. Comparison of two analgesia applied to periprostatic nerve blockage during transrectal ultrasound guided prostate biopsy. North Clin Istanb 2025;12(4):419–424.*

Prostate cancer (PCa) is the second largest cause of cancer-related mortality worldwide, making up 15% of all cancer cases [1]. Patients with PCa have been diagnosed with great success using the raised serum prostate-specific antigen (PSA) level and abnormal digital rectal examination (DRE) findings [2].

The study has been reported in abstract form at the 5th International Hippocrates Congress on Medical and Health Sciences (18–19 December 2020).

Received: June 01, 2024 *Accepted:* July 07, 2024 *Online:* August 08, 2025

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Prostate biopsy is the gold standard diagnostic method for obtaining the histopathological diagnosis [3]. Ultrasound (US)-guided transrectal or transperineal biopsy is the two main procedures used to the diagnose of PCa. Local analgesia with PPNB is the well-known technique in analgesia before prostate biopsy [4]. PPNB reduces the pain and discomfort associated with the prostate biopsy. However, the ultrasonography probe's insertion and the needle's movement during infiltration of local anesthetic (ILA) can both be uncomfortable [5]. Numerous studies have demonstrated that the administration of intrarectal local analgesia reduces discomfort during prostate biopsies [6, 7].

In a prospective, randomized trial, the main goal of this research was to assess the Visual Analog Scale (VAS) pain scores during TRUS-guided prostate biopsy while utilizing prilocaine-lidocaine cream, diclofenac suppository, or PPNB alone.

Highlight key points

- During transrectal US-guided prostate biopsy, administration of diclofenac suppository or prilocaine-lidocaine cream in addition to PPNB provides better analgesia than PPNB alone.
- Prilocaine-lidocaine cream is more effective analgesia than diclofenac suppository during US probe insertion and manipulation.

MATERIALS AND METHODS

Study Design

A randomized, prospectively controlled study design was used to compare the pain level during a TRUS-guided prostate biopsy. This study included 162 patients who had TRUS-guided prostate biopsies performed at the Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Center within a 6-month period, from April to October 2017. The Dr. Abdurrahman Yurtaslan Ankara Onkoloji Training and Education Hospital Center ethical committee approved the study protocol (No. 2017-03/01). All patients signed an informed consent form. The study was conducted in accordance with the guidelines of the Declaration of Helsinki.

Population Selection

Inclusion criteria were adult males aged 40 and over, PSA levels higher than 4 ng/mL, or solid nodules on digital rectal examination (DRE). Exclusion criteria were known sensitivity to lidocaine, prilocaine, or diclofenac; hemorrhagic diathesis; anticoagulant use; undiagnosed pain; chronic pain syndrome; and anorectal disease.

Study Protocol

Patients referred to transrectal ultrasound guided prostate biopsy (TRUS-PB) based on the inclusion criteria as well as those referred for repeat biopsies as have been enrolled. The treatment of patients taking anticoagulants has been consulted with the relevant clinics. Those who took aspirin discontinued five days before the biopsy; coumadin, clopidogrel, and other drugs were discontinued one week ago. All patients had a negative urine culture prior to the procedure. To reduce the risk of urinary infection and sepsis, all patients without a history of drug allergy were prophylactically started with ciprofloxacin 500 mg S: 2x1 one day before the biopsy and maintained for one week afterward. A fleet enema was administered half an hour before the biopsy to purify the bowels. After infection prophylaxis, group 1 received a 5 g concentration of 5% prilocaine/lidocaine cream and waited 30 minutes; whereas group 2 received a 100 mg diclofenac suppository and waited one hour. Group 3 was determined as patients who underwent PPNB only. Age, serum PSA levels, DRE findings, prostate size at TRUS, presence of diabetes mellitus (DM), and hypertension (HT) were recorded after ILA was performed.

Prostate Biopsy

Participants were placed in the left lateral decubitus position, and transrectal ultrasound was performed using an ultrasound scanner (Sonoscape SSI5500BW) with a 3-15 MHz/ R 8 mm endorectal biplane transducer. The prostate has been scanned in both the axial and sagittal planes for evidence of abnormalities. Prostate volume was measured. Subsequently, ILA was performed in all groups by injecting 5 ml of 2% lidocaine hydrochloride lateral to the junction between the base of the prostate and the seminal vesicle using a 25 cm 18-gauge spinal needle. After 5 minutes, the prostate biopsy was conducted. All patients had six core samples obtained from each lobe, for a total of 12 core samples per participant. All specimens were obtained utilizing an automated biopsy gun (Geotek Alfa-Gun) and a 25-cm, 18-gauge tru-cut biopsy needle.

Table 1. Comparison of groups' demographic datas

	Group 1 (n=54)	Group 2 (n=54)	Group 3 (n=54)	p
Age*	63.36±6.28	66.04±7.58	67.41±7.99	0.06
PSA (ng/mL)**	6.56±4.67	6.67±5.47	7.55±7.46	0.99
Prostat volume (mL)**	40±17.48	45±32.32	46.5±23.96	0.57
Diabetes mellitus (%)	22.2	13	14.8	0.39
Hypertension (%)	25.9	29.6	29.6	0.88

PSA: Prostate Specific Antigen; *: Statistically analyzed with One Way ANOVA test; **: Analyzed with Kruskal. Wallis test, others analyzed with Pearson Chi- Square test.

Table 2. Comparison of groups' Pain VAS scores

	Group 1 (n=54)	Group 2 (n=54)	Group 3 (n=54)	p
Insertion of probe (VAS 1)	4 (3.75)	5 (5)	6 (2)	**0.001**
PPNB (VAS 2)	2 (1.75)	3 (2.75)	5 (2)	**0.001**
Biopsy sampling (VAS 3)	2 (1)	2 (2)	3 (2)	**0.001**

PPNB: Periprostatic nerve block, VAS: Visual Analog Scale. Statistically analyzed with Kruskal Wallis test.

Pain Assessment and Complications

Pain was assessed using a 10-point VAS (VAS, 0=no pain, 10=severe pain) at three points: VAS 1 pain related to the probe insertion and manipulation, VAS 2 pain related to ILA, and VAS 3 pain related to prostate sampling. The performing urologist briefed patients about the various stages of the procedure. Pain was recorded following each stage. Three hours and one week after the biopsy, patients were assessed for possible complications. Complications such as hematospermia, hematuria, rectal bleeding, acute urinary retention (AUR), dysuria, and fever were documented.

Statistical Analysis

IBM Statistical Package for the Social Sciences (SPSS) version 23 was utilized for the analysis. A One-way analysis of variance (ANOVA) was used to compare patient characteristics. Kruskal – Wallis test was conducted to assess differences in pain scores; a non-parametric Mann–Whitney U test or parametric Student t-test were used to determine the comparison of binary groups. Pearson Chi-square or Fisher's Exact test to evaluate the categorical datas. Statistical significance was indicated by p-values less than 0.05.

RESULTS

Demographic Data

There were no significant differences in age (p=0.06), PSA levels (p=0.99), prostate volume (p=0.57), presence of DM (p=0.39), or HT (p=0.88) between the groups. The clinical values of the participants are shown in Table 1.

Pain Scores

Significant differences in VAS scores among all research groups are observed (p=0.001). Table 2 displays clinical values of the VAS scores. The pain scores of the groups are examined separately. A statistically significant difference was found between Group 1 and Group 2 in the VAS 1 score (p=0.01). There was no statistically significant difference in the VAS 2 and VAS 3 scores between Group 1 and Group 2 (p=0.08 and p=0.23, respectively). A significant difference in VAS scores was found between groups 1 and 3 (p=0.001). Significant differences in VAS 1, 2, and 3 scores were found between groups 2 and 3 (p=0.014, p=0.005, and p=0.008, respectively). Table 3 shows the clinical values of VAS scores between the groups.

Table 3. Comparison of pain VAS scores between the groups

	Group 1 & 2 p-value	Group 1 & 3 p-value	Group 2 & 3 p-value
Insertion of probe (VAS 1)	**0.01**	**0.001**	**0.014**
PPNB (VAS 2)	0.08	**0.001**	**0.005**
Biopsy sampling (VAS 3)	0.23	**0.001**	**0.008**

PPNB: Periprostatic nerve block; VAS: Visual Analog Scale. Statistically analyzed with Kruskal Wallis test. P<0.016 value were considered statistically significant.

Table 4. Comparison of complications

	Group 1 (n=54)	Group 2 (n=54)	Group 3 (n=54)	p
Fever (%)	1 (1.9)	0 (0)	0 (0)	0.36
Hematospermia (%)	18 (33.3)	21 (38.9)	18 (33.3)	0.78
Hematuria (%)	15 (27.8)	20 (37)	17 (31.5)	0.58
Rectal bleeding (%)	6 (11.1)	8 (14.8)	7 (13)	0.84
Acute urinary retention (%)	1 (1.9)	2 (3.7)	4 (7.4)	0.35
Dysuria (%)	3 (5.6)	3 (5.6)	4 (7.4)	0.89

Statistically analyzed with Pearson Chi-Square test.

Complications

Fever was seen in only one patient in Group 1. Other complications were seen in all of the groups. Table 4 shows that there were no significant differences in the complication incidence between groups (p>0.05).

DISCUSSION

In this study, when compared to a control group of patients who administered PPNB alone, the intrarectally applied 5% prilocaine lidocaine cream or 100 mg diclofenac suppository considerably reduced the severity of pain. This pain was assessed by VAS at the time placement and movement of the US probe, during PPNB, and at prostate sampling. It was demonstrated that 5% prilocaine-lidocaine cream significantly reduced pain during US probe placement and movement compared to 100 mg diclofenac suppositories; however, no significant difference was observed during PPNB and prostate sampling.

Previous studies demonstrated pain during prostate biopsy is associated with three factors: the insertion and manipulation of the US probe in the rectum, the core-biopsy needle puncture to the periprostatic nerve area and to multiple prostate gland tissues. Prostatic pain can originate from the inferior rectal nerve or capsule of the prostate gland. Pelvic plexus fibers go to the prostate with cavernous nerves. The inferior rectal nerve, located above the prostate apex, is a pain sensitive area [8, 9].

Nash et al. [10] reported that the PPNB technique was effective in providing anesthesia for prostate biopsy. This technique provides blocking the neurovascular bundles, having no effect on the dentate line or the anal sphincter. Previous studies have demonstrated that prilocaine–lidocaine cream when applied before PPNB, is superior to the application of PPNB alone in terms of reducing the level of pain, during ultrasound probe placement, ILA application, and biopsy taking [4]. Moreover, in another study, pain was lower during probe placement and biopsy application in patients treated with prilocaine lidocaine cream, during ILA application did not show any significant difference [6]. In this study, the use of 5% prilocaine lidocaine cream showed a significant difference compared to the PPNB alone group during US probe placement, ILA, and prostate biopsy sampling.

The analgesic function of diclofenac has been reported in previous studies using a suppository form. Diclofenac,

a non-steroid anti-inflammatory drug (NSAID), acts by inhibiting prostaglandin synthesis. Prostaglandins, along with leukotrienes and cytokines, cause edema and pain sensations in the rectal mucosa. Diclofenac acts by decreasing the effects of local mediators on pain responses both locally and systemically [11]. The analgesic function of diclofenac demonstrated that there was a significant difference in pain relief in the 100 mg diclofenac suppository group only one hour after prostate biopsy [11, 12]. Interestingly, in this study, there was a significant difference in pain level during US probe placement, ILA, and prostate biopsy in the diclofenac group compared to the PPNB only group.

Although diclofenac suppository has systemic and local effects, prilocain-lidocaine cream has a local effect by inhibiting neurovascular innervation in the rectal mucosa. The pain relief function of prilocaine-lidocaine cream was found to be significantly higher than that of the diclofenac suppository group during ultrasound probe insertion in the study of Valdez-Flores, while no significant difference was observed related to the prostate sampling [7]. In this study, the VAS pain scale was compared during PPNB in addition to probe placement and prostate sampling, no significant difference was observed.

In the current study, complication rates were recorded;post-biopsy complication rates were similar to previously reported results [13, 14]. Hematospermia, hematuria, and rectal bleeding were the most frequent complications that did not need treatment. No significant differences were observed between the groups (Table 4).

Despite the use of the PI-RADSv2 scoring system by magnetic resonance imaging (MRI), all patients do not have a PIRADS score greater than three. Patients with PSA-density >0.20 ng/mL/mL and a PI-RADS score of 3 have elevated prostate cancer risk; targeted and systematic biopsies are required [15, 16].

The limitations of this study are that PSA density was not calculated and did not include targeted biopsy. MRI-targeted prostate biopsy is commonly performed, and further prospective studies can be conducted on its use for analgesia.

Conclusion

In conclusion, this study emphasizes that the application of 100 mg diclofenac suppository or 5% prilocaine-lidocaine cream as an adjunct administration to PPNB decreased the amount of pain when compared to PPNB alone. The efficiency increased when diclofenac suppository was applied one hour ago and prilocaine-lidocaine cream 30 minutes ago before US probe insertion. Moreover, prilocaine-lidocaine cream provides significant pain reduction at the placement and movement of the US probe when compared with a 100 mg diclofenac suppository.

Ethics Committee Approval: The University of Health Sciences, Dr. Abdurrahman Yurtaslan Oncology Training and Research Hospital Ethics Committee granted approval for this study (date: 09.03.2017, number: 2017-03/01).

Informed Consent: Written informed consent was obtained from the participants and their parents.

Conflict of Interest: The authors declared that they have no conflict of interest.

Financial Disclosure: The authors declared that no support was received from any person or institution for the study.

Use of AI for Writing Assistance: Not declared.

Authorship Contributions: Concept – HCA, TNY; Design – HCA; Supervision – TNY; Data Collection and processing – HCA, TNY; Analysis and interpretation – HCA; Literature search – HCA; Writing – HCA, HB; Critical review – TNY, HB.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):425–429
doi: 10.14744/nci.2025.42402

Original Article UROLOGY

Investigation of blood parameters as predictors in diagnosing acute scrotum

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ABSTRACT

OBJECTIVE: Acute Scrotum (AS) is defined as pain associated with swelling and redness of the scrotum. This study investigates the hemogram and C-Reactive Protein (CRP) values obtained from patients diagnosed with AS, based on the hypothesis that blood parameters can be used as biomarkers in the diagnosis of Testicular Torsion (TT). The aim was to evaluate the predictability of these parameters in diagnosing AS in both pediatric and adult age groups.

METHODS: Demographic data and blood parameters including Hemoglobin (Hb), Platelet (PLT), CRP, and White Blood Cell (WBC) levels of patients who presented to our Emergency Department with the diagnosis of AS (TT, epididymitis, varicocele) and received medical and surgical treatment were analyzed. The importance of these parameters in diagnosis was assessed.

RESULTS: A total of 26 patients were included in the study. The mean age of the cases was 15.8±3.6 years. 20 of the patients (76.9%) were in the pediatric age group, while 6 (23.1%) were in the adult age group. 93.1% (27) of the cases were unilateral, while 6.9% (2) had bilateral acute scrotum symptoms. The highest incidence of cases occurred in spring with 9 cases (31%), followed by winter and summer with 7 cases each (24.1%), and autumn with 6 cases (20.7%). Significant differences in age, platelet, and hemoglobin levels were observed between the pediatric and adult age groups.

CONCLUSION: In conclusion, this study highlights significant age-related differences in the diagnosis and treatment of AS. The higher requirement for surgical intervention in pediatric patients further emphasizes the importance of early diagnosis and timely intervention. However, further studies with larger sample sizes are needed to validate these findings and better understand the etiological differences. Additionally, a more specific analysis of blood parameters in subgroups (such as epididymitis, orchitis, TT) under the term "acute scrotum" may contribute to the literature.

Keywords: C-Reactive Protein; infertility; testicular torsion; hemoglobin.

Cite this article as: *Demir B, Nogay S, Dolanbay T, Polat ME, Akdemir E. Investigation of blood parameters as predictors in diagnosing acute scrotum. North Clin Istanb 2025;12(4):425–429.*

Acute Scrotum (AS) is defined as pain accompanied by swelling and redness of the scrotum. The most common causes of AS include torsion of intrascrotal appendages, epididymitis, testicular torsion (TT) and idiopathic scrotal edema. The primary goal in the management of AS is to establish an accurate diagnosis in a timely manner without jeopardizing the viability of the affected testis. In this context, TT is a condition that requires emergency intervention. To ensure an accurate and rapid diagnosis, a thorough medical history, physical examination, and diagnostic imaging when necessary are crucial. The similarity of clinical findings among different etiological causes, the presence of excessive tenderness in the scrotal region and ethical considerations may pose challenges during clinical examination [1–3].

Received: May 14, 2025 *Revised:* June 17, 2025 *Accepted:* July 03, 2025 *Online:* August 27, 2025

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TT results from the twisting of the spermatic cord and occurs in 10–15% of AS cases [4]. Additionally, it has been reported that approximately one-third of cases may present with atypical clinical findings [3]. A delay of four to eight hours in treatment can lead to permanent ischemic damage to the testis, which may result in fertility issues or, in some cases, necessitate orchiectomy [5]. In recent years, an increase in the incidence of acute epididymitis (AE) has been observed, accounting for 10–71% of all AS cases [2].

Scrotal edema is usually one of the initial signs of AS and develops suddenly, spreading rapidly. Localized redness is generally seen along with edema. Edema often begins in one hemiscrotum and gradually extends to the entire scrotum. Redness may extend to the groin (67%), perineum (42%), penis (20%), or suprapubic region. Edema and erythema are fundamental findings in all patients, with local pain accompanying them in 80% of cases. Additionally, in 21% of cases, the disease may recur up to three times without complications [6].

Bozlu and colleagues reported that germ cell loss in TT patients is associated with testicular lipid peroxidation products, nitric oxide (NO) content and myeloperoxidase (MPO) levels [7]. In recent years, oxidative stress has been shown to play a significant role in the damage caused by TT [8].

In this study, based on the hypothesis that blood parameters may serve as biomarkers for the diagnosis of TT, hemogram and C-reactive protein (CRP) values obtained from patients diagnosed with AS were examined. The predictability of these parameters in the diagnosis of pediatric and adult age groups was evaluated.

Highlight key points

- There are age-related variations in the diagnosis and treatment of acute scrotum.
- Early diagnosis and early surgery in acute scrotum are very important
- In our study model, we identified blood parameters that can be used to predict the prognosis of patients with acute scrotum and presented them to the scientific community for evaluation.

MATERIALS AND METHODS

After obtaining ethical approval from the Non-Interventional Ethics Committee of Malatya Turgut Ozal University Faculty of Medicine on (13.03.2025) with protocol number (2025/39), the study was initiated. Our study aimed to determine the significance of demographic data and blood parameters—including Hb (12.2–18.1g/dl), PLT (142–424 10^3/uL), CRP (0–0.5 g/dl) and WBC (4.6–10.2 10^3/uL) of patients presenting to the Emergency Department of Malatya Training and Research Hospital with an AS diagnosis (TT, epididymitis, varicocele) who received medical and surgical treatment. Biochemical markers were measured using the CRP kit on the About device in our hospital's biochemistry laboratory. Hemogram parameters were also measured in our hospital's biochemistry laboratory using the SYSMEX XN-1000 hemogram device and Cell Pack DST, DCL, WNR, WDF, and SLS Lysercell kits. A total of 45 patient data were accessed, and 29 patients who met the inclusion and exclusion criteria were included in the study.

While designing our study, we acted in accordance with the Helsinki Declaration.

Inclusion Criteria

1. Patients who applied to the emergency department with complaints of acute scrotum (TT, epididymitis, orchitis, varicocele) in the 0–18 age groups (pediatric) and 18 age groups (adult).
2. Patients whose diagnosis of acute scrotum was supported by physical examination and/or ultrasonography.
3. Those whose hemogram (Hb, WBC, PLT) and CRP values were fully recorded at the time of application.
4. Patients whose full data set could be accessed through the Hospital Information Management System (HIMS).

Exclusion Criteria

1. Patients with missing hemogram and/or CRP data.
2. Patients receiving anticoagulant, immunosuppressive or drug therapy that may affect systemic inflamation.
3. Patients with known hematological disease (e.g. leukemia, anemia, thrombocytopenia, etc.).
4. Patients experiencing scrotal complaints due to a cause other than acute scrotum (e.g. trauma, tumor, inguinal hernia, etc.).
5. Patients who refused hospital follow-up or treatment and were discharged voluntarily.

Table 1. Presentation of parametric and non-parametric

	Mean±SD	Median–IQR (25–75%)
Hb (g/dL)	14.9±1.3	
PLT (10^3/uL)	280.6±60.4	
WBC (10^3/uL)		10.2 (8.2–12.4)
CRP (mg/dl)		0.2 (0.2–0.6)

SD: Standard deviation; IQR: Interquartile range; Hb: Hemoglobin; PLT: Platelet; WBC: White blood cell; CRP: C-reactive protein.

Statistical Analysis

The findings obtained in the study were evaluated using IBM SPSS Statistics 27 software (Newyork, USA) for statistical analyses. The normal distribution suitability of the parameters was assessed using the Shapiro-Wilk test. Descriptive statistical methods, including mean, median, standard deviation, percentage (25–75% Interquartile Range - IQR), and frequency, were used to evaluate study data. For comparisons of quantitative data, Student's t-test was applied for normally distributed parameters, whereas the Mann-Whitney U test was used for non-normally distributed parameters. For comparisons of qualitative data, the chi-square test (Pearson chi-square test, Fisher's exact chi-square test) was applied. A p-value of <0.05 was considered statistically significant. Our study was planned retrospectively, and all patients meeting the inclusion and exclusion criteria were included in the study.

RESULTS

A total of 26 patients were included in the study. The mean age of the cases was 15.8±3.6 years.

Among our patients, 20 (76.9%) were in the pediatric age group, and 6 (23.1%) were in the adult age group. Of the cases, 93.1% (27) had unilateral acute scrotum findings, while 2 patients (6.9%) presented with bilateral involvement. The seasonal distribution of cases was as follows: spring, 9 cases (31%); winter and summer, 7 cases each (24.1%); and autumn, 6 cases (20.7%).

Hemogram parameters such as Hb (14.9±1.3) and PLT (280.6±60.4) were reported as mean±SD.

WBC (10.2 [8.2–12.4]) and CRP (0.2 [0.2–0.6]) exhibited non-parametric distributions and were presented as median and IQR values. The mean (±SD) values for parametrically distributed data and the median (min–max) values for non-parametrically distributed data are presented in Table 1.

Table 2. Relationship between pediatric (n_p=20) and adult groups (n_a=6) in terms of hematological and biochemical markers

Parameters	Recurrence	p
Age (year)	Pediatric Adult	0.0001*
PLT (10^3/uL)	Pediatric Adult	0.016*
Hb (g/dL)	Pediatric Adult	0.027*
WBC (10^3/uL)	Pediatric Adult	0.394**
CRP (mg/dl)	Pediatric Adult	0.546**

WBC: White blood cell; CRP: C-reaktif protein; Hb: Hemoglobin; PLT: Platelet; *: Independent sample t test; **: Mann-Whitney U Test.

Table 3. The correlation analysis between age and WBC, CRP, Hb and PLT values

Parameters		Correlations	p
WBC	age (n=26)	0.183	0.342
CRP		-0.194	0.313
Hb		0.698**	0.0001
PLT		-0.418*	0.024

WBC: White blood cell; CRP: C-reaktif protein; Hb: Hemoglobin; PLT: Platelet; **: Correlation is significant at the 0.01 level (2-tailed); *: Correlation is significant at the 0.05 level (2-tailed).

Comparing treatment status between pediatric and adult patients, the chi-square test indicated no significant association (p=0.21). When classifying patients into pediatric and adult groups, Student's t-test results for age, Hb and PLT values were as follows: (p=0.0001, 0.016, 0.027), respectively. For WBC and CRP, which exhibited non-parametric distributions, p-values were (p=0.394, 0.546), respectively.

The comparative analysis of hematological and biochemical markers in pediatric and adult populations is presented in Table 2.

The correlation analysis between age and WBC, CRP, Hb and PLT values is presented in Table 3.

Table 4. Sensitivity and specificity values of Hb and PLT in pediatric and adult age groups

	AUC	p	Cut-off	Sensitivity	Specificity	Youden Index
Hb (g/dL)	0.754	0.039	14.8	0.45	0.33	0.55
PLT (10^3/uL)	0.183	0.021	270000	0.35	0.33	-0.317

Hb: Hemoglobin; PLT: Platelet; AUC: Area under curve.

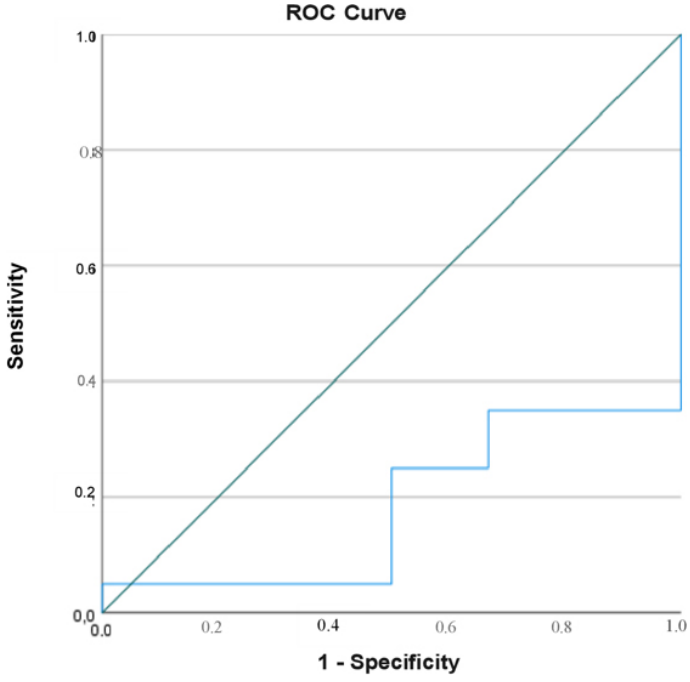

Figure 1. ROC curve graph of platelet values in pediatric and adult age groups.

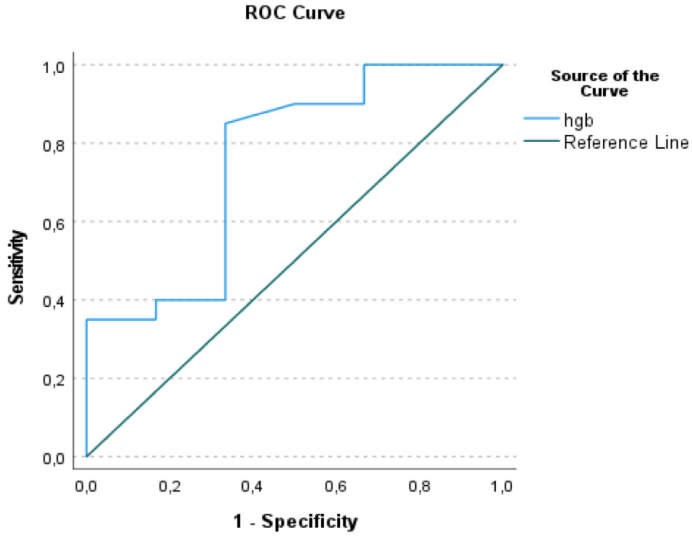

Figure 2. ROC curve graph of Hb values in pediatric and adult age groups.

The diagnostic performance of Hb (AUC 0.754, p=0.039, cut-off 14.8, Youden index 0.55, sensitivity 45%, specificity 33%) and PLT (AUC 0.183, p=0.021, cut-off 270.000, Youden index-0.317, sensitivity 35%, specificity 33%) was evaluated for pediatric and adult patient groups, with ROC curves shown in Table 4 and Figures 1, 2.

DISCUSSION

This study demonstrated significant differences between pediatric and adult patient groups in acute scrotum cases. The higher requirement for surgical intervention in pediatric patients may be associated with the increased incidence of TT in this age group. The literature suggests that TT is the most common acute scrotal condition in pediatric patients and that early diagnosis and surgical intervention are critically important [9, 10]. If TT is not treated in a timely manner, testicular loss becomes inevitable.

The seasonal analysis highlighted a higher incidence of cases in the spring. This finding suggests a potential influence of seasonal factors on the prevalence of infections. Studies indicate increased rates of viral and bacterial infections in the spring and autumn, which may be related to the incidence of AS [11, 12]. Considering the study limitations, evaluating hematological parameters specifically within AS subgroups (e.g., epididymitis, orchitis, TT) rather than collectively may contribute more effectively to the literature. We believe that conducting studies with larger patient groups will yield more objective results in understanding the connection between environmental factors and the immune response.

From a laboratory perspective, the significant age-related differences in Hb and PLT values provide important insights into the pathophysiology of acute scrotum. The finding that Hb levels are higher in pediatric patients compared to adults reflects the changing dynamics of the

hematological system with advancing age [13]. Similarly, the negative correlation of PLT levels with age is consistent with age-related changes in platelet metabolism [14]. Since our study population included patients of all age groups diagnosed with acute scrotum, this may explain the statistical differences observed in blood parameters.

Several studies have reported no significant differences in blood parameters between infectious and non-infectious acute scrotum cases. While infectious blood parameters may be elevated in cases of infectious AS, such as epididymitis, no significant differences in infectious markers have been observed in conditions like TT [15–18].

The non-parametric distribution of inflammatory parameters, such as WBC and CRP, suggests that acute scrotum encompasses multiple conditions of both infectious and non-infectious origin.

In the ROC analysis, the diagnostic performance of Hb (AUC: 0.754) was found to be higher than that of PLT (AUC: 0.183), indicating that Hb may be considered a diagnostic biomarker. However, the low sensitivity and specificity values of Hb and PLT highlight the need for these parameters to be supported by additional tests in clinical practice.

Conclusion

This study demonstrates significant age-related differences in the diagnosis and treatment of AS. The higher requirement for surgical intervention in pediatric patients underscores the importance of early diagnosis and timely intervention. However, further studies with larger sample sizes are necessary to validate these findings and to better understand the etiological differences in AS. Additionally, as a study limitation, we believe that analyzing blood parameters within specific subgroups (e.g., epididymitis, orchitis, TT) rather than under the general category of acute scrotum could contribute more effectively to the literature. Our study revealed that laboratory data may differ according to age groups, suggesting that blood parameters may be helpful in the differential diagnosis of acute scrotum.

Ethics Committee Approval: The Malatya Turgut Ozal University Clinical Research Ethics Committee granted approval for this study (date: 13.03.2025, number: E-30785963-020-291039).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The author declared that artificial intelligence-supported technologies were not used in this study.

Authorship Contributions: BD, TD, SN, EA, MEP; Design – BD, TD, SN, EA, MEP; Supervision – BD, TD, SN, EA, MEP; Fundings – BD, EA; Materials –BD, SN, MEP; Data collection and/or processing – BD, TD, MEP; Analysis and/or interpretation – BD, EA, SN; Literature review – BD, MEP, TD; Writer – BD, TD, SN, EA, MEP; Critical Review; BD, SN, EA.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):430–437
doi: 10.14744/nci.2024.44420

Original Article INTERNAL MEDICINE

Evaluation of malnutrition in patients with febrile neutropenia

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ABSTRACT

OBJECTIVE: Febrile neutropenia is a critical condition in patients with malignancy, requiring oral and/or parenteral antibiotic treatment; and a significant cause of mortality and morbidity. It is well known that nutritional status is excessively impaired in these patients due to underlying disease itself along with the chemotherapeutics used. In this study we investigated nutritional status and general characteristics of patients admitted to our internal medicine clinic with febrile neutropenia.

METHODS: Thirty patients who were followed up in the internal medicine service were included in the study. For the analysis of the data of the patients, height, weight, body mass index (BMI), weight loss in the last three months, albümin and total iron binding capacity values were recorded. Hand grip strength, mid-upper arm and mid-calf circumference measurements were obtained for the assessment of muscle strength; Mini Nutritional Assessment (MNA) and Nutrition Risk Screening 2002 (NRS 2002) scores were calculated at admission and discharge to evaluate nutritional status. Multinational Association of Supportive Care in Cancer (MASCC) score was used to identify risk and manage treatment.

RESULTS: We included thirty patients (mean age 58.27±16.52 years, 53% females). Of 30, six patients had lung cancer, four patients had myelodysplastic syndrome, three patients had stomach cancer, two patients had gastrointestinal system lymphoma, two patients had colon cancer, two patients had breast cancer, two patients had Non-Hodgkin's lymphoma.

CONCLUSION: Majority of the patients, admitted to our internal medicine clinic, with febrile neutropenia were found to be malnourished; regardless of their risk classifications. Nutritional assessment scores of the majority were in the low-risk group. In conclusion, patients hospitalized with febrile neutropenia had poor nutritional status.

Keywords: Febrile neutropenia; malnutrition; MASCC score; nutritional tests.

Cite this article as: *Akbas ES, Ucak Basak S, Sevim E. Evaluation of malnutrition in patients with febrile neutropenia. North Clin Istanb 2025;12(4):430–437.*

Febrile neutropenia (FN) is the development of fever ≥38.3 C° in a patient with neutropenia, without any other influential environmental factors [1].

The National Febrile Neutropenia Association Study Group in Turkiye set up a guideline and indicated fever as the measurement of body temperature either oral or axillary ≥38.3 C° once or 38–38.2 C° for an hour. For the same purpose, neutropenia is defined as neutrophil count less than 500/mm^3, or between 500 and 1000/mm^3 and is expected to drop below 500/mm^3 [2].

Febrile neutropenia is still one of the major complications of chemotherapy and a leading cause of morbidity in cancer patients in spite of the developments in prophylaxis treatment. Various approaches, dose reduction and delays in courses of treatment, have been tested for prevention. Mortality is around 5% in solid tumors and over 11% in hematologic malignancies [3].

The source of infection is blood in 34%, upper and lower respiratory tract in 23% and 13%, respectively, soft tissue including skin and intravascular devices in 18%,

Received: March 04, 2024 *Revised:* March 28, 2024 *Accepted:* April 09, 2024 *Online:* August 11, 2025

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and gastrointestinal tract in 7%; whereas not indicated in 56% of patients with neutropenic fever [4].

Alteration in skin integrity due to injection, venipuncture, or intravascular catheterization causes skin and soft tissue infections. Major causing microorganisms are coagulase-negative staphylococci, Gram-positive cocci mainly *Staphylococcus* aureus and Gram-negative enteric bacilli (e.g., *Escherichia coli* and *Klebsiella* species). Given the humid environment in axillary region, Gram-negative bacilli such as *Pseudomonas aeruginosa* may cause hidradenitis apart from skin flora bacteria. Intravascular catheterization is an important source of infection usually caused by coagulase negative staphylococci, *Propionibacterium* and *Corynebacterium* species, viridans streptococci found in normal skin flora; and other pathogens including *S. Aureus*, Gram-negative bacilli, and *Candida species*. Vancomycin resistant *Leuconostoc*, *Pediococcus* and *Lactobacillus* species are rare causes of infection in FN [5].

Recent epidemiological studies with solid tumors determined an increase incidence of Gram-negative bacteria infections. Gram negative bacteria, most common causes being *E. Coli*, *Pseudomonas* and *Klebsiella* species, are mainly of intra-abdominal origin and seen as polymicrobial infections involving anaerobic bacteria [6, 7].

Fungi may end up with life threatening infections in cancer patients with neutropenia. Frequently, they cause secondary infections. Prolonged and severe neutropenia along with prior antibiotic use are important risk factors for fungal infections; others include long hospital stay, glucocorticoid use, and proximity to construction areas. *Candida* and *Aspergillus* species are the most common fungi detected [4].

Risk assessment for possible complications must be done in all neutropenic patients with fever. For this purpose, "Multinational Association for Supportive Care in Cancer (MASCC)" criteria is the most commonly used assessment tool: maximum score is 26 and ≥21 is categorized as low risk [8].

Malnutrition is a major socioeconomic problem in today's health care environment, with an estimated prevalence of 30–50%. The prevalence may be even higher in long-term care facilities, reportedly as high as 85%. Furthermore, malnutrition has been associated with increased healthcare-related costs, including longer hospital stays and increased rates of major and minor complications [9].

Highlight key points

- According to NRS 2002, 59% of patients were found to be malnourished; based on MNA assessment, 56% had malnutrition, 37% were at risk of malnutrition, and 7% had normal nutritional status.
- There was a significant increase in leukocyte, neutrophil, and lymphocyte counts between admission and discharge ($p<0.05$).
- The source of infection could not be identified in 30% of the patients; among the identified sources, the most common was lower respiratory tract infection (26.7%).
- Following initial treatment, fever subsided within the first 24 hours in 64% of patients; 77% were classified as low-risk febrile neutropenia according to the MASCC score.
- No significant correlation was found between MASCC score and hand grip strength, nutritional assessment scores, or neutrophil-related parameters.

Protein malnutrition (PM) is the most common type of malnutrition and leads to various physiological consequences depending on its duration and intensity. PM primarily affects hematopoietic tissues due to continuous turnover. PM causes changes in the lymphohematopoietic organs (bone marrow (BM), spleen and thymus), anemia, leukopenia and changes in the immune system, increasing susceptibility to infections [10]. Malnutrition increases the risk of febrile neutropenia in children with malignancies by decreasing cytokine response and hormonal changes [11]. The presence of malnutrition increases treatment-related toxicities in cancer patients receiving chemotherapy. it is estimated that deaths in 10–20% of patients are due to adverse events related to malnutrition, not the tumor itself. therefore, it is recommended to assess malnutrition and ensure adequate nutrition before starting treatment [12].

Our aim in this study was to evaluate the nutritional status of patients admitted to our internal medicine clinic with febrile neutropenia.

MATERIALS AND METHODS

In our single center study, we included patients admitted to our internal medicine clinic with febrile neutropenia after receiving cancer treatment. Patients were included regardless of their malignancy types, treatments, receiving chemotherapy and/or radiotherapy, course of treatment and disease activity status. For the purpose of not to interfering with nutritional assessments, patients with

physical disabilities or failure of oral feeding were excluded. Height, weight, body mass index (BMI), weight loss in the last three months, albumin and total iron binding capacity (TIBC) levels were recorded. Anthropometric measurements could not be taken for 3 patients because they died or were sent to intensive care.

A hand grip test was performed to evaluate for sarcopenia. The hand grip test was performed using a hand dynamometer with the dominant hand [13]. A weight <16 kg (kilogram) for women and <27 kg for men was considered as possible sarcopenia [14].

Mini Nutrition Assessment (MNA), Nutritional Risk Screening 2002 (NRS 2002) scores were calculated at the time of admission and at discharge in order to evaluate nutritional status. Patients with an NRS-2002 total score ≥3 were defined as malnourished. According to MNA evaluation, patients were classified as well-nourished with a score above 24 points, at risk of malnutrition between 17–23 points, and severely malnourished with a score below 17 points.

Mid-upper arm measurement was performed at the midpoint between the tip of the shoulder and the tip of the elbow; mid-calf measurement was performed at the widest part of the gastrocnemius muscle.

We recorded general clinical characteristics; primary malignancy site and metastatic disease if present, source of infection, obtained cultures and grown microorganisms, received antibiotics, duration of the treatment, afebrile time point and following laboratory tests; lymphocyte, neutrophile, hemoglobin levels during admission and at discharge. Multinational Association of Supportive Care in Cancer (MASCC) score was used to identify risk and manage treatment.

Ethical Standards

The study was conducted in accordance with the Declaration of Helsinki. The institutional clinical research ethics committee approved this study protocol on May 6, 2016 (approval number: 6905).

Statistical Analysis

For statistical analysis, descriptive analysis was used for continuous variables (mean, median, mode, standard deviation, minimum, maximum). Normal distribution was tested with Shapiro-Wilks's test. Student's T-test was used for dependent and not normally distributed two group comparison and ANOVA was used to comparise more than two groups. Two dependent and normally distributed continuous variables were compared with Wilcoxon Signed Rank Test, and Friedman test was used to compare of more than two variables. McNemar test was used for paired nominal variables. Statistical significance was set at $p<0.05$. MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013) was used for the analyses.

Table 1. Sociodemographic data (n=30)

Gender (%)	
Woman	53.3
Male	46.7
Age (Mean±SD)	58.27±16.52
Marriage status (%)	
Married	80
Single	6.66
Widowed/divorced	13.33
Education status (%)	
Illiterate	13.33
Primary school	46.66
Middle school	23.33
High school	10
University	6.66

SD: Standard deviation.

RESULTS

We included 30 patients (mean age 58.27±16.52 years, 53% females) (Table 1). Mean body temperature measured at admission was 38.5±0.4 and mean absolute neutrophil count was 219.3±222.3. Out of 30 patients, 6 (20%) had lung cancer, 4 (13.4%) had myelodysplastic syndrome and 3 (10%) had gastric cancer. 8 patients had metastases and 22 patients had no metastases.

There was statistical significance in leukocyte, neutrophil, and lymphocyte levels of patients between measurements during admission and at discharge ($p<0.05$ for all) (Table 2). However, there was no significant change in MNA and NRS 2002 scores. Based on NRS 2002, 59% of our patients had malnutrition. When we assessed patients with MNA test; 56% had malnutrition, 37% had malnutrition risk, and %7 had normal nutritional status.

Table 2. Blood tests and nutritional assessment in patients admitted with febrile neutropenia

	Mean	SD	p
Hemoglobin – A (n=30)	8.38	2.07	0.06*
Hemoglobin – D (n=30)	9.17	1.15	
Leukocyte – A (n=30)	1810	2881	<0.01**
Leukocyte – D (n=30)	7060	8019	
Neutrophil – A (n=30)	219.3	222.28	<0.01**
Neutrophil – D (n=30)	3698.7	4622	
Lymphocyte – A (n=30)	942	1673	<0.01**
Lymphocyte – D (n=30)	2447	4489	
NRS 2002 – A (n=27)	2.81	1.47	0.180*
NRS 2002 – D (n=26)	2.88	1.68	
MNA – A (n=27)	16.2	5.1	0.160*
MNA – D (n=26)	16.98	5.32	

SD: Standard deviation; A: Admission values; D: Discharge values; NRS 2002: Nutritional Risk Screening; MNA: Mini nutrition assessment; *: Paired samples t Test; **: Wilcoxon Test.

Table 3. Blood culture analysis in patients admitted with febrile neutropenia

Bacteria (n=24)	%
Corynebacterium mucifaciens (n=1)	4.2
Escherichia coli (n=1)	4.2
Klebsiella pneumoniae (n=1)	4.2
Staphhylococcus epidermidis (n=1)	4.2
Stenotrophomonas maltophilia (n=1)	4.2
No growth (n=19)	79.1

Table 4. Antibiotic regimens in patients admitted with febrile neutropenia

Antibiotic (n=30)	%
Not used (n=1)	3.3
Imipenem, daptomycin (n=1)	3.3
Piperacillin Tazobactam, daptomycin (n=1)	3.3
Imipenem (n=1)	3.3
Imipenem, teicoplanin (n=1)	3.3
Meropenem (n=3)	10
Meropenem, vancomycin (n=1)	3.3
Meropenem, metronidazole (n=1)	3.3
Piperacillin tazobactam (n=18)	60
Piperacillin tazobactam, metronidazole (n=1)	3.3
Unknown* (n=1)	3.3

*: Patient was sent to another hospital.

Table 5. Anthropometric measurements of patients admitted with febrile neutropenia (n=27)

	Mean	SD
Height*	164.8	8.3
Weight**	64.15	9.5
Body Mass Index (kg/m2)	23.88	5.06
Weight Loss in Last 3 Months**	3.89	2.37
Mid-upper Arm*	26.96	4.66
Mid-calf Circumference*)	42.15	8.57
Hand Grip Strength**	17.59	8

SD: Standard deviation; *: Centimeter; **: Kilogram.

The source of infection could not be determined in 9 (30%) patients. The source of infection was lower respiratory tract infection in 8 (26.7%) patients, upper respiratory tract infection in 4 (13.3%) patients and urinary tract infection in 3 (10%) patients. Of 30, blood cultures were obtained in 24 patients; and blood culture analysis are shown in Table 3. Urine cultures were checked in 20 patient, 17 patients had no growth. *Enterobacter Aerogenes*, *Escherichia coli* and *Stenotrophomonas maltophilia* were grown in 1 patient. Table 4 demonstrates the antibiotic regimens used. Five patients (19%) had pyuria in urinalysis, eight patients (29%) had pulmonary infiltrates on chest X-ray, and one patient (4%) was found to have a metastatic lesion.

On follow-up, fever was reduced in the first 24 hours after initial treatment in 18 patients (64%) and during second day in 10 patients (36%). Of 30, 23 patients (77%) had low risk and seven patients (23%) had high risk febrile neutropenia based on MASCC scoring. Mean albumin level was 3.0±0.5 and CRP was 14.8±10.4.

Table 5 demonstrates mean height, weight, body mass index (BMI), weight loss in the last three months; mid-upper arm, mid-calf circumference, and hand grip strength measurements.

Table 6. MASCC score and nutritional assessment correlation in patients admitted with febrile neutropenia

	MASCC score
Neutrophil - A	0.01
Neutrophil - D	0.157
NRS 2002 - A	-0.199
NRS 2002 - D	-0.197
MNA - A	0.182
MNA - D	0.295
Neutrophil/lymphocyte	0.133
Hand grip strength	0.08

A: Admission; D: Discharge; NRS: Nutritional Risk Screening; MNA: Mini nutrition assessment; MASCC: Multinational association of supportive care in cancer.

There was no statistically significant correlation between MASCC score and hand grip strength measurement; NRS 2002 and MNA scores; neutrophil count, neutrophil to lymphocyte ratio both during admission and discharge (Table 6).

DISCUSSION

Majority of the febrile neutropenia patients in our study had malnutrition, but there was no relation between malnutrition and MASSC scores.

Antineoplastic therapy in cancer patients mostly effects rapidly proliferating bone marrow cells; reduces neutrophil count besides suppressing their function, and consequently predisposes to infections [4].

Poor nutrition, other organ system dysfunction due to underlying disease, mechanical obstruction, intervention and catheterization causing damage in anatomical barrier, destroyed epithelial and mucosal integrity following cytotoxic chemotherapy (mucositis), bleeding disorders, impaired immune function because of other treatments (e.g. monoclonal antibodies, fludarabine, and glucocorticoids), radiotherapy, flora changes due to prolonged hospital stay and antibiotic use are other significant risk factors for infection in cancer patients [4].

Neutrophil functioning is the leading defence mechanism of the body against microorganisms. Chemotherapy in cancer patients causes reduction in neutrophil count and impairment in their function. Decrease in neutrophil count is associated with increased risk of infection and severity. Bacteremia is present in 20% of patients with severe neutropenia (<100/mm^3) [15].

Chemotherapy induced severe neutropenia in patients with solid tumors lasts less than seven days. Thus, febrile neutropenia incidence in solid tumors does not exceed 5–50%. Neutropenic fever caused by multiple chemotherapeutic agents is seen in 10–50% of solid tumors, and the ratio increases up to 80% in hematologic malignancies [5, 16]. In our study, 11 patients (37%) had hematologic malignancies and six patients (20%) had solid tumors, with lung cancer being in the lead.

Although increased body temperature in cancer patients might be due to the underlying disease itself, chemotherapeutic and antibiotic agents used, or blood transfusions; infections are the reason for fever in two thirds of patients with neutropenia. More than half of those cases are diagnosed with 'fever of unknown origin' [1]. In our study group, most common infections occurred in pulmonary system with eight patients (27%), and source of infection was not determined in nine patients (30%).

Bacterial infections are the most commonly seen infections in neutropenic patients. Gram-negative bacteria used to be responsible for bacteremia about thirty years ago. However, as a result of the increased use of catheterization started in 1980s, gram-positive bacteria in normal skin flora havebeen seen more frequently since then. In the last few years, multidrug resistant Gram-negative microorganisms have been the increasing cause of infection. Recent studies showing Gram-negative bacteria ratios of about 25% extending up to 76% [5–7]. Our data reported Gram-negative bacteria growth in cultures (urine, blood and wound) of eight patients (80%), consistent with literature.

The most frequently obtained bacteria in cultures are Gram-positive coagulase negative *Staphylococci*, Viridans *Streptococci*, *S. Aureus* and *Ecnterococci* species [4, 5]. Gram-positive bacteria isolated in our study patients were Corynebacterium mucifaciens and *S. Epidermiditis*. *E. Coli*, *Klebsiella* species and *Pseudomonas aeruginosa* are the leading causes of Gram-negative infections. Particularly, extended-spectrum beta lactamase producing pathogens, *Klebsiella* species and *E. Coli*, interfere with treatment. Hospital-acquired microorganisms, *Acinetobacter* species and *Stenotrophomonas maltophilia*, show resistance to multiple drugs including carbapenems. Given the increase in the incidence of multi-drug resistant microorganisms in many hospitals; *Acinetobacter*, *Pseudomonas*, carbapenem resistant *Enterobacteriaceae*, methicillin-resistant *Staphylo-*

coccus aureus (MRSA), and vancomycin-resistant *Enterococci* (VRE) have been observed more frequently. Infections caused by these pathogens are more difficult to treat and have increased morbidity [1, 5, 7]. In our study, of eight Gram-negative bacteria grown in cultures, two were *E. Coli*, two were *Klebsiella pneumonia*, two were *Stenotrophomonas maltofilia*, and one was *P. Aeruginosa*.

The source of infection is not determined in about 56% of patients with febrile neutropenia, and the rest consists of blood (34%), upper and lower respiratory tract (23% and 13%, respectively), soft tissue including skin and intravascular devices (18%), and the gastrointestinal tract (7%) [17]. In our analysis of patients admitted with febrile neutropenia, source of infection was not identified in nine patients (30%); five patients (17%) had bacterial growth in blood cultures; and the respiratory system was the primary source of infection in eight patients (27%).

The National Febrile Neutropenia Association Study Group in Turkiye prepared a guideline regarding hospital admission and treatment recommendations, based on the criteria developed by "The Multinational Association for Supportive Care in Cancer" [18]. Point scoring systems are particularly helpful to evaluate low risk patients, whether to hospitalize or undergo an outpatient treatment. When MASCC score >21, mortality is between 1–3% and serious complications risk is below 5%; therefore, it is appropriate to treat at least half of these patients with oral antibiotics and continue with outpatient treatment after 24–48 hours of monitoring [16, 19]. In our study of 30 patients, 23 (77%) were categorized in low-risk group based on MASCC score >21; whereas seven patients were in high-risk group.

Neutropenic fever is an emergent medical condition. Thus, extended-spectrum bactericidal antibiotic treatment, arranged according to the liver and kidney functions, must be initiated immediately in order to prevent progression to sepsis and death. Initial empirical treatment should include anti-pseudomonal activity [20]. Accordingly; 20 patients received piperacillin-tazobactam, five patients received meropenem, and three patients received imipenem, initially, in our 30 patients study group.

Adding vancomycin to empirical therapy has been discussed as a result of increased Gram-positive bacterial infections in neutropenic patients. However, in a meta-analysis of randomized controlled studies, adding vancomycin to empirical therapy did not decrease mortality, on the contrary increased adverse event incidence, renal dysfunction being in the first place [21]. Of our 30 patients, vancomycin was added in only one patient unresponsive to initial meropenem treatment.

Teicoplanin, another glycopeptide antibiotic, can also be used for the same purpose as vancomycin. Nevertheless, linezolid which is effective against MRSA and VRE, and daptomycin, whichis effective against multi-drug resistant Gram-positive bacteria, have not been proven their efficient in empirical treatment yet. However, for the purpose of targeted treatment, linezolid therapy should be preferred in MRSA pneumonia by reason of its higher pulmonary tissue penetration compared to other glycopeptides. Daptomycin is commonly used because it shows rapid bactericidal activity in addition to good transition to foreign-bodies (catheter, vascular port) and other tissues [1, 5]. In our study group of 30 patients, daptomycin therapy was added considering MRSA infection in two patients (one with initial piperacillin-tazobactam and one with initial imipenem treatment).

Malnutrition in the elderly population is a major problem with many aspects resulting in biological, psychological, social, and economical issues. It presents with decreased total body and muscle mass (sarcopenia); and consequently, causes frailty, falls and hip fractures, prolonged healing time, increased susceptibility to infection, decubitus ulcers and poor wound healing in the elderly. In such circumstances; hospital stays happen to recur and last longer, drug use increases, patient care gets more difficult and expensive along with decreased quality of life. Besides, malnutrition has been proven as an independent risk factor for mortality [22].

Protein malnutrition is a condition resulting from a lack of protein intake or absorption, leading to different pathophysiological changes, such as physical and cognitive impairment. Protein malnutrition can impair all tissues, especially those with high cellular turnover, such as hematopoietic tissue. Protein malnutrition causes changes in hematopoietic organs and leads to anemia, leukopenia and impaired immune response. In addition, protein malnutrition induces arrest phase of hematopoietic progenitors and may cause bone marrow hypoplasia [23]. Malnutrition is a common problem in cancer patients and occurs in 80% of patients with advanced cancer. Although the mechanisms underlying nutritional alterations in cancer are not fully understood, the host's proinflammatory reaction and production of catabolic factors may result in weight loss and ultimately malnutrition. Therefore, individuals with high tumor burden may be more vulnerable to malnutrition and at higher risk of chemotherapy-related toxicity [24].

Weight change is alarming for malnutrition and a simple tool to assess the effectivity of treatment. Malnutrition should be considered in case of unintentional weight loss of 5% of the total body weight in the last month or 10% in the last six months. Moreover, adequate food consumption should be evaluated cautiously in terms of both calorie and protein intake [25]. In our study patients, mean weight loss in the last three months was 3.89±2.37.

Sarcopenia is the progressive loss of muscle mass, strength, and function. It is considered as a part of the aging process; thus, it is a geriatric syndrome. However, it can also develop in consequence of non-use/immobilization, malnutrition and cachexia [26]. Physical inactivity, decreased mobility, and poor physical strength usually accompanies sarcopenia. In the pathogenesis; loss of muscle mass and fibers, increased inflammation, change in hormone levels, poor nutritional status, impaired renin-angiotensin system, and many other factors play an important role [27]. The assessment of muscle strength in our patients with anthropometric measurements was in the normal range: mean mid-upper arm circumference was 26.96±4.6 cm and mean mid-calf circumference was 42.15±8.57 cm. On the other hand, hand grip strength was reduced with the mean measurement of 17.6±8 kg. Screening methods in malnutrition are easy and quick to apply, convenient for the patient, and does not require a qualified practitioner. They usually refer to presence of malnutrition alone; and more information in regards to cause and severity of malnutrition is necessary for patients under risk [28, 29]. In our study, mean MNA score was 16.2±5 and 17.0±5 (during admission and at discharge, respectively); indicating malnutrition. Based on NRS 2002 test, mean score was 2.8±1.5 and 2.9±1.7 (during admission and at discharge, respectively); not consistent with malnutrition. Taking into account the mean age of our patients (58.3) and the accuracy of MNA testing in elderly population; one can consider our patients had malnutrition.

Conclusion

Febrile neutropenia is a critical condition in patients with malignancy, requiring oral and/or parenteral antibiotic treatment and hospital admission; and a significant cause of mortality and morbidity. Prolonged hospital stays of the patients result in drug resistance in microorganisms due to inappropriate and unnecessary antibiotic use, and increased cost. We consider the malnutrition in these patients are due to underlying malignancy and lack of awareness of nutritional assessment. In our study, there was no correlation between MASCC score and nutritional assessment tools (NRS 2002, MNA, hand grip strength, mid-upper arm and mid-calf circumference). However, we believe that true assessment of nutritional status in patients with febrile neutropenia and compensation of any needs will be helpful to improve the treatment and reduce the length of stay in hospital.

Ethics Committee Approval: The Umraniye Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 06.05.2016, number: 6905).

Informed Consent: Written informed consent was obtained from the patient's family for this study and images.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: Artificial intelligence-supported technologies, chatbots, and image generators were not used at any stage of our work.

Authorship Contributions: SUB, ESA; Design – SUB, ESA; Supervision – SUB; Data collection and/or processing – ES, ESA; Analysis and/or interpretation – ES, ESA; Literature review – ES, ESA; Writing – ES, ESA; Critical Review– SUB, ESA.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):438–444
doi: 10.14744/nci.2024.19794

Original Article PATHOLOGY

TFE3 immunohistochemistry in renal cell carcinomas: Does the clone really matter?

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ABSTRACT

OBJECTIVE: TFE3 rearranged carcinomas constitute 5% of malignant tumours of the kidney in adults. TFE3 immunohistochemistry plays a crucial role in the diagnosis. TFE3 positivity in the appropriate histological context supports the diagnosis of Xp11 translocation renal cell carcinomas. However, there isn't any standardized approach to performing and interpreting immunohistochemical staining.

METHODS: A total of 51 renal cell carcinomas are included in the study. In this study, we compared the expression profiles of two different anti-TFE3 antibody clones (MRQ37, Cell Marque, and IHC627, GeneAbTM) on renal cell carcinoma samples that have conflicting morphologies and assessed the overall performance of these clones to identify TFE3 rearranged carcinomas.

RESULTS: There was a statistically significant difference in terms of immunohistochemical staining with TFE3-MRQ37 clone between TFE3 rearranged renal cell carcinomas and other subtypes, while no significant difference was found in staining with TFE3-IHC672. 47% of cases were stained with the TFE3-IHC672 clone and 9.8 % of cases were stained with the TFE3-MRQ37 clone at different staining intensities and proportions.

CONCLUSION: The TFE3-MRQ37 clone is easier to interpret because of the absence of background staining and is more reliable in identifying TFE3 rearranged renal cell carcinomas. However, because of various sensitivity and specificity rates, and immunoreactivity in many subtypes of renal cell carcinomas, there is a need for a standardised approach for TFE3 immunohistochemistry for diagnostic use in TFE3-tRCCs.

Keywords: Renal cell carcinoma; TFE3 rearrangement; immunohistochemistry.

Cite this article as: *Kokenek Unal TD, Kiran MM, Sungu N, Yazgan A, Gumuskaya B. TFE3 immunohistochemistry in renal cell carcinomas: Does the clone really matter? North Clin Istanb 2025;12(4):438–444.*

Renal cell carcinomas (RCC) constitute 80–85% of malignant tumours of the kidney. There are many subtypes defined so far, and new entities are being defined as pathological and molecular analyses increase [1]. MiT (microphthalmia-associated transcriptional factor) family translocation carcinomas are rare and account for 1-4% of renal tumors in adults and approximately half of the RCCs in children [2]. These tumours are characterized by fusions involving TFE3 or TFEB genes and were included as two subcategories in the 2016 WHO classification. However, with the 5th edition of the WHO Classification of Urinary and Male Genital Tumours (2022), these two entities were classified separately as TFE3-rearranged RCC (TFE3-

Received: April 03, 2024 *Revised:* July 06, 2024 *Accepted:* July 21, 2024 *Online:* August 14, 2025

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tRCC) and TFEB-rearranged RCC [3]. Although TFE3-tRCC has unique morphological features, it poses difficulties in differential diagnosis. These tumors usually have papillary morphology with eosinophilic cytoplasm and abundant psammoma bodies [4]. However, these tumours may have a morphology mimicking clear cell renal cell carcinoma, papillary renal cell carcinoma, multicystic renal cell carcinoma, oncocytoma, and even epithelioid angiomyolipoma [5]. TFE3 is a transcription factor that plays a role in cellular differentiation and is encoded by the TFE3 gene. The oncogenic activation of this gene is because of chromosomal translocation. This genetic alteration mostly involves Xp11 translocation [6]. FISH is considered to be more sensitive and specific than IHC in detecting TFE3 rearrangement, there is no clarity about when and in which cases FISH should be used and how it should be interpreted [7]. On the other hand, immunohistochemistry is more preferred because it is an easy, fast, and inexpensive method [8]. However, there is no consensus on how to perform immunohistochemistry or how to interpret it. A recent survey revealed that 16-20% of pathologists do not even know which TFE3 clone they are using [7]. According to the last edition of WHO classification, essential diagnostic criteria include presenting strong nuclear staining with TFE3 IHC in a clean background or identification of TFE3 arrangement by break-apart FISH or TFE3 gene fusion by RNA sequencing [3]. Molecular techniques are expensive and need expertise to interpret and not available for most of the pathologists [9]. In addition to that, considering the rarity of the tumour, TFE3 immunohistochemical staining is the easiest and cheapest method that can be applied in routine laboratory conditions. Because there is a lack of a standardised approach, the use of TFE3 immunohistochemistry can be confusing due to various fixation and interpretation problems. There are different brands and clones of TFE3 antibody on the market and there is no study in the literature showing the superiority of these clones over each other (if there is any). Its importance may be underestimated. In this study, we compared the expression profiles of different clones of TFE3 IHC (namely MRQ37, Cell Marque and IHC6272, GeneAbTM) on RCCs and assessed factors such as sensitivity, specificity, staining pattern, and overall performance in detecting TFE3 protein expression in tumour samples. In addition to that, we determined to reveal their differences and contributions to the definitive diagnosis of TFE3-tRCCs in challenging cases.

Highlight key points

- The 48% of renal cell carcinoma cases were weakly stained with TFE3-IHC672; and %10 of casesshowed strong nuclear positivity with TFE3-MRQ clone.
- Immunohistochemical staining with TFE3-MRQ clone was significantly different in TFE3-tRCC compared to other RCCs.
- Nosignificant difference in staining with the TFE3-IHC672 clone between TFE3-tRCCs and the others.
- Staining performed with different clones can yield highly variable results, highlighting the need for a standardized staining and evaluation algorithm in this area.

MATERIALS AND METHODS

The nephrectomy materials diagnosed between 2019 and 2023 in the Pathology Department of Ankara Bilkent City Hospital were retrospectively scanned. After ethical board approval (Ankara Bilkent City Hospital, decision ID: TABED 1-24-24 on 14.02.2024) a total of 51 patients with renal tumours that posed difficulties in differential diagnosis with their morphological features during diagnosis were included in the study. This study was conducted in accordance with 'Declaration of Helsinki'.

Demographic data on the patients was noted. Histopathological parameters such as tumour type, tumour size, tumour nuclear grade, presence of necrosis, renal sinus adipose tissue invasion, etc. were re-evaluated. For TFE3 immunohistochemical staining, 5 µ thick sections were taken from the appropriate tumour including paraffin blocks. After deparaffinization, immunohistochemistry was performed using DAKO's Autostainer Plus using TFE3 (monoclonal mouse antihuman antibody, MRQ37, Cell Marque; dilution 1/150, USA) and TFE3 (monoclonal mouse antihuman antibody, IHC672, GeneAb, dilution: 1:150, USA) according to the manufacturer's protocol. Nuclear staining for both clones as shown in Figure 1 was considered positive regardless of the intensity or the percentage of staining.

Statistical Analysis

The SPSS (Statistical Package for the Social Sciences) Windows 22.0 (IBM Inc., Chicago, IL, USA) package program was used for statistical analysis. The distribution of the data was analysed by histogram, qq plot, and Shapiro-Wilk test. Outliers were excluded from the study. An independent t-test was used for parametric data and Mann-Whitney U test was used for nonparametric data in comparisons between two groups. For comparisons of

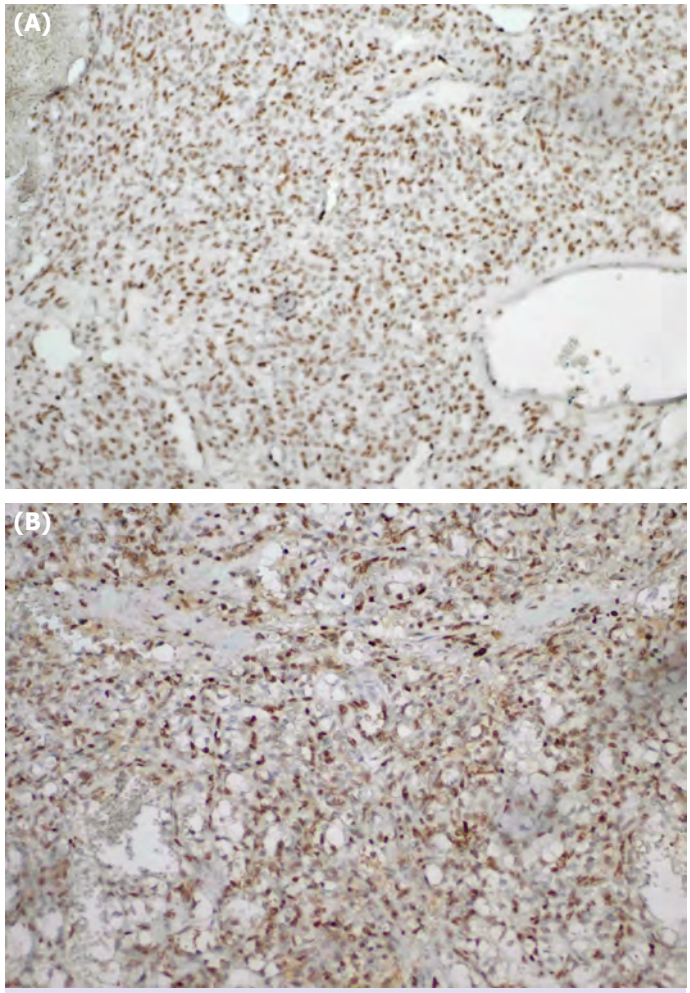

Figure 1. Strong positivity with TFE3-MRQ37 **(A)** and TFE3-IHC672 **(B)**. (x20).

three or more groups, ANOVA was used for parametric data and Kruskal-Wallis test for nonparametric data. The chi-square test was used to test for categorical variables. Sensitivity and specificity were calculated. Spearman test was used to calculate correlations between parameters. The number of units (n), median and min-max values, mean and standard deviation values were given as summary statistics. Sensitivity and specificity were calculated and the ROC curve was used to determine cutoff values. $P<0.05$ was considered statistically significant.

RESULTS

The study included 15 female (30%) and 36 male (70%) patients. The median age was 56 years (ranging from 25 to 82). 31 cases (%61) were finally diagnosed with clear cell RCC (ccRCC); 11 cases with RCC, NOS; 7 cases with chromophobe RCC (chRCC); 5 cases with papillary RCC

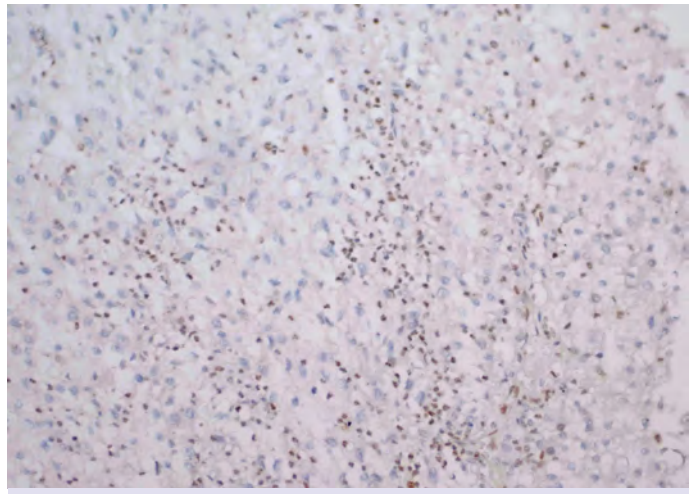

Figure 2. Tumor cells are negative but lymphocytes and stromal cells are positive with TFE3-IHC672. (x20).

(pRCC), 3 cases with TFE3-tRCC; and 2 cases with oncocytic tumour. The median tumour size was 65 mm (range: 20 to 180 mm). Radical and partial nephrectomy were performed in 39 cases (77%) and 12 cases (23%), respectively. Patient and tumour characteristics are described in Table 1. In the TNM staging, 20 (39%) cases were categorised as pT1a-b, 7 cases (13%) as pT2a-b, and 24 cases (47%) as pT3a, but none were categorised as pT4. Regional lymph nodes were neither dissected nor metastasis was present in our cases, but distant metastases were present in 12% of cases (3 ccRCC; 1 pRCC; 1 chRCC; and 1 TFE3-tRCC) at the time of diagnosis. Four cases with distant metastasis showed weak nuclear positivity with the TFE3-IHC672 clone. The median follow-up period was 33 months (min. 21 to max. 49 months. All patients were alive at the end of the follow-up period.

A total of 24 cases including 13/31 ccRCCs, 3/7 chRCCs, 2/5 pRCCs, 2/2 oncocytic tumours, and 3/3 TFE3-tRCCs showed weak nuclear staining with TFE3-IHC672. On the other hand, nuclear staining was observed with TFE3-MRQ37 in 2/31 ccRCCs, 1/5 pRCC and 2/3 TFE3-tRCC (Table 2). All TFE3-tRCCs showed strong nuclear positivity with the TFE3-IHC672 clone, while two of them showed strong nuclear staining with TFE3-MRQ37 clone. This case had been diagnosed based on the histomorphological findings and positive staining of the TFE3-IHC627 clone in addition to ancillary immunohistochemistry. Stromal cells and lymphocytes were also positively stained, regardless of tumour cells in 45/51 cases with the TFE3-IHC672 clone. Positively stained stromal cells and lymphocytes can be seen in Figure 2. In only 2 ccRCC cases, stromal cells and

Table 1. Characteristics of 51 renal cell carcinoma cases

	Mean (Min–Max) or percentage		Mean (Min–Max) or percentage		Mean (Min–Max) or percentage
Age	56 (25–82)	Nuclear grade		Renal capsule invasion	
Gender		NA	8	Present	16
Female	29	Grade 1	4	Absent	84
Male	71	Grade 2	33	Lymphovascular invasion	
Site		Grade 3	33	Present	86
Right	59	Grade 4	12	Absent	14
Left	41	pT		Renal vein invasion	
Localisation		T1a	25	Present	8
Lower pole	33	T1b	14	Absent	92
Middle pole	32	T2a	8	Perinephric tissue invasion	
Upper pole	35	T2b	6	Present	22
Operation		T3a	47	Absent	78
Parsiyel nephrectomy	24	Necrosis		Metastasis	
Radical nephrectomy	76	Present	53	Present	12
Histological subtype		Absent	47	Absent	88
RCC, NOS	6	Differentiation			
ccRCC	60	Present	4		
chRCC	14	Absent	96		
TFE3-tRCC	6	Renal sinus invasion			
pRCC	10	Present	39		
Oncocytic tumour	4	Absent	61		

Min: Minimum; Max: Maximum; RCC, NOS: Renal cell carcinoma, not otherwise specified; ccRCC: Clear cell renal cell carcinoma; chRCC: Chromophobe renal cell carcinoma; TFE3-tRCC: TFE3 rearranged renal cell carcinoma.

Table 2. The histological subtypes of renal cell carcinomas and immunohistochemical expressions of tumor cells with different TFE3 antibody clones

	RCC, NOS	ccRCC	chRCC	pRCC	TFE3-tRCC	Oncocytic tumour
TFE3-MRQ37						
Positive	0/3	2/31	0/7	1/5	2/3	0/2
Negative	3/3	29/31	7/7	4/5	1/3	2/2
TFE3-IHC627						
Positive	1/3	13/31	3/7	2/5	3/3	2/2
Negative	2/3	18/31	4/7	3/5	3/3	0/2

RCC, NOS: Renal cell carcinoma, not otherwise specified; ccRCC: Clear cell renal cell carcinoma; chRCC: Chromophobe renal cell carcinoma; TFE3-tRCC: TFE3 rearranged renal cell carcinoma.

lymphocytes were stained with the TFE3-MRQ37 clone as well as tumour cells. A background staining which is seen in Figure 3 was observed in some slides. There was a statistically significant difference in staining with the TFE3-MRQ37 clone between TFE3-tRCCs and the others (p=0.001). However, we couldn't find any significant difference in staining with the TFE3-IHC672 clone between TFE3-tRCCs and the others. Focal staining and staining at different intensities were two important and challenging situations for all RCCs in our group.

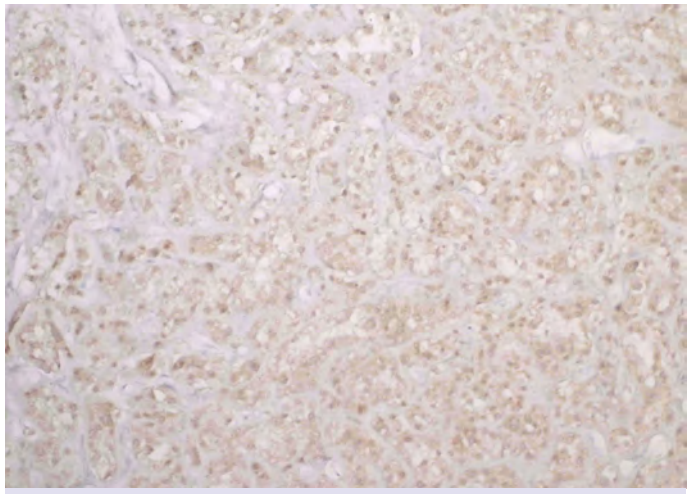

Figure 3. Background staining with TFE3-IHC372. (x20).

In this study, the specificity of the TFE3-MRQ37 clone was 94%, and the sensitivity was 67% for detecting TFE-tRCC cases. On the other hand, for TFE3-IHC672, the specificity was 56% and the sensitivity was 100%. No significant correlation was found between immunohistochemical staining with either TFE3-IHC672 or TFE3-MRQ37 clones and the other histopathological parameters examined.

DISCUSSION

In our study, we found that only staining with the TFE3-MRQ37 clone was statistically significant in TFE3-tRCCs compared to the other histological subtypes of RCCs.

TFE3-tRCCs are a group of tumour that can be easily underestimated because they can morphologically resemble many other RCC subtypes. TFE3 immunohistochemistry IHC can be used to show translocation because it detects the abnormal expression of TFE3 protein, indicating the presence of the gene rearrangement. In our study, we investigated the performance of commercially available and most commonly used antı-TFE3 clones (TFE3-MRQ37 and TFE3-IHC672).

Our results demonstrated that the TFE3-MRQ37 clone showed statistically different staining in TFE3-tRCCs compared to other RCCs. With the TFE3-IHC627 clone, there is no significant difference between TFE3-tRCCs and the other RCCs. In our study, the TFE3-IHC672 clone showed positive staining in all TFE3-tRCCs and 44% (21/48) of nonTFE3-tRCCs and therefore its specificity was low similar to the literature. An important problem with TFE3 immunohistochemistry emerges as having low specificity rates despite high sensitivity rates in the literature [10]. On the other hand, the TFE3-MRQ37 clone stained positively in only 6% (3/48) of cases of nonTFE3-tRCCs and had a high specificity rate. In the literature, different sensitivity and specificity rates were reported for TFE3 immunohistochemistry IHC [7]. Our results also reveal the different rates between antibody clones and all these findings decrease the reliability of immunohistochemistry.

Moreover, background staining and positivity in lymphocytes and stromal cells were observed in 88% of the cases when staining with the TFE3-IHC672 clone. Similarly, background staining was mentioned as an important problem in the evaluation of immunohistochemistry [7]. Our study showed that TFE3-MRQ37 is superior to TFE3-IHC627 clone in terms of ease of interpretation because of the clarity of staining and consistency of results.

In our study, we observed nuclear positivity with both antibody clones in all subtypes of RCCs at varying proportions and intensities. Similarly, it is reported that the positive staining pattern varied from weakly focal to diffusely strong staining in 111 of the 114 RCCs [7]. Moreover, Sharain et al. [10] found different staining patterns and proportions among other TFE3 rearranged tumours even with the same clone between two different laboratories. These data show us that immunohistochemical results are changeable depending on many different factors such as antibody clone and dilution, laboratory conditions, etc. Moreover, recent studies indicate that the presence of TFE3 protein overexpression in RCCs has prognostic implications regardless of the presence of TFE3 rearrangement. Tumours showing TFE3 expression have a poor prognosis [8, 11, 12]. In contrast to these studies, we couldn't find any statistically significant correlation between immunohistochemical expressions of both clones and histopathological parameters.

This study has potential limitations. As proposed in the WHO Blue Book, diagnosis of TFE3-tRCC is only possible by the demonstration of TFE3 arrangement by immunohistochemistry, FISH, or molecular testing. Firstly, we used only immunohistochemistry to diagnose cases and couldn't validate our positive cases with break-apart FISH or RNA sequencing. These tests are not available in many laboratories including ours. Although the FISH break apart probe has been validated in many studies, immunohistochemically positive but FISH-neg-

ative cases and vice versa were also reported in the literature [13]. Because available FISH probes do not cover all translocations regarding TFE3 gene, the false negative results with FISH may be explained by intrachromosomal translocations with rare partners other than XP11. FISH analysis is quite expensive and needs to Secondly, we had a small number of TFE3-tRCC cases in the study. However, TFE3-tRCC is a rare tumour and every study is important to expand our understanding of the histological and immunohistochemical properties and the behaviour of tumour. Lastly, the follow-up period was short in our study, so we didn't obtain reliable results regarding prognosis and survival.

Conclusion

In conclusion, TFE3 immunohistochemistry plays a crucial role in the diagnosis, subtyping, and prognostication of renal cell carcinoma, particularly in identifying cases with TFE3 gene rearrangement and TFE3 protein overexpression. It enhances the accuracy of diagnosis and provides valuable information for patient management. By comparing different aspects of different clones of TFE3 immunohistochemistry, we demonstrated insights into their relative performance and suitability for detecting TFE3 protein expression in RCC samples. In our study, the TFE3-MRQ137 clone gave more accurate results for the diagnosis of TFE3-tRCC and therefore may be preferred for detecting TFE3 rearrangement in routine practice. Different subtypes of RCC can be positively stained by TFE3 regardless of TFE3 rearrangement. In addition to that, various sensitivity and specificity rates of clones and different staining results depending on antibody and/or laboratory conditions are potential problems in application and interpretation of immunohistochemical staining for TFE3-tRCCs. Our results demonstrated that different TFE3 antibody clones have different staining properties. For these reasons, we hope that our results can increase the awareness of this indisputable need for developing a standardised approach for TFE3 immunohistochemistry IHC such as clones, staining procedure, interpretation of staining, and its clinical implications etc. A standardised evaluation helps guide the selection of the most appropriate clone for diagnostic or research purposes, prevents false positive diagnoses, and eliminates possible financial losses. Additionally, validation studies should be performed to ensure that the selected clone performs optimally in the intended application.

Ethics Committee Approval: The Ankara Bilkent City Hospital Ethics Committee granted approval for this study (date: 14.02.2024, number: TABED-1-24-24).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: We didn't use artificial intelligence(AI)- assisted technologies to produce the submitted paper.

Authorship Contributions: Concept – BG, TDKU; Design – TDKU, NS; Supervision – BG, AY; Fundings – TDKU; Materials – MMK, TDKU; Data collection and/or processing – MMK, TDKU; Analysis and/or interpretation – BG, TDKU, NS; Literature review – AY, MMK; Writing – TDKU, NS; Critical review – BG, AY, TDKU.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):445–452
doi: 10.14744/nci.2024.14306

Original Article GYNECOLOGY & OBSTETRICS

Mini-laparoscopy versus conventional laparoscopy for the management of endometrial cancer

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ABSTRACT

OBJECTIVE: We aimed to evaluate the feasibility of a mini-laparoscopic surgical approach versus standard laparoscopy.

METHODS: 75 patients with endometrial cancer treated by mini-laparoscopic (n=25) or conventional laparoscopic surgery (n=50) at a tertiary-care university-based teaching hospital and academic affiliated private hospital were included.

RESULTS: There was no significant difference between the mini-laparoscopy and the conventional laparoscopy group regarding surgical procedures. The mean operation time and the median estimated blood loss were similar (p=0.671 and p=0.158, respectively). No difference was found in terms of the number of lymph nodes removed. No intraoperative complications were observed in both groups. Return to daily routine and the rate of additional analgesia requirement were similar in the groups. The mean duration of hospitalization was 3.6±1.2 days in the mini-laparoscopy group and 4.9±3.6 days in the conventional laparoscopy group (p=0.025).

CONCLUSION: We demonstrated that mini-laparoscopic staging could be a competent technique performed regardless of harm by talented surgeons using state-of-the-art instruments. Mini-laparoscopic surgery appears to be a further possibility to minimize surgical trauma by reducing the size of the ports without decreasing the extent and effectiveness of the procedures.

Keywords: Endometrial cancer; laparoscopy; mini-laparoscopy; staging.

Cite this article as: *Giray B, Vatansever D, Misirlioglu S, Arslan O, Manici M, Arvas M, Taskiran C. Mini-laparoscopy versus conventional laparoscopy for the management of endometrial cancer. North Clin Istanb 2025;12(4):445–452.*

Endometrial cancer (EC) is the most common gynecologic malignancy globally, with an incidence of 12 cases per 100,000 susceptible people [1]. Approximately 80% of endometrial carcinoma have been found to harbor a couple of risk factors, including high body mass index (BMI), diabetes, and metabolic syndrome. Patients with early-stage EC have been shown to have a better five-year survival rate, around 90%, compared to those with advanced stages. Standard surgery (total hysterectomy and bilateral salpingo-oophorectomy), whether lymphadenectomy or not, due to risk factors leading to decreased survival and treatment resistance, has also been demonstrated in several studies.

Received: December 29, 2023 *Revised:* July 06, 2024 *Accepted:* July 23, 2024 *Online:* August 27, 2025

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As per current literature, the safety and effectiveness of the laparoscopic approach for malignant procedures have been proven in different settings, particularly in gynecological cancers [2–6]. It is associated with rapid recovery, less pain, and improved quality of life judged against open surgery. Standard laparoscopic surgery is performed using 5-mm and 10-mm diameter instruments along with 10-mm optics. On the contrary, mini-laparoscopic surgery is a procedure that leverages below 5-mm diameter instruments (≤3.5 mm) concomitant with either 5-mm or 3.5-mm optics. Recently, the mini-laparoscopic approach has gained tremendous popularity in the era of gynecological surgery [7, 8]. Several studies have reported astonishing results favoring mini-laparoscopy for benign and malignant conditions without detrimental surgical quality [9–11]. Today, myomectomy, radical hysterectomy, and lymphadenectomy can be carried out using mini-laparoscopy with minimal surgical trauma and better cosmetic outcome [11–13]. Furthermore, mini-laparoscopy is associated with low pain scores, diminished wound complication rates, de-escalated port-site hernia cases, and improved beautifying consequences compared to standard laparoscopy [10]. Notably, 5-mm laparoscopes are not routinely preferred due to their lack of resolution and image quality.

Using propensity-matched comparison, we sought to evaluate the feasibility of a mini-laparoscopic surgical approach (5-mm scope and 3-mm/5-mm instruments) versus standard laparoscopy (10-mm scope and 5-mm instruments).

Highlight key points

- Endometrial cancer (EC) is the most common gynecologic malignancy globally.
- Mini-laparoscopic surgery is a procedure for leverages below 5-mm diameter instruments (≤3.5 mm) concomitant.
- Today, myomectomy, hysterectomy, and lymphadenectomy can be carried out using mini-laparoscopy with minimal surgical trauma.
- Mini-laparoscopy is associated with low pain, diminished wound complication, de-escalated port-site hernia, and improved cosmetic consequences compared to standard laparoscopy.

MATERIALS AND METHODS

Study Population

Patients with EC treated at a university hospital by mini-laparoscopic (n=25, Group A) or conventional laparoscopic (n=50, Group B) surgery were enrolled in this study. All operations were performed by gynecologic oncology surgeons with equal expertise in minimally invasive surgical approaches in the field. Informed consent was obtained before surgery after a discussion of the surgical risks in obedience to the declaration of Helsinki. Gynecologic examination, pelvic ultrasonography, cervical cytology, pre-operative endometrial sampling, adjacent and distant organ metastasis screening (abdominal magnetic resonance imaging, computerized tomography of thorax), and blood sampling were performed on all participants. Groups were compared in terms of age, menopausal status, gravida, parity, BMI, previous abdominal surgery, American Society of Anesthesiology (ASA) score, surgical procedure, operating time, estimated blood loss, complications, analgesia requirement, hospitalization, return to daily routine, and final pathology report. Low molecular weight heparin and compression stocking were applied to prevent thromboembolic complications. The study was approved by the Koc University Ethical Committee (approval date: 24.04.2020 approval number: 2020.159.IRB2.049).

Data Collection

Data was extracted from the institution-based electronic medical records, including preoperative examination notes, operative reports, discharge summaries, pathology documents, and outpatient follow-up charts. In addition, clinical research associate team members jotted down a customized gynecologic oncology worksheet. Baseline characteristics, including age, BMI (kg/m^2), parity, previous history of abdominal surgery, and menopausal status, were recorded. Operative time and intra-operative complications were re-reviewed by observing full-length video of all operations. Total operative time was the interval between the start of abdominal insufflation and skin closure.

Surgical Technique

All procedures were performed under general anesthesia in the dorsal lithotomy position. First, the abdominal cavity was insufflated with carbon dioxide, and pneumoperitoneum (12 mm/Hg) was achieved, then the visualization was obtained with a 30o high-definition scope. Surgical technique was performed using an optical trans-umbilical 5-mm or 10-mm trocar and 5-mm ancillary trocars in the conventional laparoscopy group. An optical trans-umbilical 5-mm and 3.5-mm ancillary trocar on the left side and 3.5-mm and 5-mm ancillary trocars (just one 5 mm

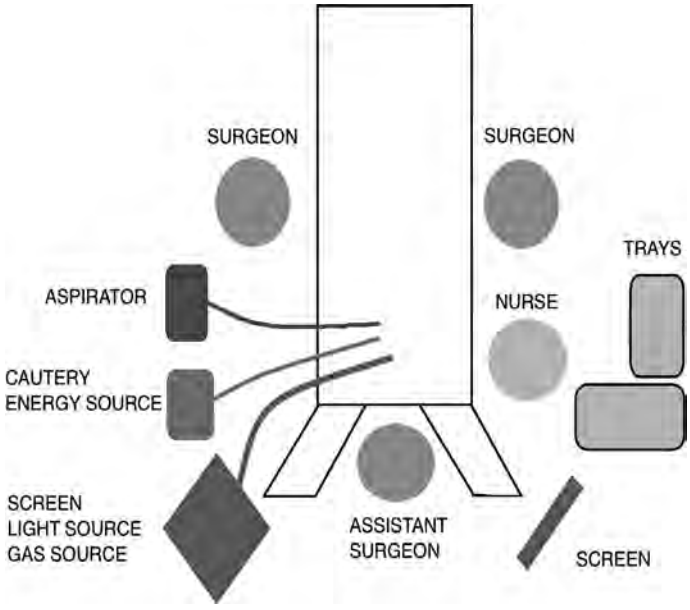

FIGURE 1. Schematic representation of the operating room.

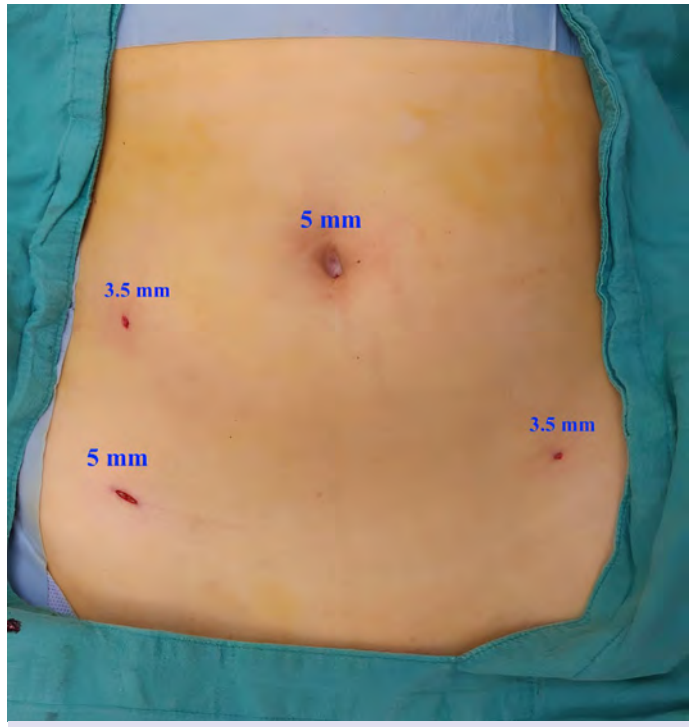

FIGURE 2. Postoperative incisional scars.

to use an energy device) on the right side were used in the mini-laparoscopy group (Karl Storz, GmbH & Co. KG., Tuttlingen, Germany) [8]. A 5 mm trocar to use a cutting and coagulation device was inserted at the right lower abdominal wall near the anterior superior iliac spine in the mini-laparoscopy group. Figure 1 shows the schematic representation of the operating room [14]. For all procedures, a uterine manipulator was used to make uterine manipulation easier. All of the patients underwent a hysterectomy and bilateral salpingo-oophorectomy. Lymph node evaluation was performed based on the risk factors. In our institution, the sentinel lymph node (SLN) mapping technique with indocyanine green (ICG) has been widely used since 2014. The standard technique is to inject 4 ml of ICG diluted in aqueous solvent into the uterine cervix at the 3 and 9 o'clock positions, submucosally and deep of the cervix, and the Pinpoint® Endoscopic Fluorescence Imaging System (Pinpoint®, Novadaq Technologies, Bonita Springs, FL, USA) was opted out intraoperatively to locate the SLN. Pelvic lymphadenectomy was defined as removing lymphatic tissue around the obturator nerve accompanied by common, external, internal iliac arteries and veins. Paraaortic lymphadenectomy was defined as removing lymphatic tissue around the aorta and vena cava up to the inferior mesenteric artery or renal vein. The vaginal vault was closed with the V-Loc™ wound closure device. The 3.5-mm and 5-mm ancillary incisions were approximated using strips without suturing, while 10-mm incisions were closed with sutures instead. Postoperative incisional scars are shown in Figure 2. For postoperative analgesia, patients were given ibuprofen (400 mg), paracetamol (1 gr), and tramadol (1 mg/kg) approximately 30 minutes before the skin closure. In the first 24 hours, postoperative pain was relieved with diclofenac potassium (50 mg) administered orally every 8 hours.

Statistical Analysis

Data was analyzed by SPSS (Version 26.0. 2011, IBM SPSS Statistics for Windows; IBM Corp. Armonk, NY, USA). Since this is a retrospective comparison between two groups, we adopted a propensity-matched comparison to reduce the covariate imbalance in measured baseline patient characteristics between surgical groups. Patients who underwent mini-laparoscopic surgery were matched 1:2 to a group of patients who underwent conventional laparoscopic surgery. Median, mean, standard deviation, frequency, and ratio values were used for descriptive statistics. The variables were investigated using Kolmogorov-Simirnov/Shapiro-Wilk's test to determine whether or not they are normally distributed. The χ^2 or Fisher's exact test was used to analyze categorical variables. The Student's t-test and Mann-Whitney U test were used to analyze continuous variables. A p-value <0.05 was used as the cutoff for significance.

Table 1. Baseline characteristics of the patients who underwent mini-laparoscopy and conventional laparoscopy[a]

	Mini-laparoscopy (n=25)	Conventional laparoscopy (n=50)	p
Age, years	62.2±9.9	60.9±13.2	0.645
Menopausal status			0.189
Premenopausal	4 (16%)	15 (30%)	
Postmenopausal	21 (84%)	35 (70%)	
Gravida	2 (0–7)	2 (0–8)	0.670
Parity	2 (0–6)	2 (0–8)	0.632
BMIb, kg/m^2	28±4.6	29.2±4.5	0.855
Previous abdominal surgery	17 (68%)	42 (84%)	0.111
ASAc score	2 (1–3)	2 (1–3)	0.317

a: Data are presented as number (%), mea±SD or median (range); b: BMI, body mass index; c: ASA, American Society of Anesthesiologists.

RESULTS

Records of 25 patients with EC who underwent mini-laparoscopy and 50 patients with EC who underwent conventional laparoscopy were analyzed. The median age was 61 years (range, 37–87 years), the mean BMI was 29 kg/m^2 (range, 21–39.8 kg/m^2), the median gravida was 2 (range, 0–8), the median parity was 2 (range, 0–8), 59 of 75 patients (78.7%) had a history of previous abdominal surgery, and the median ASA score was 2 (range, 1–3) without significant difference between the groups (Table 1). There was no significant difference between the mini-laparoscopy and the conventional laparoscopy group regarding surgical procedures (Table 2). The mean operation time and the median estimated blood loss were similar (p=0.671 and p=0.158, respectively) (Table 2). Tumor characteristics in the two groups were reported in Table 3. No difference was found in mean uterine diameter, mean tumor diameter, tumor histology, grading, staging, the number of lymph nodes removed, and lymph node involvement (Table 3). No intraoperative complications were observed in both groups. As a postoperative complication, umbilical wound infection was seen in one patient in both groups, and ileus was observed in one patient in the mini-laparoscopy group. Umbilical wound infections of the patients were superficial and treated with oral antibiotic agents. Postoperative ileus occurred at postoperative day 3 and resolved with conservative treatment at postoperative day 5. Return to daily routine and the rate of additional analgesia requirement were similar in the groups. The mean duration of hospitalization was 3.6±1.2 days in the mini-laparoscopy group and 4.9±3.6 days in the conventional laparoscopy group (p=0.025). Five patients (20%) received adjuvant therapy in the mini-laparoscopy group, while 7 (14%) received adjuvant therapy in the conventional laparoscopy group. The median follow-up was 29.1 months (15–52 months) for the mini-laparoscopy group and 46.7 months (14–107 months) for the conventional laparoscopy group. Recurrence was detected in 2 patients in the conventional laparoscopy group. No death was detected in either group.

DISCUSSION

In the last decade, mini-laparoscopic surgical approaches have drawn remarkable popularity in gynecological and non-gynecological surgery [15, 16]. The industry has provided a wide range of mini-laparoscopic instruments for laparoscopic surgeons to enhance their utilization of those cutting-edge technologies [17]. Of course, technological advances have facilitated the application of minimally invasive techniques in gynecological cancer treatment. For instance, decreasing wound size was found to be potentially associated with abatement of incisional hernias as well as other wound complications. Moreover, the smaller trocars do not require vigorous force to penetrate through the abdominal wall, resulting in a dropped rate of injuries to vessels and visceral organs.

To the best of our knowledge, limited studies investigated the safety and feasibility of mini-laparoscopy in EC staging [18–21]. Our study indicates that staging of EC could be performed using mini-laparoscopic instruments without mischievous outcomes similar to conventional laparoscopy. There was no significant difference between

Table 2. Surgery related parameters of the patients[a]

	Mini-laparoscopy (n=25)	Conventional laparoscopy (n=50)	p
Surgical procedures[b]			0.154
TLH + BSO	1 (4%)	9 (18%)	
TLH + BSO + SLNB	0 (0%)	1 (2%)	
TLH + BSO + BPLND	0 (0%)	6 (12%)	
TLH + BSO + BPLND + SLNB	16 (64%)	23 (46%)	
TLH + BSO + BPPALND	1 (4%)	3 (6%)	
TLH + BSO + BPPALND + SLNB	7 (28%)	7 (14%)	
Radical hysterectomy + BPLND + SLNB	0 (0%)	1 (2%)	
Mean operation time (min)[b]			
TLH + BSO	110	150±49	0.456
TLH + BSO + BPLND	183±58	177±58	0.771
TLH + BSO + BPPALND	266±52	305±87	0.284
Radical hysterectomy + BPLND	–	220	–
Estimated blood loss (ml) (median, range)	60 (20–200)	80 (30–400)	0.158
Intraoperative complications	–	–	
Port site-related complications			
Umbilical wound infection	1 (4%)	1 (2%)	1
Postoperative complications			
Ileus	1 (4%)	–	0.333
Additional analgesia requirement	4 (16%)	10 (20%)	0.763
Mean duration of hospitalization (day)	3.6±1.2	4.9±3.6	**0.025**
Return back to daily activity (day)	7.8±3.1	8.9±3.7	0.205
Rehospitalization	–	–	–
Adjuvant treatment[c]			0.101
None	20 (80%)	43 (86%)	
BrT	1 (4%)	5 (10%)	
ERT	4 (16%)	1 (2%)	
Chemoradiotherapy	0 (0%)	1 (2%)	

a: Data are expressed as number (%) or mean±SD; b: BPLND, bilateral pelvic lymph node dissection; BPPALND, bilateral pelvic-paraaortic lymph node dissection; BSO, bilateral salpingoophorectomy; SLNB, sentinel lymph node biopsy; TLH, total laparoscopic hysterectomy; c: BrT, brachytherapy; ERT, external radiotherapy.

the groups regarding baseline characteristics and history of previous abdominal surgery. Seventeen out of 25 patients (68%) and 42 out of 50 patients (84%) had a history of previous abdominal surgery in the mini-laparoscopy and conventional laparoscopy groups, respectively. Cianci et al. [22] reported 46 EC cases, which were done using mini-laparoscopic instruments. It is worth mentioning that half of the patients had a history of previous abdominal surgery in their study. Consequently, mini-laparoscopic surgery seems suitable for patients with previous surgery.

This study found no difference between the groups regarding the surgical procedures and the mean operation times. The management of EC staging, including bilateral pelvic-paraaortic lymphadenectomy (BPPALA), was performed successfully in all patients. No conversions were needed from mini-laparoscopy to open surgery as bilateral pelvic-paraaortic lymphadenectomy was indicated. Ghezzi et al. [19] encountered no technical difficulties with the smaller instruments during lymphadenectomy in patients with endometrium cancer and indicated that bleeding was controllable even with the small diameter coagulation devices.

Table 3. Final pathological findings of the patients who underwent mini-laparoscopy and conventional laparoscopy[a]

	Mini-laparoscopy (n=25)	Conventional laparoscopy (n=50)	p
Mean uterine diameter (mm)	71±13.7	73.4±17.7	0.524
Mean tumor diameter (mm)	23.6±19.8	21.1±15.6	0.550
Histology			0.659
Endometrioid	24 (96%)	46 (92%)	
Non-endometrioid	1 (4%)	4 (8%)	
Grade			0.816
G1	12 / 25 (48%)	26 / 50 (52%)	
G2	10 / 25 (40%)	18 / 50 (36%)	
G3	3 / 25 (12%)	6 /50 (12%)	
Stage[b]			0.793
I A	19 (76%)	41 (82%)	
I B	5 (20%)	8 (16%)	
II	1 (4%)	0 (0%)	
III C1	0 (0%)	1 (2%)	
Number of removed lymph nodes[c]			0.082
TLH + BSO + SLNB	–	3	
TLH + BSO + BPLND	–	18.8±8.8	
TLH + BSO + BPLND + SLNB	22±14.1	17±11.6	
TLH + BSO + BPPALND	78	39.3±18.8	
TLH + BSO + BPPALND + SLNB	31.3±7.1	41.14±15.3	
Radical hysterectomy + BPLND + SLNB	–	21	
Lymph node involvement	0 (0%)	1 (2%)	1

a: Data are expressed as number (%) or mean±SD; b: According to the FIGO 2009 classification; c: BPLND, bilateral pelvic lymph node dissection; BPPALND: Bilateral pelvic-paraaortic lymph node dissection; BSO: Bilateral salpingoophorectomy; SLNB: Sentinel lymph node biopsy; TLH: Total laparoscopic hysterectomy.

SLN mapping, in conjunction with minimally invasive techniques, has been accelerated in the management of endometrial cancer. Accordingly, SLN mapping was performed successfully in both groups. In a study conducted on 38 patients with early-stage endometrium cancer, Uccella et al. [23] obtained bilateral sentinel node detection in 11 patients out of 15 using a mini-laparoscopic approach without complication.

None of the patients were converted to laparotomy or required blood transfusion in both groups. There was no statistical difference between the groups regarding port site-related or postoperative complications. Our data and the findings of trials comparing mini-laparoscopy and conventional laparoscopy are alike, which means no statistically significant difference has been reported related to intraoperative or early postoperative complications [18, 20].

There is clear evidence that mini-laparoscopic surgery is associated with less postoperative pain, better cosmetic outcomes, and less hospitalization than conventional laparoscopy [24, 25]. In this study, the rates of additional analgesia requirement were sequentially found to be 13% and 17% in the mini-laparoscopy group and the conventional laparoscopy group. A previous randomized study indicated that the necessity for postoperative analgesics was lower when laparoscopy is performed with 3-mm instead of 5-mm ancillary ports [24].

Acton et al. [18] found no difference in length of hospital stay between 5-mm and 10-mm laparoscopic surgery. The mean time of return to daily routine was similar between the groups, while the mean duration of hospitalization was lower in the mini-laparoscopy group than in the conventional laparoscopy group.

As we know, the use of smaller instruments has not limited the ability of gynecologic surgeons to perform staging surgery. Therefore, the mini-laparoscopic route does not compel a learning curve for trained conventional laparoscopic surgeons. Overall, our promising results demonstrate the feasibility and reliability of mini-laparoscopic surgery in EC staging.

Study Limitations

The present study has some limitations which must be pointed out. Our study is limited by its relatively small sample size. Retrospective design can also be criticized; however, we strongly believe that our sample size handicap has been adjusted by propensity matching comparison. As a result, it reduces the covariate imbalance in measured baseline patient characteristics between surgical groups. Another strength of this study is our highly experienced surgeons. Given that, there was no potential bias in the practices of different surgeons.

Conclusion

We demonstrated that mini-laparoscopic endometrial cancer staging could be a competent technique that is performed regardless of harm by talented surgeons using state-of-the-art instruments. Mini-laparoscopic surgery, of note, appears as a further possibility to minimize surgical trauma by reducing the ports' size without decreasing the procedures' extent and effectiveness. Undoubtedly, additional prospective studies are necessary to get external and internal validation of our findings.

Ethics Committee Approval: The Koc University Clinical Research Ethics Committee granted approval for this study (date: 24.04.2020, number: 2020.159.IRB2.049).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The author declared that artificial intelligence-supported technologies were not used in this study.

Authorship Contributions: Concept – BG, DV, SM, OA, MM, MA, CT; Design – BG, DV, SM, OA, MM, MA, CT; Supervision - BG, DV, SM, OA, MM, MA, CT; Fundings – BG, DV; Materials– BG, SM; Data Collection and processing – BG, MA; Analysis and interpretation – BG, MM; Literature search – BG, CT; Writing – BG, OA; Critical review – BG, DV, SM, OA, MM, MA, CT.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):453–460
doi: 10.14744/nci.2025.97415

Original Article HISTOLOGY & EMBRYOLOGY

Hydroxytyrosol has a protective effect on the kidneys through dardarin and spexin

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ABSTRACT

OBJECTIVE: In this study, the possible role of dardarin and spexin in the protective effect of hydroxytyrosol (HT) against corn syrup-induced renal injury in rats was investigated.

METHODS: Rats were categorized into four groups (n=6) as control, HT, corn syrup, and corn syrup+HT. Over 6 weeks, rats were administered water infused with 30% corn syrup, 4 ml/kg/day solution containing HT was administered, both independently and in conjunction with corn syrup, throughout the 6 weeks. The molecular parameters of dardarin and spexin in the renal tissue were assessed through histopathological examination. Biochemical parameters were also examined with the ELISA Method.

RESULTS: In this study, it was observed that the dardarin and spexin levels increased in the control group as a result of the administration of corn syrup. After HT treatment, it was observed that the dardarin and spexin levels decreased. The increase in glucose, amylase, and lipase levels because of corn syrup consumption decreased with hydroxytyrosol consumption. The increase in erythrocyte extravasation, exudate accumulation, and fibrosis in kidney tissue observed as a result of corn syrup decreased as a result of HT administration.

CONCLUSION: It is thought that the protective effect of HT against damage to the renal due to corn syrup consumption may be mediated by dardarin and spexin.

Keywords: Corn Syrup; dardarin; hydroxytyrosol; kidney; spexin.

Cite this article as: *Kocaman N, Onat E, Hancer S. Hydroxytyrosol has a protective effect on the kidneys through dardarin and spexin. North Clin Istanb 2025;12(4):453–460.*

It has been determined that the likelihood of chronic kidney disease increases by 60% in people who consume high-calorie and high-fructose drinks [1]. The underlying cause of kidney disease due to high fructose intake; It is thought that there may be an increase in serum uric acid, an increase in vasopressin level, and postprandial hypertension due to fructose intake [2, 3]. It has been observed that ingestion of foods containing high fructose corn syrup (HFCS) increases renal vascular resistance and increases renal vasoconstriction due to sympathetic system activation [4]. Consumption of beverages containing HFCS may aggravate renal vasoconstriction tone and therefore increase the risk of nephropathy due to renal ischemia. For these reasons, long-term consumption of large amounts of fructose is thought to be associated with kidney diseases [3].

Extra virgin olive oil (EVOO) is the most important antioxidant component in the Mediterranean diet [5, 6]. The protective effect of EVOO on the kidneys due to its antioxidant properties has been confirmed in experimental nephropathy models [7, 8]. The most important of

Received: November 25, 2024 *Revised:* March 10, 2025 *Accepted:* April 02, 2024 *Online:* August 26, 2025

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the polyphenolic compounds in EVOO is hydroxytyrosol (HT), which has a strong antioxidant effect [9]. It has been shown that the administration of extra virgin olive oil rich in polyphenols to patients with chronic kidney disease improves the renal analytical profile more than in patients given extra virgin olive oil poor in polyphenols. This nephroprotective effect is thought to be related to the antioxidant property of HT [10].

Dardarin (LRRK2), which has a protein structure, is found in the brain, lungs, and to a lesser extent, in some other tissues. Known as a protein with multiple domains, LRRK2 has GTPase and protein kinase activities. They also have important roles in many cellular events and signaling pathways, including the cytoskeleton, vesicle transport, mitochondrial metabolism, and the regulation of endocytosis and autophagy [11]. LRRK2 is known to have many functions in the body, but its full functional role is not elucidated yet.

Spexin is a peptide hormone that is a member of the spexin/galanin/kisspeptin group [12, 13]. Spexin is found in many tissues such as ovaries, testicles, heart, skeletal muscle, kidney, lung, liver, pancreas, brain, thyroid, adrenal gland, spleen, adipose tissue, stomach, and gastrointestinal tract [14]. Spexin stimulates galanin receptor types 2 (GALR2) and 3 (GALR3) in target cells [15]. It also plays a role in many functions in the body, such as feeding, energy regulation, lipid accumulation, blood pressure, water/salt balance, cardiovascular, and renal functions, and cardiorenal responses [16–18]. Various studies have shown that this hormone can regulate inflammatory processes, but the underlying mechanism is not yet clear [19].

In this study, it was investigated whether LRRK2 and spexin molecules play a role in the protective effect of HT against kidney damage caused by corn syrup consumption in rats.

Highlight key points

- Corn syrup consumption may cause damage to the kidneys.
- HT may have a protective effect on kidney damage caused by corn syrup consumption.
- Dardarin may be an important molecule in the diagnosis of kidney damage.
- Spexin is a new peptide and may be a precursor of kidney damage.
- Dardarin and spexin may be important target molecules in the treatment of kidney diseases.

MATERIALS AND METHODS

Animals and Experimental Design

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [20]. The Animal Ethics Committee of Adıyaman Unıversity approved the study protocol (Protocol no: 2024/031). The experiments were performed according to the "Guide for the Care and Use of Laboratory Animals". The study was conducted in accordance with the Declaration of Helsinki. In the study, 24 male Sprague-Dawley rats, weighing 200-250 g, aged 8-10 weeks, obtained from the Adıyaman University Animal Experimental Research Center were used. The rats were housed in a fixed environment. They were provided with standard food and water consumption during the study. The rats were divided into four groups, 6 animals in each group: Group I: control, Group II: HT, Group III: corn syrup, Group IV: corn syrup + HT. No application was made to the control group during the experiment. Hydroxytyrosol (HT) was supplied by Kale Naturel Herbal Products Company in Turkey. HT was given orally to rats in Groups II and IV at a dose of 4 ml/kg/day for 6 weeks. 30% corn syrup was added to the drinking water of rats in Groups III and IV for 6 weeks [21]. At the end of this period, rats were administered intraperitoneal ketamine (75 mg/kg) + xylazine (10 mg/kg) and blood was taken from their hearts. Kidney tissues were placed in 10% formaldehyde solution for immunohistochemical examinations.

Serological Analysis

Cardiac blood samples of the nonfasted rats were centrifuged at 4° and 10.000 g for 30 min. Serum samples were immediately stored at -80° until the samples were assayed. Analysis for glucose, amylase, lipase, insulin and uric acid levels was performed spectrophotometrically on an Architect c16000 biochemistry autoanalyzer (Abbott Diagnostics, USA) with commercially available kits from Abbott Diagnostics.

Histochemical Examination

Histological follow-up series were applied to the kidney tissues taken and then embedded in paraffin blocks. Sections were taken from these blocks (5 μm). They were stained with Hematoxylin & Eosin, Masson Trichrome, and Immunohistochemically.

TABLE 1. Levels of various biochemical parameters in blood serum of rats

Groups	Control	HT	Corn syrup	Corn syrup+HT
Glucose (mg/dl)	178.33±4.08	203.67±50.62	460.33±63.53[ab]	194±22.18[c]
Amylase (U/L)	1583.3±40.82	1731.5±40.42	2572.2±281.38[ab]	1763.8±139.23[c]
Lipase (U/L)	9.17±0.41	11.33±1.03	15±2.45[ab]	11.5±0.84[c]
Insulin (uIU/ml)	0.03±0.02	0.028±0.02	0.06±0.02	0.04±0.01
Uric acid (mg/dl)	0.75±0.05	1.15±0.5	3.77±3.45	1.35±0.3

Error bars indicate SD; HT: Hydroxytyrosol; a: P<0.05 compared to the control group; b: P<0.05 compared to HT group; c: P<0.05 compared to corn syrup group.

Immunohistochemical Examination

Kidney tissues from rats were embedded in paraffin blocks after undergoing a histological follow-up series and 5 μm thick sections were taken from these blocks for immunohistochemical staining [22]. Histological tissue microarray slides of 3 μm thickness were used for immunohistochemical staining (IHC). LRRK2 primary antibody (orb500678; Biorbyt Ltd., Cambridge, England) and spexin primary antibody (A04088-1, booster biology technology, Pleasanton, CA, USA) were used as antibodies. The evaluation was performed on a Zeiss Axio Scope A1 microscope (Carl Zeiss Microscopy GmbH 07745 Jena, Germany). After immunohistochemical staining, histoscoring was performed for LRRK2 and spexin. As a result of microscopic evaluation of staining intensity: Negative colored areas were given a value of 0, areas showing less than 25% staining were given a value of 0.1, areas showing 26–50% staining were given a value of 0.4, areas showing 51–75% staining were given a value of 0.6, and areas showing staining close to homogeneity (76–100%) were given a value of 0.9. The formula used in the histoscore was as follows.

Histoscore = Distribution x Intensity [22].

Statistical Analysis

Power Analysis

In this study, G power 3.1.9.7v program ANOVA fixed effects procedure was used to calculate the sample sizes of the groups. Considering Effect size: 0.90, statistical power (1 - β): 0.90 and significance level 0.05 as bidirectional, actual power was determined as 0.90, and 6 animals for each group (4 groups) for a total of 24 animals.

Statistical analyses were performed using SPSS 22 (SPSS Statistics 21.0 (Armonk, New York: IBM Corp.). The conformity of quantitative data to normal distribution was evaluated with the Shapiro-Wilk test. The One-Way ANOVA Test and the Tukey HSD Test were used for post-hoc multiple comparisons. Study results are presented as mean±SD, indicating the level of statistical significance ($p<0.05$).

RESULTS

Biochemical Findings

Plasma glucose, amylase, and lipase levels in rats given corn syrup were significantly increased compared to both groups (control, HT) ($p<0.001$). Plasma glucose, amylase, and lipase values were lower in the corn syrup group as a result of HT administration ($p<0.001$). Plasma insulin and uric acid values were higher in rats given corn syrup than in both groups (control and HT) but were not significant. Plasma insulin and uric acid values were lower in rats given corn syrup as a result of HT administration, although not significant (Table 1).

Histochemical Findings

As a result of the staining of the groups with Hematoxylin-Eosin and Masson trichrome, the control and HT groups were seen as normal (Table 2, Fig. 1, 2). In the Corn Syrup group, there was an increase in erythrocyte extravasation, exudate accumulation, and fibrosis compared to the control and HT groups ($p<0.001$). Compared to the Corn Syrup Group, a significant decrease in erythrocyte extravasation, exudate accumulation, and fibrosis was observed in the Corn Syrup+HT Group ($p<0.001$) (Table 2, Fig. 1, 2).

Immunohistochemical Findings

In the kidney tissue with immunohistochemical staining, LRRK2 immunoreactivity increased sig-

Table 2. Histopathologic findings of the renal tissues (hematoxylin and eosin-Masson trichrome)

Parameters	Control	HT	Corn syrup	Corn syrup+HT
Erythrocyte extravasation	1.43±0.53	1.71±0.49	5.57±0.79^{ab}	2.29±0.49^{c}
Exudate accumulation	1.43±0.53	1.29±0.49	6.71±0.49^{ab}	1.71±0.49^{c}
Fibrosis	1.43±0.53	1.29±0.49	4.71±0.49^{ab}	2.86±0.69^{abc}

Error bars show SD; HT: Hydroxytyrosol; a: P<0.05 compared to control; b: P<0.05 compared to HT; c: P<0.05 compared to corn syrup.

Table 3. Immunohistochemical results for dardarin in the renal tissues

Groups	Control	HT	Corn syrup	Corn syrup+HT
Dardarin	0.21±0.07	0.2±0.06	0.4±0.07^{ab}	0.27±0.05^{c}

Error bars indicate SD; HT: Hydroxytyrosol; a: P<0.05 compared to the control group; b: P<0.05 compared to HT group; c: P<0.05 compared to corn syrup group.

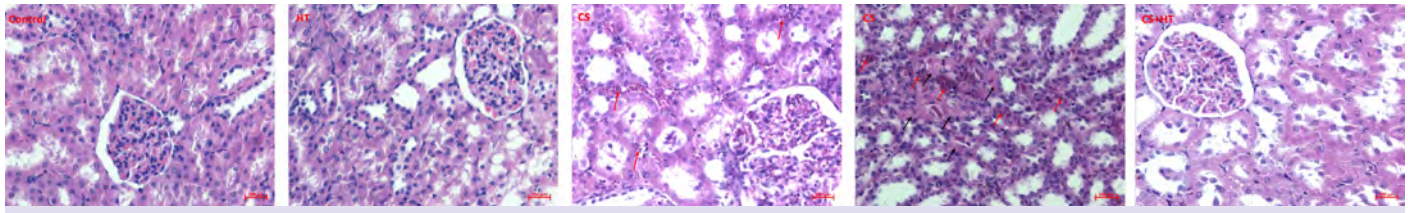

Figure 1. The histopathological findings of renal tissues of observation (hematoxylin and eosin).

HT: Hydroxytyrosol; CS: Corn syrup; CS+HT: Corn syrup+ Hydroxytyrosol.

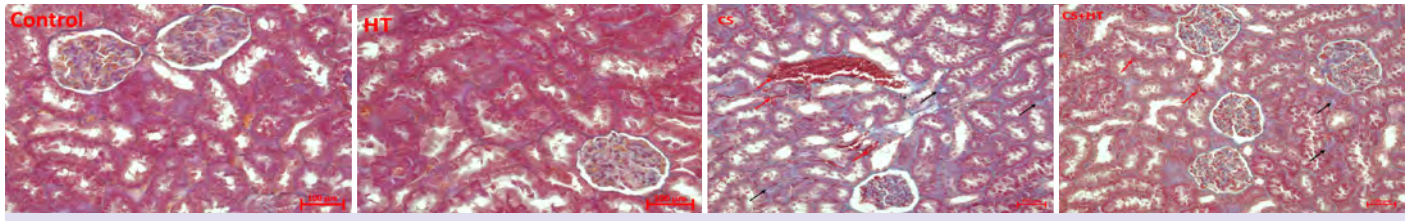

Figure 2. The histological findings of renal tissues of observation (Masson trichrome staining).

nificantly as a result of corn syrup application compared to the two groups (control, HT) (p<0.001). In contrast, LRRK2 immunoreactivity was lower in the corn syrup group as a result of HT application (p=0.005) (Table 3, Fig. 3).

Specin immunoreactivity increased as a result of corn syrup application compared to two groups (control, HT) (p<0.001). Specin immunoreactivity was lower in the corn syrup group as a result of HT application (p<0.001) (Table 4, Fig. 4).

DISCUSSION

In the study, the effect of HT, which is thought to have a protective effect on rats whose kidneys were damaged by consuming corn syrup, and whether new proteins such as LRRK2 and spexin were involved in this effect was examined. As a result of this examination, it is thought that HT has a protective effect against the negative changes caused by corn syrup in the kidneys and that LRRK2 and spexin may also play a role in this effect.

TABLE 4. Immunohistochemical results for SPX in the renal tissues

Groups	Control	HT	Corn syrup	Corn syrup+HT
SPX	0.36±0.08	0.34±0.07	0.94±0.21[ab]	0.51±0.08[c]

Error bars indicate SD; HT: Hydroxytyrosol; SPX: Spexin; a: P<0.05 compared to the control group; b: P<0.05 compared to HT group; c: P<0.05 compared to Corn Syrup group.

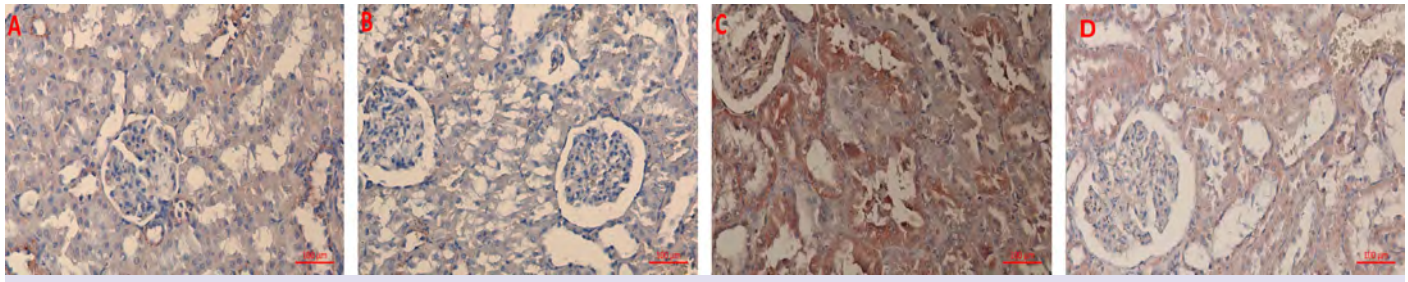

FIGURE 3. Immunohistochemical findings for dardarin in renal tissues (control, HT, corn syrup, corn syrup+HT).

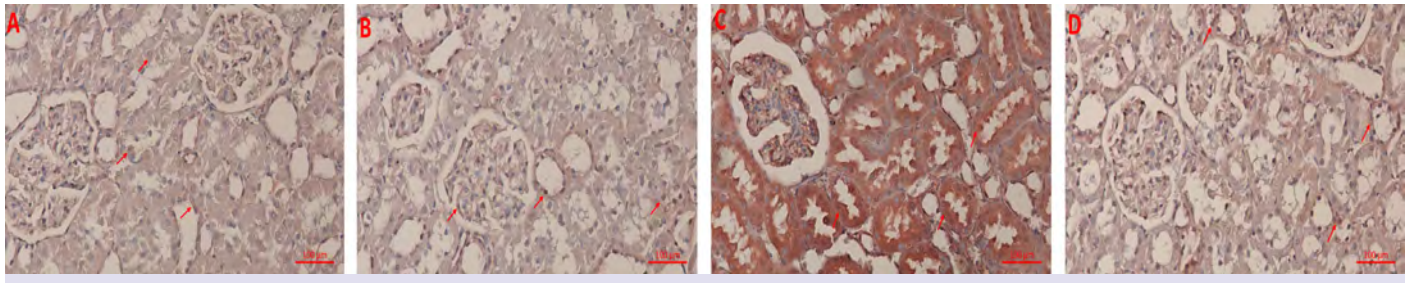

FIGURE 4. Immunohistochemical findings for spexin in renal tissues (control, HT, corn syrup, corn syrup+HT).

It has been suggested that the Mediterranean diet, which has important antioxidant properties, has a protective effect on diabetic nephropathy [23]. When the structure, hydrophilic properties, metabolism, and transformations of the HT molecule, the most important antioxidant component in EVOO, were examined, it was observed that its conjugated metabolites were mainly excreted by the kidneys [24]. HT accumulates in the kidneys until it is excreted from the body [25] and in the meantime, it may have a nephroprotective effect thanks to its antioxidant properties [26]. These antioxidant properties explain the protective effect of HT on the kidneys from a morphological and functional perspective. A previous study showed that it has been shown that the administration of extra virgin olive oil rich in polyphenols to patients with chronic kidney disease improves the renal analytical profile more than in patients given extra virgin olive oil poor in polyphenols [5]. In our study, consistent with these findings, plasma glucose, amylase, lipase, insulin, and uric acid levels increased with corn syrup and decreased as a result of HT application, proving once again the protective effect of HT on blood glucose-insulin regulation and the kidney. In addition, it was observed that the increase in erythrocyte extravasation, exudate accumulation, and fibrosis observed in the renal tissue as a result of corn syrup application was significantly reduced as a result of HT application.

Numerous studies have revealed that LRRK2 is functionally involved in a wide variety of cellular events, including inflammation, autophagy, apoptosis, synaptogenesis, and proliferation [27, 28]. It has been shown that LRRK1 and LRRK2 play important roles in regulating protein homeostasis and that the LRRK2 −/− kidney undergoes a large loss of LRRK compared to other organs, thus aging. LRRK2-dependent molecular and cellular changes are likely to be

responsible for increased cell death and inflammatory responses [29]. Drugs that inhibit LRRK2 to treat PD have been shown to induce pathologies in peripheral organs, especially the kidneys and lungs [30]. Since LRRK2 is found at high levels in papillary renal cell carcinoma (pRCC), inhibition of LRRK2 kinase is important for its effect on the kidney [31, 32]. Disruption of LRRK2 expression in pRCC cells leads to cell cycle arrest and inhibition of crucial cell signaling pathways due to impaired growth factor receptor-dependent stimulation. Moreover, genetic deletion of LRRK2 in mice caused significant pathologies in the lung and kidney, suggesting that long-term inhibition of LRRK2 enzymatic activity in Parkinson's disease may be particularly detrimental to these organs and therefore this treatment approach is not appropriate. LRRK2 amplification and overactivity are implicated in the subgroup of type I papillary renal cell carcinoma, which accounts for approximately 10% of kidney cancer [33]. In our study, the increase in the LRRK2 molecule in the kidney due to corn syrup intake suggests that LRKK2 may play a role in inflammation and apoptotic pathways in the kidney. The decrease in the amount of LRRK2 in rats receiving HT at the same time as corn syrup suggests that LRRK2 may play a role in the healing properties of HT against the kidneys. However, more studies are needed on the effect of LRRK2 in this pathology to say this.

It has been shown that SPX can reduce oxidative stress and inflammation in those who develop kidney failure due to excess weight [14]. It has been shown that SPX treatment stimulates an inflammatory response by regulating cytokine and chemokine levels in chronic renal failure [34]. In another study, it was observed that as a result of SPX application, oxidative stress in the kidneys decreased, and inflammation in renal dysfunction caused by obesity decreased [14]. Additionally, a protective effect on kidney damage was observed as a result of intracerebroventricular administration of SPX [35]. However, in chronic renal failure due to adenine, SPX application showed an inhibitory effect on renal damage and inflammation [36]. In another study, it was observed that spexin-based (galanin receptor) GALR2 agonists improved diabetic nephropathy without changing metabolic syndrome parameters [37]. A recent study observed pathological changes in kidney tissue, increased oxidative stress and apoptosis, and elevated SPX levels as a result of exposure of rats to aluminum. Additionally, it has been observed that pathological changes and elevated SPX levels caused by aluminum are reduced by NAC administration, and nephrotoxicity is prevented prophylactically [38]. In another study, increased SPX immunoreactivity in kidney tissue was observed in ADR-induced nephrotoxicity. However, a significant decrease in SPX levels was observed in the ADR + NAC group in response to the decrease in oxidative stress and apoptosis [39]. In our study, we think that the increase in SPX levels in rats given corn syrup may help prevent damage to the kidney tissue due to oxidative stress and apoptosis caused by corn syrup. The decrease in SPX levels after HT treatment suggests that HT may have a protective effect on nephrotoxicity by affecting SPX levels, which are thought to maintain the balance between oxidative stress and apoptosis.

This study suggests that LRRK2 and spexin may be effective in the healing properties of HT against the pathological processes induced by corn syrup in the kidneys. However, the mechanisms involved in this effect need to be further investigated. In addition, these results need to be supported by analysis tests such as Western Blot and PCR and their clinical aspects must also be investigated.

Conclusion

It is thought that HT has a healing effect on corn syrup-induced kidney damage and that newly discovered molecules such as LRRK2 and spexin may play a supporting role in this effect. LRRK2 and spexin may be important markers in screening and monitoring treatment protocols for kidney diseases.

Ethics Committee Approval: The Adiyaman University Animal Experiments Ethics Committee granted approval for this study (date: 02.05.2024, number: 2024/031).

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The authors declared that during the preparation of this work the author(s) did not use AI and AI-assisted technologies.

Authorship Contributions: Concept – NK, EO, SH; Design – NK, EO, SH; Supervision– NK, EO,SH; Fundings – NK, EO, SH; Materials – NK, EO; Data collection and/or processing – NK, EO, SH; Analysis and/or interpretation – NK, EO, SH; Literature review – NK, EO, SH; Writing – NK, EO, SH; Critical review – NK, EO, SH.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):461–467
doi: 10.14744/nci.2025.48902

Original Article ONCOLOGY

Impact of progression sites and line of therapy on survival outcomes in patients with HER-2 positive metastatic breast cancer treated with T-DM1

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ABSTRACT

OBJECTIVE: Ado-trastuzumab emtansine (T-DM1) is a key treatment for HER2-positive metastatic breast cancer (HER2+ MBC), yet the influence of progression sites and therapy lines on outcomes remains unclear.To assess the relationship between progression sites and the line of T-DM1 therapy with survival outcomes in HER2+ MBC.

METHODS: We retrospectively analyzed 123 patients with HER2+ MBC treated with T-DM1. Data on metastatic progression sites (brain, liver, bone, lung, lymph nodes), line of T-DM1 therapy (2nd-line vs ≥3rd-line), and death status were examined. Due to limited survival time data, mortality was used as the primary outcome. Death rates were compared across subgroups using descriptive statistics.

RESULTS: Brain and lung progression were associated with the highest mortality rates (76.7% and 73.1%, respectively). Liver and bone progression also showed elevated death rates (70.0% and 64.3%). Notably, more patients who used T-DM1 as the second-line therapy had a higher mortality rate at 66.7% compared to those treated with it in the third line or after (45.1%).

CONCLUSION: Progression to brain and lung during T-DM1 treatment correlates with higher mortality. Early-line use of T-DM1 may be linked with worse outcomes, possibly due to more aggressive disease biology. The obtained data could inform the decision-making process when treating patients with HER2+ MBC and predict their prognosis.

Keywords: Ado-trastuzumab emtansine; brain metastases; HER2-positive breast cancer; liver metastases; metastatic progression; therapy line.

Cite this article as: *Surmeli H, Buyukmurat N. Impact of progression sites and line of therapy on survival outcomes in patients with HER-2 positive metastatic breast cancer treated with T-DM1. North Clin Istanb 2025;12(4):461–467.*

Approximately 15 to 20 percent of cases of breast cancer are HER2+ or have aggressive tumor biology and poor disease outcomes but are responsive to targeted therapies. Her2-directed therapies have completely changed the prognosis of HER2+ MBC, with the patients getting more life years and better outcomes for the disease [1]. Considering its use in the management of HER2+ MBC, ado-trastuzumab emtansine (as T-DM1), T-DM1 is especially important.

The drug T-DM1 detects a trastuzumab and cytotoxic emtansine (DM1) combination in an antibody-drug conjugate configuration, thereby enabling targeted chemotherapy to predominantly affect HER2-expressing cancer cells [1]. Its experience in the second line setting has been backed by the EMILIA trial, though it is commonly used in the subsequent training, third line, or later. Although patients widely use T-DM1, response to the drug varies based on different clinical situations. The number of residual diseases, the types of past therapies, and specific locations of cancer spread contribute to the fact that prognosis and effectiveness of treatment can be patient-specific.

Received: July 15, 2025 *Revised:* July 28, 2025 *Accepted:* August 01, 2025 *Online:* August 26, 2025

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The location at which the disease advances during T DM-1 treatment may strongly affect survival. Brain metastases are prevalent in HER2+ MBC— affecting up to 30–50% of patients during their disease course — and associated with impaired quality of life and survival [2]. Similarly, the invasion of disease into internal organs, such as the liver or lungs is a usual occurrence of advanced illness and poorer health outcomes. However, real-world data exploring how the site of disease progression affects survival in patients treated with T-DM1 is still quite limited. Timing the administration of T-DM1 differently along the sequence of treatments could also affect overall survival. The advantages of T-DM1 in the second-line situation have been well established in trials, but in everyday life T-DM1's benefit to initiate before versus after that stage remains unclear. Early initiation of T-DM1 may possibly signify a more aggressive disease, and, similarly, a late initiation could possibly reflect increased treatment resistance. Determinants of whether survival is affected by the line of t-DM1 therapy may also be used to inform the planning of personalized treatment strategies.

The main purpose of this study is to find out whether the location where the disease progresses and the stage of therapy can influence overall survival in HER2+MBC patients treated with T-DM1. Our aims involve the establishment of whether disease progression at critical metastatic areas such as the brain, liver, bones, lung, as well as distant lymph nodes is associated with higher mortality. and investigate survival outcome differences with reference to T-DM1 initiation as second, third, or later-line therapy [2]. Lacking sufficient time-to-event data among our participants, we decided to analyze survival by observing death status – our binary outcome.

Analysis of such associations can help clinicians to identify at-risk populations and design better therapeutics. The finding of the present report takes on additional value in the age of personalized oncology, where decisions on therapy are driven by data. Through new considerations of T-DM1's effectiveness, our work adds value to prognostic models predictive of those with HER2+ metastatic breast cancer.

Highlight key points

- Brain and lung metastases were the strongest predictors of mortality during T-DM1 therapy.
- Second-line use of T-DM1 showed higher mortality than later use, suggesting more aggressive disease.
- Progression site and therapy line are key factors for prognosis and treatment planning.
- Findings highlight the need for prospective studies to validate real-world outcomes.

MATERIALS AND METHODS

Study Design and Population

As a retrospective, observational study the aim was to analyze the relationship between locations of metastatic progression and therapy line and its influence on survival in HER2 positive metastatic breast cancer (HER2+ MBC) patients treated by ado-trastuzumab emtansine (T-DM1). The study recruited women diagnosed with HER2 + MBC, and who had undergone T-DM1 treatment at any point in therapy. Analysis of the institutional records between [insert date range] saw 123 patients qualify for inclusion in the study.

Data Collection

Institutional records processed in an anonymized database served as the data for analysis. To carry out the study, data on the characteristics of patients; metastatic sites during T-DM1 treatment; therapeutic line introducing T-DM1, and overall survival were gathered. Specific evaluation of progression at the following sites was made:

- Brain
- Liver
- Bone
- Lung
- Distant lymph nodes

Each variable determined whether a particular site had progressed during T-DM1 treatment; 0 for no progression and 1 for progression.

Treatment sequencing data were used to create two line-of-therapy groups: 2nd-line or later; and 3rd-line or later. Total mortality (binary variable 1=dead, 0=alive) was the main outcome measure. Because overall survival (OS) and progression-free survival (PFS) were recorded as time-to-event variables in the dataset and were highly incomplete, these measures were not included in the final analysis.

Statistical Analysis

This study was approved by the Kartal Dr. Lutfi Kirdar City Hospital Ethics Committee (Approval No: 2025/010.99/1619, Date: 27.05.2025).

TABLE 1. Mortality by progression site

Progression site	Progression	Patients (n)	Deaths (n)	Death rate (%)
Brain	Yes	30	23	76.7
	No	50	23	46.0
Lung	Yes	26	19	73.1
	No	54	27	50.0
Liver	Yes	20	14	70.0
	No	60	32	53.3
Bone	Yes	28	18	64.3
	No	52	28	53.8
Lymph nodes	Yes	17	8	47.1
	No	61	37	60.7

Descriptive statistics gave an overview of more or less how often each site of progression, therapy line, and death status occurs. The primary consideration was the comparison of death rates for patients by progression sites and lines of therapy. For all variables, we calculated the occurrence and percent of the number of patients who died. Numberically the death rates were indicated in tabular structure and graphically with bar charts.

Patients were grouped based on:

- There was progression at all metastatic sites or none at all.
- Stage of therapy (initial second line of therapy vs. later lines).

Because data was categorical and time-to-event information was unavailable, inferential statistical procedures such as Chi-square or logistic regression were not focalized. Therefore, the analysis focused on representing not only mortality trends with the help of tables but also in graphical forms.

All statistical analyses were performed using SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patient Characteristics

The analysis included a cohort of 123 HER2-positive metastatic breast cancer (HER2+ MBC) patients receiving treatment with ado-trastuzumab emtansine (T-DM1). From the total, 100 patients provided adequate survival information and were included in the primary outcome analysis. Data for metastatic progression sites were available for ranges of 88–91 patients, with a line of therapy noted for 108 patients, contingent upon the site.

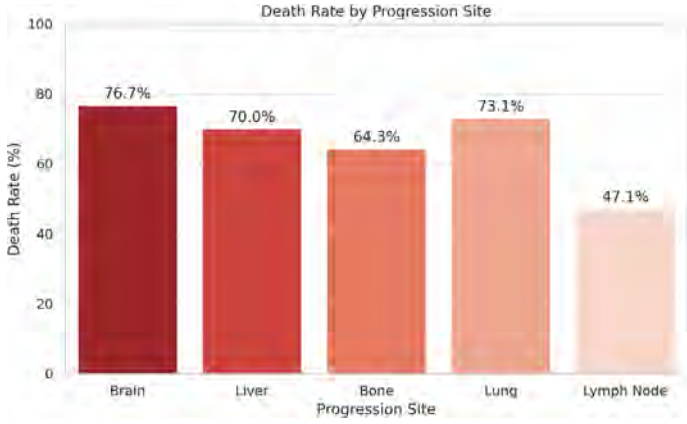

FIGURE 1. Death rate by progression site.

Among patients with a recorded sequence of their treatment, 39 (36.1%) received T-DM1 forth time. For the remainder, data regarding their treatment sequence either were missing or were not adequately defined.

Mortality by Metastatic Progression Site

Survival outcomes among the patients were significantly varying based on where their disease progressed first during T-DM1 therapy. The highest mortality rates were observed for the patients with metastases to the brain or lungs, while patients with metastases in the liver or bones were at lower mortality risk. Lymph node progression was correlated with the lowest death rates among the patients.

Figure 1 which is the Death Rate by Progression Site represents a breakdown of patients who died of their respective diseases in relation to the location where they progressed during a T-DM1 treatment: brain, liver, bone and lung, and distant lymph nodes.

Table 1. Mortality by Progression Site. A much greater percentage of patients died (76.7% with brain metastasis and 73.1% with lung metastasis), which could indicate that T-DM1 is less effective or that the underlying disease biology is more advanced when metastasis occurs in these areas.

Mortality by Line of Therapy

The in-patient death rate for patients undergoing T-DM1 in second-line therapy (66.7%) was higher than in the third-line or further periods (45.1%).

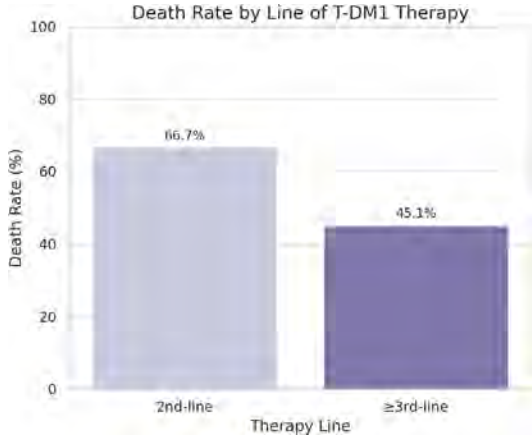

FIGURE 2. Death rate by line of therapy.

TABLE 2. Mortality by line of therapy

T-DM1 therapy line	Patients (n)	Deaths (n)	Death rate (%)
2nd-line	39	26	66.7
≥3rd-line	51	23	45.1

Figure 2. Death Rate by Line of Therapy. According to the data, 66.7% of patients receiving second-line T-DM1 died, whereas only 45.1% of those who started the treatment were in third or later lines.

Table 2. Mortality by Line of Therapy may be explained by the tumor aggressiveness: the patients with more aggressive disease were treated earlier with T-DM1 and there was a paradoxical higher death rate in the second-line group.

DISCUSSION

Here, the study addressed the interplay between metastatic progression sites, T-DM1 treatment sequence, and mortality among patients with HER2-positive metastatic breast cancer. Our findings suggest that progression to the brain and lungs during T-DM1 treatment is associated with markedly higher mortality, while the timing of T-DM1 therapy—whether administered in the second line or third line and beyond—may influence survival outcomes in ways that challenge prior assumptions derived from clinical trials.

The relationship between the progression of CNS disease and high mortality (76.7%) underscores the difficulties clinicians experience treating metastatic breast cancer that is HER2 positive. For patients with advanced HER2-positive disease, up to 50%, of brain metastases develop, and brain metastases management is still a major clinical challenge. Even with systemic effectiveness, poor CNS penetration of T-DM1 suggests that it may not be as effective against intracranial disease. Research by Montemurro et al. [3–5] revealed that T-DM1 has reduced effectiveness among untreated or growing brain metastase cases with pronounced exceptions where selected CNS responses are seen [3]. Our data reinforce the notion that patients with CNS involvement represent a high-risk subgroup and may benefit from alternative or combinatorial strategies involving CNS-active agents such as tucatinib or neratinib.

Along the same lines, the high mortality rate, 73.1%, in patients with lung involvement becomes additional evidence of the aggressive character of visceral metastases. Having lung disease with a significant tumor load and rapid progression concurrent with lung disease may cause pulmonary dysfunction and increase patient morbidity. As expected, patients with liver (70.0%) or bone (64.3%) metastases also had increased mortality rates, with the trend being less pronounced. This is consistent with published data noting the substantial correlation between metastatic spread to the visceral organs and unfavorable survival in patients with breast cancer [6, 7]. T-DM1 continues to be effective for visceral and non-visceral metastatic disease, although its clinical benefits might be blunted in those with advanced organ involvement especially when associated with poor overall health or prior resistance to treatment.

Contrastingly, lymph node progression did not confer a worse prognosis in our dataset. In fact, patients with nodal involvement exhibited a lower mortality rate (47.1%) compared to those without (60.7%). While this may seem counterintuitive, it is consistent with the hypothesis that isolated nodal progression represents a more indolent form of metastatic disease. Because the lymph nodes are usually amenable to local therapy and do not quickly impair the function of major organs, this mode of spread implies a more favorable short-term prognosis. This finding needs further study, particularly in light of increased treatment strategies focusing on oligometastatic disease such as stereotactic body radiation [8].

One of the most provocative findings of this study was the higher observed mortality among patients who

received T-DM1 as second-line therapy compared to those treated in later lines (66.7% vs. 45.1%). This result contradicts the results of the EMILIA trial in which T-DM1 used as second-line treatment was associated with a clear survival benefit, as compared to lapatinib and capecitabine [1]. There are a number of feasible reasons for this surprising result. First, confounding by indication is likely at play: patients who received T-DM1 earlier may have done so due to rapid progression on first-line therapy, indicating more aggressive disease biology. Second, there is a possibility that selection bias is tilted toward T-DM1 usage in sicker patients or those who are unable to tolerate other treatments. Third, because the inclusion of patients in everyday clinical practice when they are in worse health status, with underlying conditions, or have reasons to avoid some treatments is present, differences in clinical trial outcomes are established.

Additionally, among patients who received T-DM1 in the third-line setting or later, the second-line treatments administered before initiating T-DM1 included a combination of pertuzumab and trastuzumab with a taxane (docetaxel or paclitaxel) in most cases. A smaller subset had received dual HER2 blockade followed by capecitabine-based regimens. This sequence reflects evolving treatment landscapes where newer agents such as trastuzumab deruxtecan or tucatinib were either not accessible or reserved for later stages. These observations suggest that prior exposure to standard HER2-directed regimens might influence the efficacy of subsequent T-DM1 therapy. However, due to the retrospective nature of this study and the heterogeneity of treatment strategies, we recommend cautious interpretation of these sequences. Future studies with detailed therapy mapping and temporal analysis will help clarify the effect of prior regimens on T-DM1 outcomes.

Treatment sequencing might also contribute to these differences. Novel therapies at some medical centers such as trastuzumab deruxtecan or tucatinib along with capecitabine are saved for the following lines of treatment especially when T-DM1 had been previously efficacious. The limited access to the less frequent use of newly introduced therapies in specific environments would skew the results of survival analysis, making it pillow to precisely determine how the order of treatment influences therapeutic benefit [9]. Overall, our findings show that the process of establishing treatment order in HER2+ MBC is sophisticated because it contemplations clinical standards, tumor progression, health state, and therapeutic options.

Our findings can further enlarge the current evidence base on how T-DM1 performs in clinical practice outside the clinical setting. Observational studies such as ours are still essential for confirming efficacy, and controlled studies continue to be vital for establishing efficacy, but observational studies can also provide light on how treatment works in different and representative populations [10, 11]. Through this, they create visibility on ignored challenges – like insufficient CNS treatment or sub-optimal schedules – and remove the discussion over mitigating important research questions.

Although our findings prove to be helpful we have to keep in mind that our study has limitations. Lack of access to precise time-to-event statistics (such as PFS and OS) was an important obstacle since it limited the use of non-parametric statistic Kaplan-Meier survival curves or multivariable Cox proportional hazards models. Consequently, survival outcomes were assessed using a binary mortality endpoint, limiting the granularity of interpretation. Moreover, our analysis failed to gather crucial clinical factors such as ECOG performance status, tumor extent, hormone receptor status, and previous treatments that may affect the interpretation of the survival outcomes. Retrospective analysis can also result in information bias, particularly with the manner in which the progression of disease is recorded. We relied on recorded binary indicators of progression rather than radiographic progression-free intervals or RECIST-based metrics [12–14]. Furthermore, treatment heterogeneity—including dose modifications, delays, and duration of T-DM1 therapy—was not captured in the current dataset.

Despite this, the study provides valuable information on how metastatic patterns and time for treatment affect the outcome in patients who are on T-DM1 therapy with HER2 + MBC. The study points out the need to individualize treatment plans for patients who experience the advancement of central nervous system or visceral disease and questions the possible prognostic value of CD-DM1 treatment timing [15, 16].

Conclusion

The results of this study show the clinical relevance of metastatic locations and the use priority of treatments in HER2+ patients with metastatic breast cancer maintained on T-DM1. Among the metastatic sites assessed, progression to the brain and lungs emerged as the strongest predictors of mortality, with

death rates exceeding 70% in both groups [17]. These findings reinforce the clinical understanding that central nervous system and visceral involvement represent biologically aggressive disease states and may signal resistance to standard HER2-targeted therapies, including T-DM1.

The limited ability of T-DM1 to effectively penetrate the blood-brain barrier may partly explain its diminished efficacy in patients with CNS involvement. This underscores the urgent need to incorporate CNS-active HER2-targeted agents—such as tucatinib-based regimens—into the treatment pathway for patients at high risk for, or with established brain metastases [18]. Similarly, the poor outcomes observed with lung and liver progression highlight the need for more tailored systemic strategies and potential combinations in heavily burdened visceral disease.

Another striking result was that the use of T-DM1 as a second-line treatment showed faster mortality in patients receiving T-DM1, compared to patients who received T-DM1 in an earlier stage. Unlike data from the clinical trials, this finding is possibly swayed by real-world scenarios in that in the more aggressive conditions, more treatment escalation is likely to follow sooner.

This evidence does not support the idea that clinicians must consider the biology of the disease but also the course of patient treatment in deciding when and where to introduce such T-DM1. Future prospective studies are critical in supporting these observations and improving upon the rationale order of T-DM1 treatment in HER2+ MBC.

Ethics Committee Approval: The Kartal Dr. Lutfi Kirdar Sehir Hastanesi Ethics Committee granted approval for this study (date: 27.05.2025, number: 2025/010.99/1619).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: No AI-based technologies (including large language models such as ChatGPT or similar tools) were used in the preparation of this manuscript.

Authorship Contributions: Concept – HS; Design – HS; Materials – HS; Data collection and/or processing – HS; Analysis and/or interpretation – HS; Literature review – HS; Writing – HS; Critical review – NB.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):468–474
doi: 10.14744/nci.2025.08455

Original Article NEUROLOGY

Epidemiological study of congenital myasthenic syndromes based on national electronic health database of Turkiye

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ABSTRACT

OBJECTIVE: Congenital myasthenic syndromes (CMS) represent a group of genetically heterogenous disorders characterized by defective signal transmission at the neuromuscular junction. Although global prevalence of CMS remains uncertain, regional studies have reported varying prevalence rates. This study aimed to define the incidence and prevalence of CMS in Turkiye utilizing data from the national electronic health registry. Additionally, the rate of pyridostigmine prescriptions among patients with CMS was assessed.

METHODS: The study was a retrospective national cohort study, and patients with at least three G70.2 ICD-10 code entries between 1 January 2015 and 22 May 2024 were included. While calculating incidence and prevalence rates official census data from the Turkish Statistical Institute were used.

RESULTS: A total of 406 patients were included in the study, with females comprising 48.8% of the cohort. The mean age at diagnosis was 20.59±21.65 years (median: 12.00, min-max: 0-86). Among the cohort, 58.6% were diagnosed before the age of 18, and 12.3% before the age of one. Pyridostigmine was prescribed at least once to 68.2% of the patients. The annual incidence of CMS ranged from 0.28 to 0.59 per million between 2016 and 2023. In 2023, the incidence and prevalence rates of CMS were calculated as 0.63 and 4.49 per million, respectively.

CONCLUSION: This study represents the first comprehensive nationwide epidemiological analysis of CMS in Turkiye utilizing the national electronic health registry. The study enhances the understanding of the epidemiological landscape of CMS in the country by reporting the current incidence, prevalence, and pyridostigmine prescription rates and underscores the significance of this rare but impactful neuromuscular disorder.

Keywords: Congenital myasthenic syndromes; epidemiology; incidence; prevalence.

Cite this article as: *Inan B, Ozturk B, Ata N, Taskiran E, Birinci S, Sonkaya R, et al. Epidemiological study of congenital myasthenic syndromes based on national electronic health database of Turkiye. North Clin Istanb 2025;12(4):468–474.*

Congenital myasthenic syndromes (CMS) comprise a group of genetically heterogenous disorders characterized by defective signal transmission at the neuromuscular junction [1]. Based on the localization of mutated protein, CMS are classified into presynaptic, synaptic, and postsynaptic subtypes [2]. The clinical onset typical-

Received: April 04, 2025 *Accepted:* May 11, 2025 *Online:* August 08, 2025

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ly occurs at birth or during early childhood with fatigable weakness of the ocular, bulbar, and limb muscles. However, milder cases may remain undiagnosed until early adulthood. Late-onset cases predominantly affect axial and proximal limb muscles [3, 4].

Although the global prevalence of CMS remains unknown, various regional studies have reported prevalence rates ranging from 9.2 to 22.2 per million in pediatric population [5–7] and from 1.8 to 3.1 per million in the general population [7–9]. Early diagnosis of CMS is essential due to the potential for effective therapeutic interventions [2, 10]. However, treatment strategies must be tailored to specific CMS subtypes, as medications beneficial for one form may exacerbate symptoms in another.

In this study, we primarily aimed to elucidate the epidemiological profile of CMS in Turkiye. Secondarily, we assessed the rate of pyridostigmine prescriptions among patients with CMS.

Highlight key points

- This study represents the first comprehensive nationwide epidemiological analysis of CMS in Turkiye, utilizing the national electronic health registry.
- In 2023, the prevalence rate of CMS was 4.49 per million in the general population and 10.00 per million among children.
- Between 2016 and 2023, the annual incidence of CMS ranged from 0.28 to 0.59 cases per million in the general population, and from 0.63 and 1.35 per million in the pediatric population.

MATERIALS AND METHODS

This study was conducted in collaboration with the Health Policy Development Working Group of the Republic of Turkiye Ministry of Health (RTMH). We identified patients with CMS based on International Classification of Diseases, 10th revision (ICD-10) coding within the e-Nabiz and Health Record Reporting System (HRRS) registries, both managed by RTMH.

e-Nabiz and HRRS Registries

In Turkiye, national health data are mainly managed by RTMH via e-Nabiz and HRRS, while the Social Security Institution administers the state-funded universal health insurance system that provides a coverage for most of the population. HRRS serves as a platform for recording and reporting healthcare data, facilitating more effective health service management. e-Nabiz is a digital interface enabling patients and healthcare providers to access medical records collected across healthcare facilities. For this study, all data were anonymized and handled in strict adherence to the data protection regulations.

Case Identification, Incidence and Prevalence Calculations

The HRRS database was queried for ICD-10 code G70.2 (congenital and developmental myasthenia) entries between 1 January 2015 and 22 May 2024. As diagnostic codes, whether preliminary, definitive or used for prescription, are permanently stored in the database, a case of CMS was defined as having at least three G70.2 code entries. This threshold was adopted from previous epidemiological studies of autoimmune myasthenia gravis [11, 12] to enhance diagnostic reliability (Fig. 1).

Demographic and clinical variables including sex, date of birth, age at diagnosis, province of residence, and the total number of G70.2 ICD-10 code entries were collected. The initial entry date of the G70.2 code in HRRS was designated as the date of diagnosis. Prescription data for pyridostigmine were identified using Anatomical Therapeutic Chemical (ATC) code N07AA02.

Since e-Nabiz was implemented in 2015 and prior data were retrospectively integrated that same year, prevalence estimates were calculated without temporal restrictions. However, incidence analyses were confined to the 2016–2023 period to avoid bias from retrospective data aggregation in 2015. Population data including total and sex-disaggregated provincial and Turkiye census data, and total and sex-disaggregated census data for children (<18 years) were obtained from the Turkish Statistical Institute website [13] for the corresponding years.

Statistical Analysis

Descriptive statistics were expressed as mean±standard deviation or median (minimum–maximum) for continuous variables and as frequency (percentage) for categorical variables. Statistical analyses were performed using IBM SPSS Statistics version 23.0 for Windows (Armonk, New York: IBM Corp.), and graphical visualizations were generated using GraphPad Prism version 10.2.3 for Mac, GraphPad Software, Boston, Massachusetts, USA (www.graphpad.com). A p-value <0.05 was considered statistically significant.

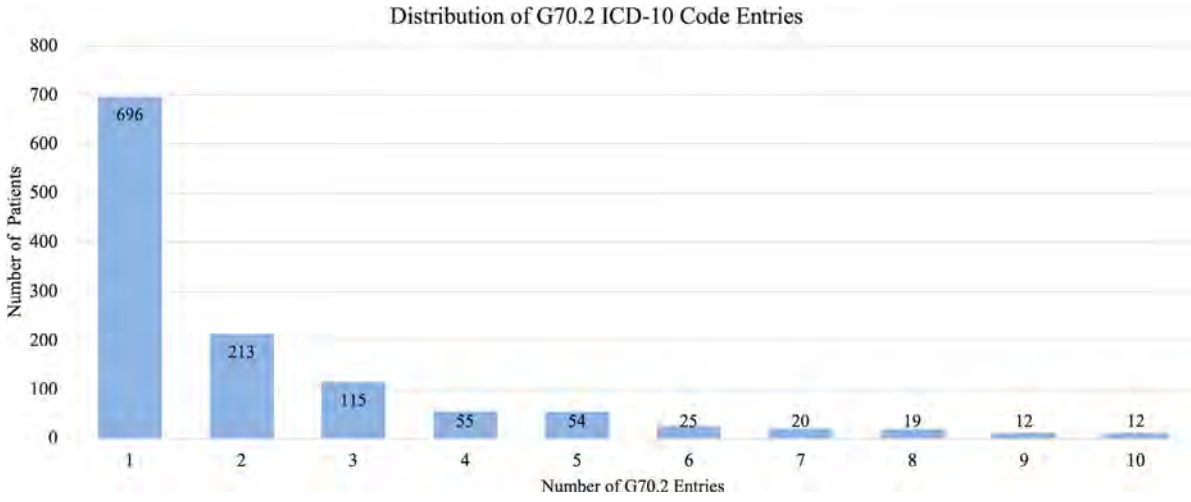

FIGURE 1. The graph showing the number of patients according to the number of G70.2 code entries.
ICD: International classification of diseases.

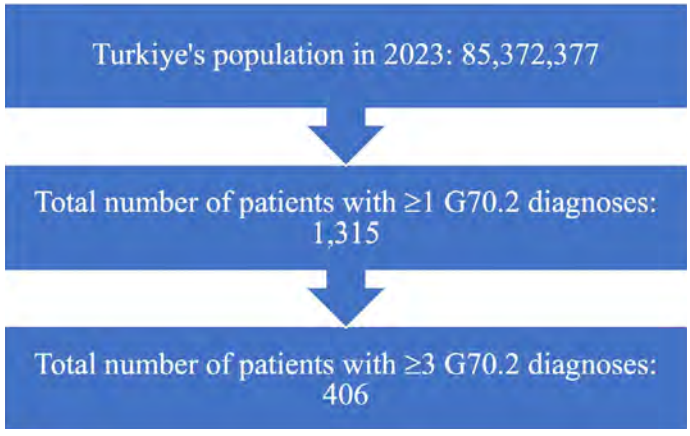

FIGURE 2. Study flowchart.

Ethical Approval

This study was conducted in line with the principles of the Declaration of Helsinki. Ethical approval was obtained from both the Republic of Turkiye Ministry of Health and University of Gulhane ethics committee (approval number and date: 2024-365, 28/06/2024). The requirement for informed consent was waived due to the retrospective nature of the study.

RESULTS

A comprehensive search of the RTMH database revealed 1,315 individuals with at least one G70.2 code entry. Among these, 406 individuals (198 females, 208 males) had ≥3 G70.2 entries and were thus included in the study cohort (Fig. 2). The female-to-male ratio was 0.95, indicating no notable sex predominance. The mean age at the diagnosis was 20.59±21.65 years (median: 12.00, min–max: 0–86). Of these, 238 patients (58.6%) received a diagnosis before the age of 18, and 50 (12.3%) were diagnosed before one year of age. Demographical characteristics and age at the time of initial diagnosis are presented in Table 1 and Figure 3.

Regional differences in diagnostic age were observed, with the lowest mean age at diagnosis reported in the Southeastern Anatolia region and the highest in the Mediterranean region (Fig. 4). Pyridostigmine was prescribed at least once to 277 patients (68.2%) overall. Subgroup analyses revealed prescription rates of 58.4% (n=139) among pediatric patients and 82.1% (n=138) among adults.

For nationwide prevalence calculation, individuals diagnosed in 2024 and those who had died prior to 2023 were excluded. We excluded three additional cases due to missing provincial data during regional prevalence calculations.

Between 2016 and 2023, the annual incidence of CMS in the general population ranged from 0.28 to 0.59 cases per million, while incidence in the pediatric population varied between 0.63 and 1.35 per million. The peak incidence occurred in 2017, with the lowest rate observed in 2023. As expected, incidence rates were consistently higher in children compared to the total population (Fig. 5a, b). The prevalence of CMS in 2023 was calculated as 4.49 per million in the general population, 10.00 per million in children, and 2.55 per million in

TABLE 1. Classification of the patients according to the age at the diagnosis

	Female		Male		Total	
	n	Age at diagnosis Mean±SD (Median, Min–Max)	n	Age at diagnosis Mean±SD (Median, Min–Max)	n	Age at diagnosis Mean±SD (Median, Min–Max)
Children	125	5.73±4.63 (5.00, 0–16)	113	6.27±4.77 (5.00, 0–17)	238	5.99±4.67 (5.00, 0–17)
Adults	73	39.11±17.88 (38.00, 18–86)	95	42.96±20.19 (38.00, 18–86)	168	41.29±19.26 (38.00, 18–86)
All population	198	18.04±19.77 (10.00, 0–86)	208	23.03±23.09 (13.50, 0–86)	406	20.59±21.65 (12.00, 0–86)

SD: Standard deviation; Min: Minimum; Max: Maximum.

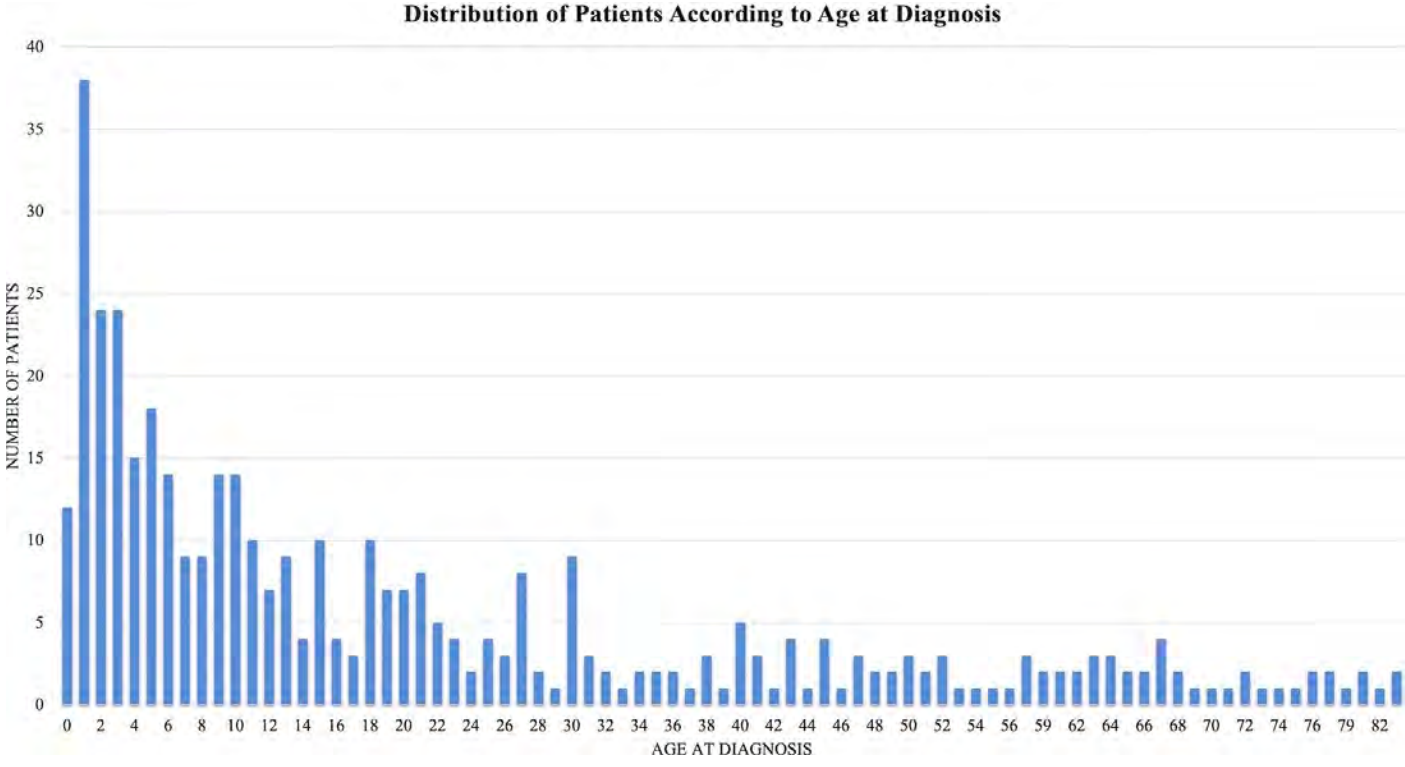

FIGURE 3. The graph showing the distribution of the patients according to the age at the initial diagnosis.

adults (Table 2). The cities with the highest number of patients were Istanbul, Izmir and Ankara, respectively. When examined by geographical regions, the Aegean region exhibited the highest prevalence rate, while the Black Sea region reported the lowest (Fig. 6).

DISCUSSION

CMS are exceedingly rare disorders, and their exact global prevalence remains undetermined. However, several epidemiological studies from different countries have reported prevalence rates ranging from 2.8 to 22.2 per million in pediatric age group [5–7], and from 1.8 to 3.1 per million in the general population [7–9]. Although several genetically confirmed, institution-based CMS studies have been reported from Turkiye, this study represents the first nationwide epidemiological investigation utilizing national electronic health records. It also constitutes one of the largest population-based studies on CMS globally [2, 5–7, 9].

In 2023, the incidence of CMS in individuals under 18 years of age was 0.63 cases per million, while it was 0.28

Table 2. Prevalence of congenital myasthenic syndromes according to the age groups in 2023

	Female		Male		Total	
	n	Prevalence (per million)	n	Prevalence (per million)	n	Prevalence (per million)
Children	116	10.73	106	9.30	222	10.00
Adults	72	2.26	89	2.84	161	2.55
All population	188	4.41	195	4.56	383	4.49

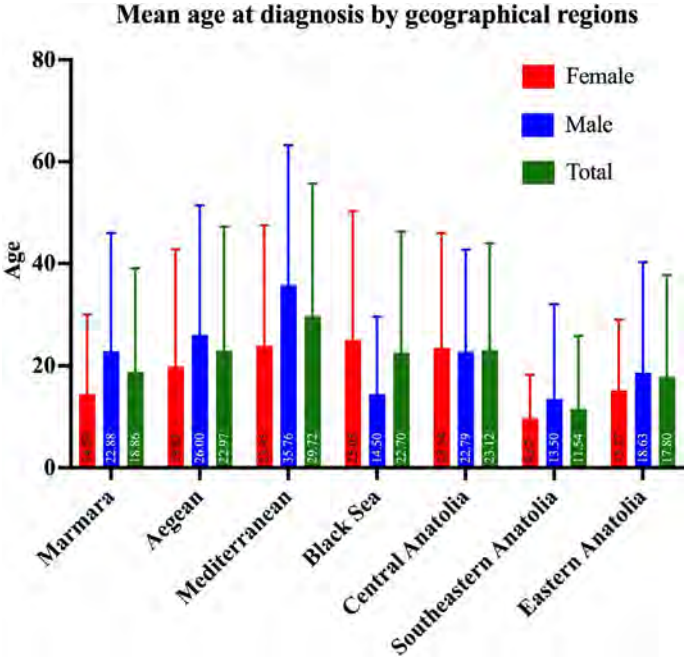

Figure 4. The graph showing the mean age at the initial diagnosis of patients by geographical regions in Turkiye

per million in the overall population. The corresponding prevalence rates were 10.00 per million in children and 4.49 per million in the general population. The pediatric prevalence in Turkiye aligns closely with that of Austria (10.5 per million) [7] and United Kingdom (9.2 per million) [5], but remains significantly lower than that reported in Slovenia (22.2 per million) [6]. For the general population, the prevalence in Turkiye exceeds that of Belgium (3.2 per million) [2], Austria (3.1 per million) [7], Brazil (1.8 per million) [8] and Spain (1.8 per million) [9].

No significant sex-based difference in prevalence was observed in this study, with females comprising 48.8% of the cohort. This is consistent with findings from previous Turkish studies [14, 15], as well as those from the

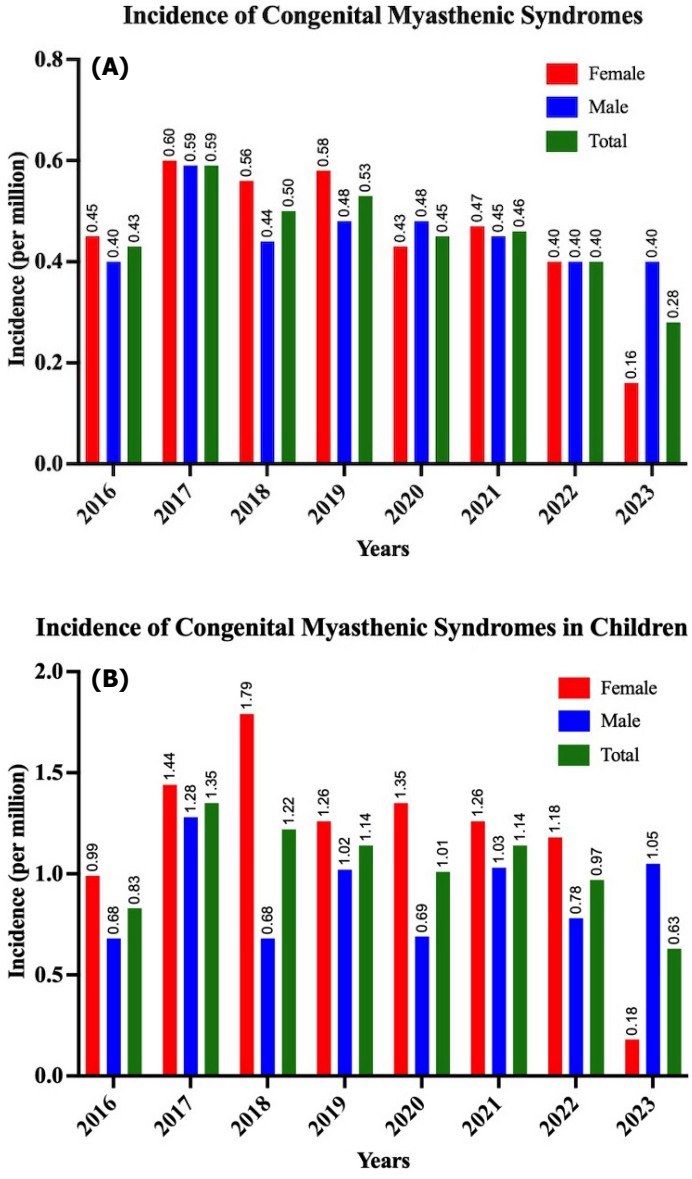

Figure 5. The graphs showing the incidence rates of congenital myasthenic syndromes. **(A)** In all population **(B)** In children.

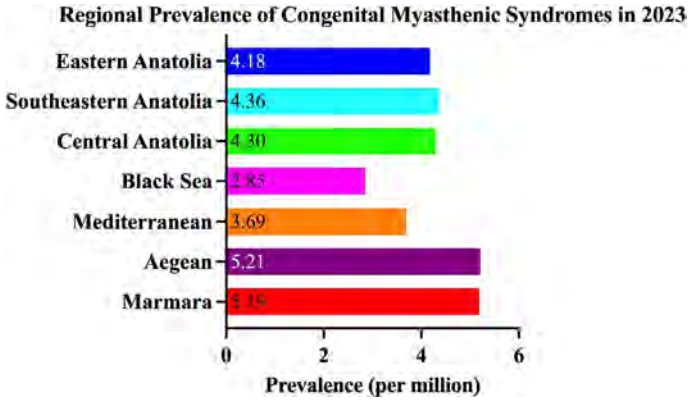

FIGURE 6. The graph showing the prevalence rates of congenital myasthenic syndromes in 2023 by geographical regions in Turkiye.

United Kingdom [5] and Japan [16]. However, other studies have reported a slight female [6–9, 17, 18] or male [10] predominance.

In this study, the mean age at the initial diagnosis was 20.6 years (median: 12), with 58.6% of the patients diagnosed before the age of 18 and 12.3% within the first year of life. In contrast, previous single-center studies from Turkiye reported lower mean diagnostic ages, ranging from 4.8 to 7.8 years [10, 14, 17, 18], and over half of the patients were diagnosed during the first year of life [10, 15]. This discrepancy may be attributed to the nature of those studies, which were conducted in tertiary care centers with a focus on genetically confirmed cases, potentially limiting their generalizability to the broader population. Furthermore, the rate of patients diagnosed before the age of 18 in Belgium (81%) [2], and those diagnosed before the age of one in Austria (50%) [7] were also higher than the rates observed in our cohort.

Although there is no curative treatment for CMS, early diagnosis remains critical, as the majority of patients respond favorably to symptomatic pharmacological treatment [3, 4, 19]. Pyridostigmine, an acetylcholinesterase inhibitor, is the most frequently used treatment and acts by enhancing acetylcholine (Ach) availability at the neuromuscular junction [3, 4, 20]. Pyridostigmine is generally effective in cases of Ach receptor deficiency, fast-channel syndrome, rapsyn deficiency, and glycosylation defects [19]. However, it may worsen symptoms in subtypes such as slow-channel syndrome, collagen Q, DOK7, and muscle-specific kinase [3, 4]. Unfortunately, genetic data were not available in this study. Nevertheless, 68.2% of patients in our cohort received at least one prescription for pyridostigmine, a rate comparable to the Austrian cohort (71.4%) [7], but lower than the rates reported in other studies, which ranged from 83.3 to 100% [2, 6, 16].

This study has several limitations that should be acknowledged. First, the identification of the cases was based solely on G70.2 diagnostic code entries without access to clinical and electrophysiological findings, or genetic test results. Additionally, any diagnoses entered into the registry, preliminary or definitive or for prescription purposes, remain unchanged even if a patient later receives a different final diagnosis. Therefore, to mitigate this limitation and to increase diagnostic accuracy, we applied a conservative inclusion criterion as having at least three G70.2 code entries. However, this approach may have resulted in the exclusion of some recently diagnosed cases.

Conclusion

This study is the first comprehensive nationwide epidemiological analysis of CMS in Turkiye based on national electronic health records. The study enhances the understanding of the epidemiological profile of CMS in the country by reporting the current incidence, prevalence, and pyridostigmine prescription rates and highlight the importance of awareness and early detection, particularly given the impact of CMS on neurodevelopment during childhood. These data are expected to contribute to the development of regional and national health policies and the planning of healthcare services to improve patient care.

Ethics Committee Approval: The Gülhane Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 28.06.2024, number: 2024-365).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study was not funded by any financial or governmental authority.

Use of AI for Writing Assistance: The authors declared that during the preparation of this manuscript, the authors utilized ChatGPT-4.0 to aid in rephrasing complex scientific content for enhanced clarity and readability. The use of AI-assisted tools was limited to language refinement and did not influence the originality or scientific content of the manuscript.

Authorship Contributions: Concept – BI, BO, RS, EE, OK, ETan, ZO; Design – BI, BO, RS, EE, OK, ZO; Supervision – ZO; Materials – NA, SB; Data collection and/or processing – BI, BO, NA, ET, SB; Analysis and/or interpretation – BI, BO, ET, ETan, ZO; Literature review – BI; Writing – BI; Critical review – BO, NA, ET, SB, RS, EE, OK, ETan, ZO.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):475–482
doi: 10.14744/nci.2025.62592

Original Article MICROBIOLOGY

The role of parvovirus B19 infection in frequently ill children

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ABSTRACT

OBJECTIVE: Parvovirus B19 is a very common infection, especially in school-age children, with its rapid spread. In the present study, Parvovirus B19 infection was detected in frequently ill nursery children. The present study aimed to determine the seroprevalence value, to determine whether there is an active infection and to determine to what extent it affects the relevant immune system parameters, to determine whether the obtained data will contribute to the epidemiology of childhood Parvovirus B19 infections in our country and also, to determine whether it is a reason for children to become ill frequently.

METHODS: Parvovirus B19 DNA test results of 112 children aged 2–6 years who were grouped as frequently ill and infrequently ill and who went to nursery and kindergarten were examined quantitatively with the Real-Time PCR Method. Parvovirus B19 IgG and Parvovirus B19 IgM antibody presence was investigated with the ELISA Method. Flow Lymphocyte subgroups were analyzed with the Cytometry Method.

RESULTS: Among the 112 patients who were included in the study, 105 (93.7%) Parvovirus B19 DNA results were negative and 7 (6.3%) were positive. Parvovirus B19 IgG test results were negative in 108 (96.4%) patients and positive in 4 (3.6%). When the Parvovirus B19 IgM results were evaluated, 109 (97.3%) were determined as negative and 3 (2.7%) positive. Natural Killer Cells (NK) from patients with positive Parvovirus B19 DNA, Parvovirus B19 IgG, and IgM were detected outside the normal limit value ranges in CD25, CD19, HLA DR, CD3, CD45RO, and CD8 values.

CONCLUSION: No significant relationships were detected between frequent illness and Parvovirus B19 infection, the infection did not significantly affect the immunodeficiency parameters, and although it is already known that Parvovirus B19 infection peaks every 3–4 years, the study did not coincide with this period of Parvovirus B19 infection.

Keywords: Children; diagnosis; ELISA; flow cytometry; parvovirus B19.

Cite this article as: *Gurkan Kiraz F, Varkal MA, Kirkoyun Uysal H, Demirci M, Soguksu P, Sarsar K, et al. The role of parvovirus B19 infection in frequently ill children. North Clin Istanb 2025;12(4):475–482.*

Human Parvovirus B19 (HPV B19) infection is a common infection on a global scale, with no geographical boundaries or ethnic origins, and is more common in childhood than in adults with adult seroprevalence rates between 70–85% [1]. HPV B19 is a single-stranded DNA virus that multiplies in erythroblasts in the bone marrow. In addition to causing acute infection, it can persist in different cell types throughout life [2].

HPV B19 is a respiratory virus that causes symptoms such as fever, headache, malaise, and myalgia as well as

Received: December 25, 2024 *Revised:* February 20, 2025 *Accepted:* May 22, 2025 *Online:* August 28, 2025

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clinical manifestations such as Erythema Infectiosum (EI) (Fifth Disease, Slapped Cheek Syndrome) and oligoarthritis [3–5]. Infection is more common at the end of winter and the beginning of summer. Seroprevalence might vary between 2–10% in children under five years of age [4]. It has been reported that childhood rashes are frequently detected in schools and nurseries because of the easy spread of the virus, affecting approximately 50% of school children and 20% of school personnel [6, 7].

HPV B19 was first discovered in 1974 in England by Cossart et al. [8], who evaluated the tests for Hepatitis B Virus Surface Antigen (HBsAg) in the sera samples that were taken from 11 individuals and identified a novel virus similar to existing Parvoviruses under electron microscopy, which was described as a Parvovirus-Like Agent. The virus was later named Parvovirus B19 because it was discovered in the B19 well of the microtiter plate in the study. Initially described as a Parvovirus-Like Agent or Human Parvovirus, this virus was called Parvovirus B19 (PV B19) by the International Committee on Taxonomy of Viruses (ICTV) in 1985 when it was identified as a member of the Parvoviridae family [9]. HPV B19 is the only accepted member of the Erythroparvovirus genus that shows tropism with erythroid progenitor cells [10].

The seroprevalence range of IgG antibodies in HPV B19 infection commonly seen in humans is 2–15% in children aged 1–5 years and 15–60% in children aged 6–19 years [1, 11]. This rate is close to 60% in adults and increases with age [12]. Infection is more common in late winter and early spring. The infection rate reaches epidemic levels every 3–4 years, and transmission of the infection to seronegative individuals is quite common during outbreaks. For this reason, an increase in the number of children with EI and transient aplastic crisis may be detected in the community. The recurrence rate of HPV B19 infection outbreaks in school or home environments is approximately 50% in children and approximately 20–30% in teachers [13, 14]. The virus is usually transmitted through close contact. Although the transmission rate is 50% in household contacts, this is 10–60% in children with school or daycare contacts [15, 16].

Virus-specific IgM antibodies are found during the transient aplastic crisis when the reticulocyte count is at its lowest level and IgG is formed during the 10 days after the aplastic crisis [11]. IgM antibody begins to be produced 8–12 days after infection and plays an active roles in clearing viremia. The presence of IgM-type antibodies continues for 3–6 months [5, 17]. The production of IgG antibodies begins a few days after the production of IgM antibodies, after IgM antibodies. Although the level of IgM antibodies decreases after months or weeks, IgG antibodies continue to exist [18]. Virus-specific anti-HPV B19 IgG begins to be produced on the 16th day following the onset of infection, which is evidenced by the emergence of arthralgia and EI, which are partly mediated by the accumulation of antigen-antibody complexes. It is already known that IgG, which is specific to the virus, controls HPV B19 infection and restores erythroid cell production, might be detected in blood samples of individuals for a lifetime and protects the individual against a secondary infection [5, 18, 19]. Itching, rash, and arthralgia can be seen 17–18 days after the infection. The recovery process, which begins with the appearance of an IgM antibody 10–12 days after the infection, coincides with the period when the viral load is at its highest [11]. The humoral immune response occurs in healthy individuals who are infected with HPV B19 [20]. The reticulocyte count is quite low at the time of viremia during which a temporary decrease in hemoglobin level also occurs. Reticulocytes reach normal levels after 7–10 days [21]. With the use of PCR tests, HPV B19 DNA might be detected in blood samples for months or even years from the beginning of the infection in individuals with healthy immune systems, despite the presence of neutralizing antibodies [22–26].

Along with humoral immunity, cellular immunity also contributes to the control of infection [27]. After the infection, Classification determinant 8 (CD8) T lymphocytes, which present antigens and recognize the infected cell-like dendritic cells, are detected on the surface of the infected cell. Once activated, CD8 T cells serve as memory cells for a long time [28]. The activation of CD8 T-Cells against HPV B19 epitopes and their detection for up to 2 years following the infection contributes to the T-Cells keeping pathogenic cells un-

Highlight key points

- No significant relationships were detected between frequent illness and Parvovirus B19.
- Children with Parvovirus B19 infection had lower CD25 levels compared to those without the infection.
- The majority of children in the study (93.7%) tested negative for Parvovirus B19 DNA.

der control for a long time [29]. During the HPV B19 infection period, Classification determinant 36 (CD36) EPCs show cytopathic properties [30]. Although HPV B19 infects various tissues and cells, including erythroid Classification determinant 34 (CD34) cells, synovial tissue, hepatic tissue, myocardial endothelial cells, and tonsil tissue, it does not cause an active viral infection in these cells [31–35].

MATERIALS AND METHODS

The Istanbul University Faculty of Medicine Clinical Research Ethics Committee granted approval for this study (date: 16.01.2022, number: 1411396) and this study was conducted in accordance with the Declaration of Helsinki.

In the present study, HPV B19 DNA presence was quantitatively investigated by Real-Time PCR method, HPV B19 DNA test was done using the Artus Parvo B19 RG PCR (QIAGEN GmbH, Hilden Germany) PCR kit on the ROTOR-GENE Q (QIAGEN GmbH, Hilden Germany) device in a total of 112 pediatric patients aged 2–6 years who applied to the General Pediatrics Clinic of the Department of Child Health and Diseases of Istanbul University, Istanbul Faculty of Medicine, were evaluated as frequently ill and infrequently ill, and who went to nursery and kindergarten in blood samples brought to the laboratory of the Department of Medical Microbiology, Virology and Basic Immunology, Istanbul University, Istanbul Faculty of Medicine. The presence of HPV B19 IgG and HPV B19 IgM antibodies was investigated with the ELISA Method using the SERION ELISA Classic Parvovirus B19 IgG/IgM (Serion GmbH, Würzburg, Germany) kit on the DiaSorin ETI-Max3000 (DiaSorin S.p.A. Saluggia, Italy) Device. Lymphocyte subgroups were analyzed with Flow Cytometry using Navios EX Software (Beckman Coulter, Brea, CA, USA). The data of the children were recorded in the anamnesis form during the interview with the parents. Frequent illness criteria in children were determined as fever over 38 degrees and 5 or more fevers per year.

Data Analysis

The IBM SPSS 21 for Windows (IBM Corp., Armonk, NY, USA) was used to analyze the study data. The p-value obtained was considered significant when it was less than 0.05.

Table 1. Percentage distribution of patients' HPV B19 DNA, IgG, and IgM results according to whether they become ill frequently or not

	Frequently ill (n=58)	Not frequently ill (n=54)	Percentage (%)
HPV B19 DNA			
Positive	3	4	6.3
Negative	55	50	93.7
HPV B19 IgG			
Positive	1	3	96.4
Negative	57	51	3.6
HPV B19 IgM			
Positive	1	2	2.7
Negative	57	52	97.3

DNA: Deoxyribose nucleic acid; HPV B19: Human parvovirus B19; n: Number of patients.

RESULTS

Patients who applied to the General Pediatrics Clinic and were included in the study had applied to the clinic with complaints of cough, fever, runny nose, sore throat, shortness of breath, rash on the body, redness on the cheeks, frequent illnesses, and itching on the face and trunk. Based on the clinical data, 11 of the 112 patients had allergies, 10 had a known disease (heart murmur, asthma, multicystic kidney, hepatosplenomegaly, meningitis 1 year ago, allergic rhinitis), and 14 patients had a history of previous hospitalization.

Among the children who were included in the study, 36 (32.1%) were girls and 76 (67.9%) were boys. Based on the information received from the parents, it was learned that 58 (51.8%) of the children had frequent infections and 54 (48.2%) did not have frequent infections.

When the HPV B19 DNA results of 112 patients were evaluated, 105 (93.7%) were negative and 7 (6.3%) were positive. The percentage distribution of the patients according to sex, whether they had frequent infections or not, HPV, B19 DNA PCR, HPV B19 IgG, and HPV B19 IgM results are given in Table 1.

As seen in Table 1, the analysis of whether there was a significant difference between the frequently ill and not frequently ill groups in terms of HPV B19 DNA positivity (Chi-Square) could not be done because of the in-

Table 2. The percentage distribution of those who had HPV B19 infection (n=112)

	Percentage
Negative	91.1
Positive	8.9
Total	100.0

HPV B19: Human parvovirus B19; n: Number of patients.

Table 3. The number of those who had HPV B19 infection according to the frequency of becoming ill

Frequent illness	Negative	Positive	Total
Yes	49	5	54
No	53	5	58
Total	112	10	112

HPV B19: Human parvovirus B19.

sufficient number of cases. The analysis of whether there was a significant difference between the frequently ill and not frequently ill groups in terms of HPV B19 IgG positivity (Chi-Square) could not be done because of the insufficient number of cases. The analysis of whether there is a significant difference between the frequently ill and not frequently ill groups in terms of HPV B19 IgM positivity (Chi-Square) could not be done because of the insufficient number of cases.

Patients with positive HPV B19 DNA, HPV B19 IgG, and HPV B19 IgM values were defined as those with HPV B19 infection. Information about these patients is given in Tables 2 and 3.

In the evaluation made by using the Chi-Square Test for children who were positive for any of the HPV B19 DNA, HPV B19 IgG, and HPV B19 IgM values (who had HPV B19 infection) and separated according to whether they had frequent infections or not, no statistically significant differences were detected (p=0.9). In this context, the fact that the children had the HPV B19 virus at any point in their lives did not cause them to have frequent infections.

Among the 112 patients, 105 (93.7%) had negative HPV B19 DNA results and 7 (6.3%) had positive HPV B19 DNA. When the HPV B19 IgG results of 112 patients were evaluated, 108 (96.4%) were negative and 4 (3.6%) were positive. Among the patients with HPV B19 IgG positivity, 3 were male and went to kindergarten and 1 was a female and went to daycare. When the HPV B19 IgM results of 112 patients were evaluated, 109 (97.3%) were negative and 3 (2.7%) were positive. Among the 3 patients with HPV B19 IgM positivity, 2 were male and went to kindergarten and 1 was a female and went to daycare. Only 1 of the 3 patients who had HPV B19 IgM positivity had a history of frequent illness. The female patient who had a positive HPV B19 IgM test and was going to nursery school had positive HPV B19 DNA and HPV B19 IgG tests. Three patients had positive HPV B19 IgG values and positive HPV B19 DNA. Two of these three patients were attending kindergarten and one was attending nursery school. Two of those who went to kindergarten were boys and the one who went to nursery school was a girl.

Lymphocyte subgroups of 112 patients were examined. The results outside the normal ranges were detected in natural killer cells (NK), Classification determinant 25 (CD25), Classification determinant 19 (CD19), Human leukocyte antigen DR isotype (HLA DR), Classification determinant 3 (CD3), Classification determinant 45RO (CD45RO), and Classification determinant 8 (CD8) values of patients with positive HPV B19 DNA, HPV B19 IgM, and IgG. These results are detailed in Tables 4, 5 and 6.

The Independent Samples T-test was used for frequently ill and not ill groups and no significant differences were detected between the ages. Frequent illness was not associated with age (p=0.33). The mean CD25 value of frequently ill children was 1.84±0.75 IU/mL. The mean CD25 value of children who do not frequently become ill was 1.94±0.76 IU/mL. No significant differences were detected between the groups in terms of CD25 (p=0.23). The mean IgG value in children who become ill frequently was 957.57±254.993 IU/mL. The mean IgG value in children who do not frequently become ill was 971.71±204.723 IU/mL. No significant differences were detected between the groups in terms of IgG (p=0.37). The mean IgM value among children who became ill frequently was 117.591±49.28 IU/mL. The mean IgM value among children who did not become ill frequently was 123.806±37.67 IU/mL. No significant difference was observed between the groups in terms of IgM (p=0.23).

Table 4. HPV B19 DNA-positive patients' lymphocyte subgroup results

	Sex	Frequent illness	NK (10–20)	CD25 (2–6)	CD19 (9–25)	HLA DR (18–38)	CD3 (50–75)	CD45RO (16–38)	CD8 (15–35)
P1	Male	No		1	27				
P2	Male	No				17			
P3	Female	No	3	1					
P4	Male	Yes	29			9			42
P5	Male	Yes		1		17			
P6	Female	No	2	1		17		13	
P7	Female	Yes		1					

NK: Natural killer cells; CD: Classification determinant; HLA DR: Human leukocyte antigen-DR isotype; HPV B19: Human parvovirus B19.

Table 5. HPV B19 IgM-positive patients' lymphocyte subgroup results

	Sex	Frequent illness	NK (10–20)	CD25 (2–6)	CD19 (9–25)	HLA DR (18–38)	CD3 (50–75)	CD45RO (16–38)	CD8 (15–35)
P6	Female	No	2	1		17		13	
P8	Male	Yes	6	1					42
P9	Male	No	7	1			76		

NK: Natural killer cells; CD: Classification determinant; HLA DR: Human leukocyte antigen-DR isotype; HPV B19: Human parvovirus B19.

Table 6. HPV B19 IgG-positive patients' lymphocyte subgroup results

	Sex	Frequent illness	NK (10–20)	CD25 (2–6)	CD19 (9–25)	HLA DR (18–38)	CD3 (50–75)	CD45RO (16–38)	CD8 (15–35)
P1	Male	No		1	27				
P2	Male	No				17			
P6	Female	No	2	1		17		13	
P10	Male	Yes	7				77	40	

NK: Natural killer cells; CD: Classification determinant; HLA DR: Human leukocyte antigen-DR isotype; HPV B19: Human parvovirus B19.

Based on the T-test results of the groups that had and did not have HPV B19 infection, the CD25 average of children with HPV B19 infection is 1.30±0.48 IU/mL. The CD25 average of children without HPV B19 infection is 1.95±0.75 IU/mL. In this context, the CD25 value was observed to be lower in children with HPV B19 infection than in children without HPV B19 infection. This difference was statistically significant ($p<0.05$).

DISCUSSION

In the present study, HPV B19 DNA was quantitatively evaluated in 112 patients by using the RT-PCR Method. A total of 112 patients were classified according to whether they became ill frequently and whether they went to nursery or kindergarten. HPV B19 DNA was detected as positive in seven of these patients (6.3%). One of the seven patients who were detected as HPV

B19 DNA positive went to nursery, and two went to nursery and became ill frequently. Only three (5.1%) of the 58 patients who became ill frequently had HPV B19. Since the number of cases in which HPV B19 DNA was detected between the patient groups who became ill frequently and those who did not become ill statistically was not suitable for the Chi-Square Test, this test could not be used.

In the present study, when the HPV B19 IgG and HPV B19 IgM test results of 112 patients were evaluated, only four (3.5%) had positive HPV B19 IgG, and three patients (2.6%) had positive HPV B19 IgM tests and only one patient (0.9%) had positive HPV B19 IgM and HPV B19 IgG at the same time. The seroprevalence rate of HPV B19 IgG antibodies (3.5%) in the present study was consistent with the literature data when age ranges were also taken into account [1, 11].

One of the four patients with positive HPV B19 IgG was a male who was frequently ill and went to kindergarten, one was a female who was not frequently ill and went to kindergarten, and the other two patients were male patients who were not frequently ill. Only one of the 60 patients who were frequently ill was positive for HPV B19 IgG (1.6%) and had had the infection before. Since the number of HPV B19 IgG positive cases between the patient groups who were frequently ill and those who were not, statistically, was not suitable for the Chi-Square Test, this test could not be used.

One of the 3 patients with positive HPV B19 IgM was a female patient who was frequently ill, attending kindergarten, the other two were male, one was a male patient who was frequently ill and attending nursery school, and the remaining male patient was a male patient who was not frequently ill and attending nursery school. Only one of the 60 patients with frequent illness was positive for HPV B19 IgM (1.6%) and was in the early stages of infection. Since the number of HPV B19 IgM-positive cases between the patient groups with frequent illness and those without illness was statistically inappropriate for the Chi-Square Test, this test could not be used.

Lymphocyte subgroups of 112 patients were evaluated by Flow Cytometry. The results of CD25 values in 7 patients with positive HPV B19 DNA were evaluated and the CD25 values of patients with HPV B19 infection were significantly lower than those without ($p<0.05$).

In a previous study that was conducted by Türk Dağı et al. [36] to determine the presence of HPV B19 IgG in children aged 0–17, they aimed to determine HPV B19 seroprevalence in children and healthy blood donors using the ELISA test. They found the seroprevalence rate to be 20.7% in children aged 0–17 and 36% in adults aged 18–60. They found the HPV B19 IgG seropositivity rate of pediatric patients aged 0–4 to be 15.8%. In this context, they suggested that the reason why the seroprevalence rate in children aged 0–4 was higher than in studies conducted both in the world and in Turkey and the rates in other age groups were lower was because of population differences.

In the study that was conducted by Vilibick-Cavlek et al. [37] in Croatia between 2010–2021, they aimed to determine HPV B19 IgG and IgM antibodies from blood samples taken from 1538 patients and to analyze the seroprevalence value of HPV B19 infection. They found the rate of HPV B19 IgG antibodies in the participants as 64.1%. They found the HPV B19 IgG positivity rate in children aged 6 months to 9 years as 30% and in adolescent and child patients as 42.4%. They found the HPV B19 IgM positivity rate as 4% in the youngest age groups. They also reported that the seroprevalence value increased with age.

In their study, Bouafsoun et al. [38] aimed to evaluate the seroprevalence of HPV B19 IgG and HPV B19 IgM antibodies in 257 children aged 7–15 years with fever and rash. They found the seroprevalence rates of HPV B19 IgG antibodies to be 38.2% in children aged 19 months-4 years and 53.5% in children aged 4.5–9 years. They found the seroprevalence rates of HPV B19 IgM antibodies to be 7.9% in children aged 19 months-4 years and 17% in children aged 4.5–9 years. They suggested that viral infection is acquired in early childhood and that the seroprevalence value increases with age. We believe that the reason why the HPV B19 IgG positivity rate was found to be 3.5% and the HPV B19 IgM positivity rate was found to be 2.6% in the present study is that they have not encountered the HPV B19 virus before or recently, the pediatric patients are young, and families and children do not come into contact with many people because of the isolation measures and mask use because of the pandemic we have recently experienced, which prevents the transmission of HPV B19 infection.

In their study conducted between 2006 and 2009, Exindari et al. [39] aimed to determine the epidemiological and clinical characteristics of HPV B19 infections seen

in the northern region of Greece. In their study in 2006, only three out of 17 patients (17.6%) and in 2007, 16 out of 29 patients (55.2%) were diagnosed with acute infection with HPV B19 DNA and HPV B19 IgM antibodies. In 2009, they determined this rate as 14.3%. In their study, they observed that the incidence of HPV B19 varies annually. We believe that the reason why HPV B19 IgM antibodies were detected in only three out of 112 pediatric patients (2.7%) in the present study is because of the attention paid to protective measures during the pandemic process and the small number of cases.

The present study suggests that children with HPV B19 infection may be prone to transmitting the infection if their CD25 value is significantly low. However, multicenter studies with a larger number of cases are needed to strengthen the level of evidence.

Isa et al. [40] made an immunological examination of individuals with acute HPV B19 infection to explain the persistence of the infection and to determine whether they suffered from immune deficiency. They detected low NK and CD19 cell levels in some patients. However, they could not reach conclusions indicating a general immune deficiency disorder in the patients as a result of this evaluation. In the present study, NK values were detected to be low in five patients with HPV B19 infection. When the literature studies conducted so far were reviewed, no literature study related to HPV B19 infection was found in children who became ill frequently and children who did not become ill frequently.

Conclusion

The present study was conducted to determine the seroprevalence of HPV B19 in children in kindergartens and nurseries who became ill frequently, to evaluate whether the virus was already present, and to determine whether this virus caused immune deficiency disorder. However, because of the single-center nature of the present study, the small number of cases, and the narrowness of our case group, limited data were obtained as a result of the study.

In conclusion, no significant relationships were detected between frequent illness and HPV B19 infection, the infection did not significantly affect the immune deficiency parameters, and although it is already known that HPV B19 infection peaks every 3–4 years, the study did not coincide with this period of HPV B19 infection. However, we believe that conducting studies with a large number of cases on the subject will contribute to obtaining better data to improve the results obtained.

Ethics Committee Approval: The Istanbul University Faculty of Medicine Clinical Research Ethics Committee granted approval for this study (date: 16.01.2022, number: 1411396).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The author declared that artificial intelligence-supported technologies were not used in this study.

Authorship Contributions: Concept – FGK, MAV, MO; Design – FGK, MAV, HKU; Supervision – MO, AA; Fundings – FGK, MO; Materials – PS, FGK, KS; Data collection and/or processing – FGK, MO, MAV, HKU, PS, AA, KS; Analysis and/or interpretation – FGK, MO, MAV, HKU, PS, AA, MD, KS; Literature review – FGK, MO, MAV, HKU, PS, AA; Writing – FGK, MAV, HKU, MO; Critical review – FGK, MO, MAV, HKU, PS, AA, MD.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):483–489
doi: 10.14744/nci.2024.76390

Original Article DERMATOLOGY

Antihistamines and omalizumab combination treatment in patients with chronic spontaneous urticaria: Real-world experience from a tertiary care hospital

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ABSTRACT

OBJECTIVE: Chronic spontaneous urticaria is characterized by recurrent hives and/or angioedema that persists for more than six weeks, with unknown triggers. This study aimed to gather and analyze real-world data from adult patients diagnosed with chronic spontaneous urticaria who were receiving omalizumab treatment.

METHODS: This retrospective observational study included adults who received omalizumab between September 2022 and February 2024.

RESULTS: A total of 64 patients were included in the study, with a mean age of 44.3 years. Among them, 40 (62.5%) were female, and 24 (37.5%) were male. The mean duration of urticaria diagnosis was 46.6 months, with a mean omalizumab use of 23.6 months. Prior to omalizumab treatment, the most commonly used treatments were the highest dose of second-generation antihistamines (60.9%), and combination therapy with antihistamines and oral corticosteroids (31.3%). All patients received omalizumab 300 mg once every four weeks from the start of treatment and continued using antihistamines. No significant correlation was observed between the antihistamine dosage and treatment response (p=0.06). An observed interval extension and/or dose increase was noted in 23.4% of the patients. The mean Urticaria Control Test (UCT) score, weekly Urticaria Activity Score (UAS7), and Dermatology Life Quality Index (DLQI) scores significantly improved from the first visit before omalizumab treatment to the last visit after treatment (all p<0.001). Of the patients, 98.4% responded moderately or above to the treatment, 26.6% responded thoroughly, and 46.9% responded well. Only three patients (3.1%) experienced myalgia as a side effect of omalizumab therapy, with no severe adverse events reported.

CONCLUSION: Combination therapy with antihistamine and omalizumab is a reliable and beneficial therapy for managing chronic spontaneous urticaria.

Keywords: Antihistamine; chronic spontaneous urticaria; omalizumab; treatment.

Cite this article as: *Mentesoglu D, Kurmus GI, Kartal SP. Antihistamines and omalizumab combination treatment in patients with chronic spontaneous urticaria: Real-world experience from a tertiary care hospital. North Clin Istanb 2025;12(4):483–489.*

Urticaria, characterized by symptoms such as wheals and angioedema, is an inflammatory disease that can manifest as acute or chronic (lasting > six weeks) [1]. CSU, a type of chronic urticaria, causes a significant decrease in an individual's quality of life, with unpredictable attacks and unknown triggers [2].

Treatment guidelines recommend a stepwise approach for the treatment of CSU as follows: standard-dose second-generation H1 antihistamines (sgAHs), high-dose sgAHs, standard-dose omalizumab treatment, high-dose omalizumab treatment, and cyclosporine treatment [3]. Omalizumab is recommended for adults and ado-

Received: April 02, 2024 *Accepted:* June 22, 2024 *Online:* August 08, 2025

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lescents with chronic spontaneous urticaria who do not respond to antihistamines. To evaluate its effectiveness, scales such as the Urticaria Activity Score (UAS) (ranging from 0 to 6 points), weekly Urticaria Activity Score (UAS7) (ranging from 0 to 42 points), and Dermatology Quality of Life Index (DLQI) (ranging from 0 to 30 points) were used [4]. The effectiveness of omalizumab can vary depending on the individual. This unpredictability adds to the difficulties encountered indisease management [2].

Data on the use of omalizumab have been available in literature since 2006 [5]. Although the effectiveness and safety of this drug are still being studied, it is generally considered safe. Real-life data are expected to guide physicians in managing challenging patients in clinical practice, particularly those who require higher doses [5]. Furthermore, an increase in real-life data will help resolve the uncertainty surrounding the timing and methods of discontinuing omalizumab treatment [3].

This study aimed to assess the overall effectiveness and side effects of omalizumab in patients with chronic spontaneous urticaria in a real-world clinical setting.

MATERIALS AND METHODS

This study included patients aged ≥18 years who were diagnosed with CSU and were treated with omalizumab at our dermatology outpatient clinic between September 2022 and February 2024. These patients received omalizumab 300 mg at least every four weeks and were evaluated at least one month after the start of treatment. Patients with missing data or poor data reliability were excluded from the study. Approval with the number AEŞH-EK1-2023-050 was received on April 26, 2023, from the Ethics Committee of Ankara Etlik City Hospital. This study was conducted in accordance with the guidelines of the Declaration of Helsinki.

Urticaria severity was assessed using the Urticaria Control Test (UCT) and weekly Urticaria Activity Score (UAS7). The impact on the quality of life was evaluated using the Dermatology Life Quality Index (DLQI). The scores on these scales were compared before the patient's first application of omalizumab and after the last application. The final response to omalizumab treatment was categorized as follows: complete response if there was complete improvement in urticaria, good response if there was a reduction of at least 90%, moderate response if there was a reduction of 30–89%, and poor response if there was a reduction of less than 30%.

Highlight key points

- Omalizumab is used in patients with chronic spontaneous urticaria who do not respond to antihistamine therapy.
- Before starting omalizumab treatment, the most commonly used treatments were the highest dose of second-generation antihistamines alone, and combination therapy with antihistamines and oral corticosteroids.
- Patients receiving omalizumab continued their antihistamine therapy, and no significant relationship was found between antihistamine dose and treatment response.
- Most patients treated with omalizumab showed a moderate or above response to treatment, and no serious side effects were observed during omalizumab treatment.
- Combination therapy with antihistamines and omalizumab can be used as a safe and effective treatment for chronic spontaneous urticaria.

Statistical Analysis

The statistical analysis in this study was conducted using two software tools: JASP (Version 0.18.3.0, Computer Software, Amsterdam, The Netherlands) and Jamovi (Version 2.3.28, Computer Software, Sydney, Australia). Continuous (numeric) variables are presented in tables, which include the mean±standard deviation, median, minimum, and maximum values depending on their distribution. Descriptive statistics were analyzed using cross-tabs and chi-squared tests. Additionally, we performed a paired t-test to compare patients' evaluation scores from their initial and final visits. Categorical variables were summarized as numbers and percentages. We conducted Kolmogorov-Smirnov and Shapiro-Wilk tests to assess the normality of the numerical variables. Statistical significance was set at $p<0.05$.

RESULTS

Of the patients, 62.5% (n=40) were female and 37.5% (n=24) were male. Their ages ranged from 20 to 75 years, with an average of 44.3±13.7 years. However, the mean age at which urticaria first appeared was 46.6±40.7 years. The duration of urticaria ranged from 7 to 198 months, with a mean of 46.6±40.7 months. The average duration of omalizumab use by the patients was found to be 23.6±30.3 months. The mean follow-up period for the patients was 27.0±31.0 months. At the time of admission, the average blood eosinophil % value was 1.74±1.31. Additionally, 40.6% (n=26) of the patients had a history of angioedema and 21.9% (n=14) had elevated total IgE levels (Table 1).

Table 1. Clinical and demographic characteristics of all patients

	Mean±SD	Min–Max	n	%
Age	44.3±13.7	20–75		
Age of onset of urticaria	40.2±13.7	12–71		
Urticaria duration, months	46.6±40.7	7–198		
Omalizumab use duration, months	23.6±30.3	1–192		
Patient follow-up period, months	27.0±31.0	1–192		
Blood eosinophil levels, %	1.74±1.31	0–5.9		
Gender				
Female			40	62.5
Male			24	37.5
Age category, years				
18–65			61	95.3
>65			3	4.7
Urticaria duration category				
6–12 months			4	6.3
12–36 months			30	46.9
3–5 years			11	17.2
>5 years			19	29.7
Increased total IgE			14	21.9
Presence of angioedema			26	40.6

SD: Standard deviation; Min: Minimum; Max: Maximum.

Before starting omalizumab therapy, patients received dapsone, oral corticosteroids (OCS), anti-leukotrienes (ALKs), and second-generation antihistamines (sgAHs). Among patients, the most frequently used treatment was sgAH alone at the maximum dose, accounting for 60.9% (n=39). The second most commonly used treatment was the combination of sgAHs and OCS, which accounted for 31.3% (n=20) of patients. Of the patients treated with omalizumab, 96.9% (n=62) used sgAHs, whereas 3.1% (n=2) used a combination of sgAHs and dapsone. The sgAH doses used by patients receiving omalizumab were as follows: 1X dose (39.1%, n=25), 4X dose (34.4%, n=22), 2X dose (21.9%, n=14), and 3X dose (4.7%, n=3). No significant correlation was observed between the antihistamine dosage and treatment response (p=0.06). During the follow-up period, changes were made to the dose or duration of omalizumab in 23.4% (n=15) of patients. These changes included extended intervals (12.5%, n=8) and increased doses (10.9%, n=7) (Table 2).

Seven patients (10.9%) discontinued the omalizumab treatment. The reasons for discontinuation were transfer to another center (4 patients, 6.3%), completion of treatment (2 patients, 3.1%), and treatment failure (1 patient, 1.6%). Moreover, discontinuation occurred most often before six months. During follow-up, myalgia was observed as a side effect of omalizumab in 4.7% (n=3) of the patients (Table 2).

The final response status of patients to omalizumab treatment, in decreasing order of frequency, was as follows: good response (≥90% reduction, n=30, 46.9%), complete response (complete recovery, n=17, 26.6%), moderate response (30–89% reduction, n=16, 25%), and poor response (<30% reduction, n=1, 1.6%) (Table 2).

During the evaluation of the patients on their first visit before receiving omalizumab treatment and on their last visit after receiving omalizumab treatment, a statistically significant difference was observed in the follow-up assessments of UCT, UAS7, and DLQI scores (all p<0.001) (Table 3). Data on the UCT, UAS7, and DLQI scores for all patients at the first and last visits are presented in Figure 1 and Table 3.

Table 2. Detailed data on the treatment of all patients (n=64)

	%
Previous treatments	
sgAHs (highest dose)	60.9
sgAHs+ OCS	31.3
sgAHs combination	3.1
sgAHs + ALKs	1.6
sgAHs+ Dapsone	1.6
sgAHs + ALKs + OCS	1.6
Concurrent treatments	
sgAHs	96.9
sgAHs + Dapsone	3.1
Use of sgAHs dose	
sgAHs + Dapsone	3.1
1X dose	39.1
2X dose	21.9
3X dose	4.7
4X dose	34.4
OMA dose or duration change	
Present	23.4
Treatment change type	
Extended intervals	12.5
Increasing dose	10.9
Discontinuation of treatment	
Present	10.9
Reasons for discontinuing treatment	
Treatment failure	1.6
Completion of treatment	3.1
Transfer to another center	6.3
Timing of discontinuing the OMA	
<6 months	6.3
6 months – 1 year	1.6
1–2 years	3.1
Omalizumab side effect	
Present	4.7
Absent	95.3
Omalizumab side effect type	
Myalgia	3.1
Arthralgia	–
Headache	–
Regional injection site reaction	–
Other	–
Final response to treatment	
Other	–
Complete response (complete recovery)	26.6
Good response (≥90% reduction)	46.9
Moderate response to treatment (30–89% reduction)	25
Poor response to treatment (<30% reduction)	1.6

sgAHs: Second generation antihistamines; OMA: omalizumab; ALKs: Anti-leukotrienes; OCS: Oral corticosteroids.

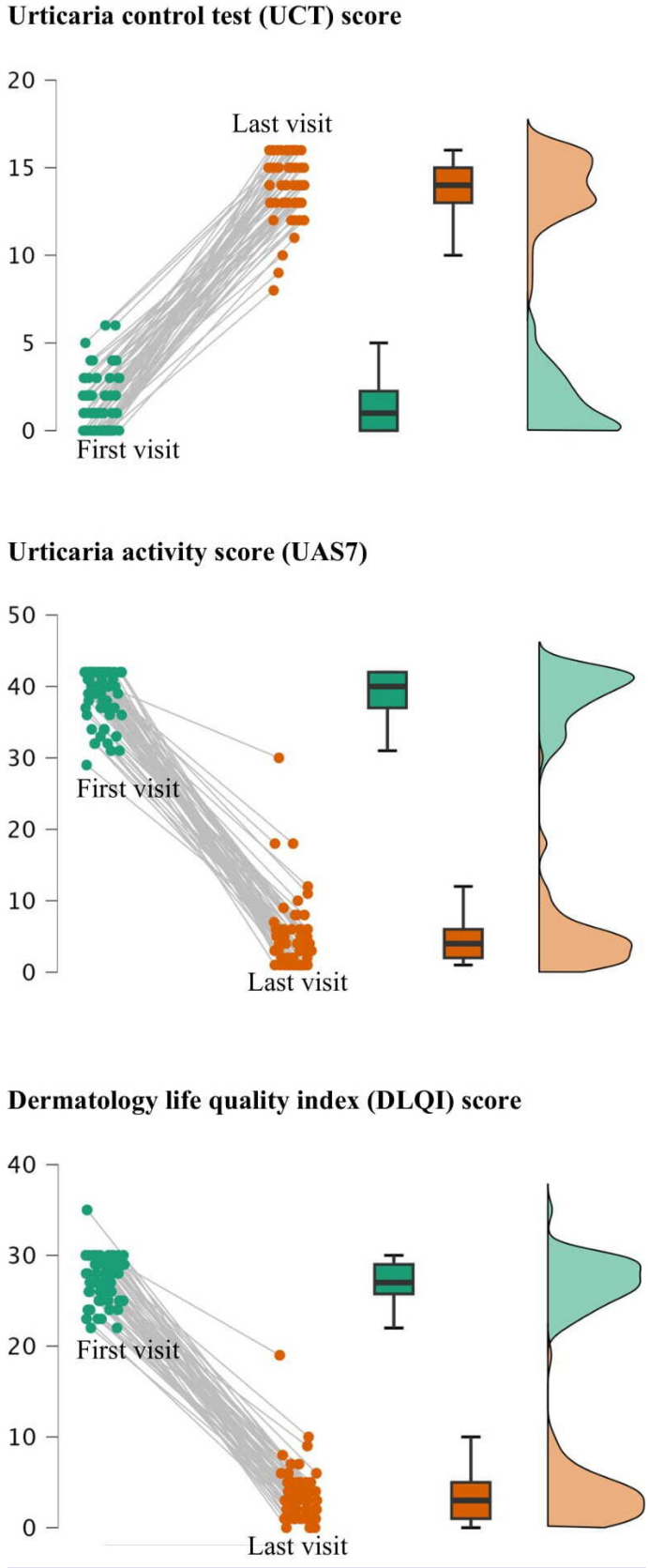

Figure 1. Changes in patients' UCT, UAS7, and DLQI scores at the first and last visit.

TABLE 3. Data on all patients' UCT, UAS7, and DLQI scores at the first and last visit (n=64)

Category, (%)	First visit	Last visit	p
UCT score, mean±SD	1.47± 1.61	13.9±1.76	**<0.001**
0–7	100	–	
8–11	–	7.8	
12–16	–	92.2	
UAS7 score, mean±SD	38.7±3.57	4.86±4.79	**<0.001**
0–6	–	82.8	
7–15	–	12.5	
16–27	–	3.1	
28–42	100	1.6	
DLQI score, mean±SD	27.1±2.45	3.52±3.00	**<0.001**
0–1	–	29.7	
2–5	–	54.7	
6-10	–	14.1	
11–20	–	1.6	
21–30	100	–	

*: Paired t-test; SD: Standard deviation; UCT: Urticaria control test; UAS7: Weekly urticaria activity score; DLQI: Dermatology Life Quality Index.

DISCUSSION

An initial dose of 300 mg, administered once every four weeks, is recommended for treating CSU with omalizumab, according to international guidelines. The current licensed doses for this medicine are 150 mg and 300 mg. A dose of 300 mg has been suggested to result in a faster, stronger, and longer-lasting response [6, 7]. However, the efficacy of omalizumab varies from patient to patient, and there are variations in the rate of recurrence following treatment, despite it being the sole and first known biological agent. Therefore, it is important to remember that patient-based illness and treatment management should serve as the foundation [2]. A study examining 84 publications revealed that approximately 60% of the published research on the use of omalizumab had a starting dose of 300 mg. In approximately 35% of studies, the initial dose was 150 mg. These findings confirm that omalizumab, when administered at the prescribed dosage, is both safe and effective [8]. In our study, we observed that all patients were treated with 300 mg every four weeks, as recommended in the literature.

A recent study by Wang et al. [9] examined 235 patients with CSU treated with omalizumab at a dosage of either 150 or 300 mg. The study found that approximately 96% of patients (n=226) experienced a rapid response to treatment within the first month. Furthermore, approximately 75% (n=180) of patients achieved a complete response to treatment. By the end of the third month, approximately 99% of the patients (n=232) had responded to the treatment. Notably, all age groups showed a positive response to treatment. The study also observed a significant improvement in UAS7 and the DQLI scores upon starting treatment (both $p<0.001$). Only six patients experienced side effects, including injection site reactions (n=3), weight gain (n=2), increased hair loss (n=2), and arthralgia (n=1). In our study, we observed a remarkably high response rate among patients. Specifically, 98.4% of the patients demonstrated a moderate or higher response. Approximately half of the patients experienced a good response, whereas a quarter achieved a complete response. When evaluating treatment response, we noticed a significant difference in the UAS7, UCT, and DLQI scores before and after omalizumab treatment ($p<0.001$ for all three scores) during the last visit. However, unlike this study, the most common side effect observed in patients was myalgia, which occurred in three patients (3.1%).

In 2023, Hide et al. [10] published a study that examined the data of 280 patients who received omalizumab for CSU. Of these patients, 274 were administered a dose of 300 mg, while six received a lower dose. The average duration of omalizumab treatment was 195 days and the mean follow-up period was 330 days. The study observed that over 90% of the patients responded positively to treatment, but approximately 23% experienced relapse after treatment discontinuation. Approximately 4% (n=11) of patients reported drug-related side effects, although none of these events were deemed serious. The patients in this study had an average duration of omalizumab use of 23.6 months, and the average follow-up period was 27.0 months, which was longer than the duration reported in the present study. No significant drug-related adverse effects were observed.

Recommended approaches to treatment during follow-up include reducing the dose and increasing the interval of drug use after patients respond well to treatment with 300 mg every four weeks. It is argued that this treatment management contributes to patients' spontaneous remission in the early period and is cost-effective [11]. This study revealed that 15 individuals experienced

changes in both dosage and duration of treatment during the follow-up period. Almost half of the patients required a longer dosage interval, whereas the rest required an increased dosage. However, no dose reductions were required during the follow-up period.

While guidelines are available for treating chronic urticaria, there is still a lack of clear information about the ongoing use of omalizumab therapy and antihistamines. Currently, there is no consensus on the most effective way to administer antihistamine treatment with omalizumab, including determining the appropriate dosage, duration of treatment, and discontinuation of treatment [12, 13].

A multicenter study conducted in patients undergoing omalizumab treatment suggested that second-generation antihistamines (sgAHs) could be a good choice for symptom management. This study demonstrated that sgAHs were effective in reducing symptoms in patients who responded well to treatment, but were not completely responsive [12]. In our study, we administered sgAH in combination with omalizumab to all the patients. Approximately 40% of patients received the lowest dose of sgAH, whereas approximately 35% received the highest dose of sgAH with omalizumab. However, we found no significant correlation between the AH dosage and treatment response.

Another multicenter study published in 2022 included 298 CSU patients treated with omalizumab. The study found that CSU patients without inducible urticaria who responded well to omalizumab could maintain control of their symptoms even without antihistamines [13]. Another study reported that omalizumab treatment resulted in a rapid response in patients. However, there was no difference in treatment response between omalizumab alone and in combination with antihistamines. Additionally, it has also been suggested that immunomodulatory agents, such as dapsone and colchicine, can be used in certain patients [14]. In our study, we found that dapsone was added to the treatment regimen of two patients who had already received omalizumab and sgAH.

In a study investigating the effects of CSU on patients' mental health, it was found that approximately 41% of the patients exhibited depressive symptoms. Furthermore, those who responded to omalizumab treatment experienced a significant decrease in depression assessment scores [15]. To evaluate the impact of CSU treatment on patients' lives, we used the DLQI. The results indicated a significant difference ($p<0.001$) in the DLQI scores before and after omalizumab treatment, suggesting a positive effect on patients' lives.

The limitations of our study include the relatively small number of patients from a single center, insufficient data on relapse, and lack of detailed comparison data for each visit, except for the first visit before omalizumab treatment and the last visit after omalizumab treatment. We believe that further studies on the use of antihistamines in combination with omalizumab treatment could have a positive impact on reducing the required dose of omalizumab over time and extending the interval between treatments. This could be a significant factor in making the drug more accessible and cost effective for patients.

Conclusion

Our study showed that omalizumab is a reliable and beneficial therapeutic agent for combination therapy with antihistamines for the treatment of CSU.

Ethics Committee Approval: The Ankara Etlik City Hospital Ethics Committee granted approval for this study (date: 26.04.2023, number: AESH-EK1-2023-050).

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used during the writing of this article.

Authorship Contributions: Concept – DM, GIK, SPK; Design – DM, GIK, SPK; Supervision – DM, SPK; Data collection and/or processing – DM, GIK, SPK Analysis and/or interpretation – DM, SPK; Literature review – DM; Writer – DM; Critical Review; DM, SPK.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):490–495
doi: 10.14744/nci.2024.42890

Original Article ORTHOPEDICS & TRAUMATOLOGY

Evaluation of area and volume changes in the costoclavicular region in patients treated nonoperatively after mid-shaft clavicle fracture

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ABSTRACT

OBJECTIVE: The aim of this study is to radiologically compare area and volume changes in the costoclavicular region with the unaffected side in patients treated nonoperatively after unilateral midshaft clavicle fracture and to evaluate functional outcomes.

METHODS: This study included 16 patients (14 males, 2 females) with midshaft clavicle fractures who were admitted between 2017–2018 and union was achieved with conservative methods. Magnetic resonance imaging (MRI) of the shoulder including the costoclavicular region was performed after union. Area and volume calculations of the fractured and unaffected costoclavicular region of the patients were performed on the standard MR sections under the guidance of a specialist radiologist. The Short Version of Disabilities of the Arm, Shoulder and Hand (QDASH) score was used for functional assessment. Range of motion was measured on the affected and unaffected sides at the last follow-up visit.

RESULTS: The mean age of the patients was 30.4±20.8 years (5–69) and the mean follow-up was 8.3±1.3 (6–10) months. The mean shortening was 14.3 mm±8.2 (3–29). The area measurements of the costoclavicular region were divided into 3 levels in axillary section: acromioclavicular joint, mid 1/3 of the clavicle, and sternoclavicular joint level. The median area measurements were 1115 (364–3675) mm^2, 1495 (365–4199) mm^2, and 1201 (197–3812) mm^2 on the unaffected side and 895.5 (351–3670) mm^2, 1098.5 (340–3191) mm^2, and 1037.5 (166–3237) mm^2 on the fractured side, respectively (p=0.905, p=0.491, p=0.888). In volume measurements, the median volumes of the unaffected side and the fractured side were 34.3 (10.7–69.7) mm^3 and 28.9 (8.1–60.9) mm^3, respectively (p=0.268). No significant difference was found in the statistical analysis of area and volume measurements. At the end of the follow-up period, the QDASH score and functional outcome of the patients were good.

CONCLUSION: Conservative treatment of midshaft clavicle fractures did not result in significant area and volume changes in the costoclavicular region. The inability to clinically demonstrate the theoretical expectation of decreased area and volume on the fractured site suggests that other biomechanical factors are involved in the healing process of the human body.

Keywords: Clavicle fracture; conservative treatent; costoclavicular region.

Cite this article as: *Gulnahar K, Okkan M, Buyukmurat N, Karadeniz E. Evaluation of area and volume changes in the costoclavicular region in patients treated nonoperatively after mid-shaft clavicle fracture. North Clin Istanb 2025;12(4):490–495.*

Received: March 21, 2024 *Accepted:* April 17, 2024 *Online:* August 08, 2025

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Clavicle fractures are among the most common problems among skeletal system injuries. Clavicle fractures account for approximately 2.6% to 4% of all fractures. They constitute approximately 44% of fractures around the shoulder [1]. Although various conservative and surgical approaches have been described for the treatment of clavicle fractures, no standard treatment has been established. Regardless of the treatment chosen, the primary goal is to achieve a painless and functional shoulder joint [2]. The aim of clavicle fracture treatment is to minimise deformity and pain at the fracture line while restoring shoulder joint movements to normal levels. Middle third clavicle fractures with less than 20 mm of shortening and intact cortical alignment or displaced fractures with less than 100% displacement can be treated conservatively [3–5] A study found that more than 100% displacement of midshaft clavicle fractures was the strongest radiographic determinant of persistent symptoms and negative sequelae in patients [6]. Many authors who reported non-surgical treatment of clavicle fractures described very satisfactory union rates and functional results [7–9].

In addition to the radiological and functional results of clavicle fractures in both adolescents and adults, biomechanical studies have also been performed [10–14]. Although these studies theoretically suggest that the causative factor is a narrowing in the costoclavicular region, no data have proven this. No biomechanical study has been found in the literature that mentions the area and/or volume change in the costoclavicular region after clavicle fracture.

Considering all the data in the literature, in this study we investigated whether there is an area and/or volume change in the costoclavicular region due to the shortening that occurs after clavicle fracture. We evaluated functional outcomes by radiographic comparison of the fractured side with the unaffected side.

Highlight key points

- Approximately 44% of shoulder fractures are clavicle fractures.
- Although there is a theory that the costoclavicular region may be constricted after a midclavicular fracture, there is no data to support this.
- There is no statistically significant difference in the area and volume of the costoclavicular region in patients treated conservatively after displaced midclavicular fracture.
- The biomechanical factors influenced the radiological and functional results by tolerating the shortening.

MATERIALS AND METHODS

Data Collection

The study was performed following the ethical standards of the Declaration of Helsinki. Ethical approval for the study was obtained from the Institutional Ethics Committee for Clinical Research on 11/07/2018. (No: 2018/227). The study was designed as a prospective cohort study. 16 patients (14 males, 2 females; mean age: 37.4±17.8 years; range, 18 to 69 years) were included in the study. The mean follow-up was 8.3±1.3 months; range, 6 to 10 months. Patients between December 2017 and July 2018, diagnosed with midshaft clavicle fracture and applied eight bandages with closed reduction, and whose last outpatient clinic check was after July 2018 were included. Routine outpatient clinic check-ups of the patients were performed on the 3rd day, 10th day, 1st month, 3rd month, and 8th month. At the 8th month follow-up, the last outpatient visit, informed consent was obtained to perform MRI of the costoclavicular region of the fractured side and the healthy side, including axial, sagittal, and coronal sections. Open fractures, operated clavicle fractures, proximal and distal 1/3 end fractures, patients under 18 years of age were not included in the study.

Radiological and Functional Assessments

Fractures of the middle third of the clavicle were identified by bilateral anteroposterior (AP) shoulder radiographs using the Neer classification [15]. The resulting shortening was measured by comparison with the clavicle on the unaffected side. All patients participated in the final radiologic and functional evaluation. Images were obtained on a 3T MRI scanner (Philips, Einthoven). Sequences and parameters acquired during scanning: coronal T1 TSE TR:543, TE:28, slice thickness 3 mm, slice spacing 3 mm, coronal STIR TR4150 msec, TE 30 msec, slice thickness: 3 mm, slice spacing 3 mm, coronal T2 TSE TR 3828 msec, TE 120 msec, slice thickness: 3 mm, slice spacing 3 mm, axial T1 TSE TR 665 msec, TE 15 msec, slice thickness: 3 mm, slice spacing 3 mm, axial T2 TSE EPI TR 5242 msec, TE 100, slice thickness: 3 mm, slice spacing taken as 3 mm. The average scanning time was 25 minutes. Measurements were performed on the Vital Vitrea workstation by evaluating the axial T1 TSE and axial T2 TSE EPI sequences of the MRI images acquired during the scans. Measurements were performed by a radiologist experienced in area and volume measurements.

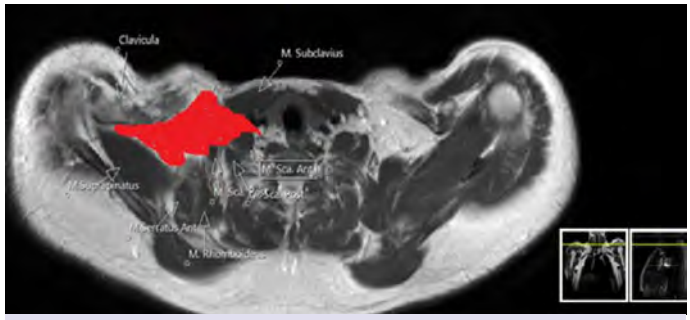

Figure 1. Comparative view of area 1 (colored in red). The proximal part starts at the level of the acromioclavicular joint and the yellow line indicates the corresponding cross-sectional position of area 1 in both the coronal and sagittal planes.

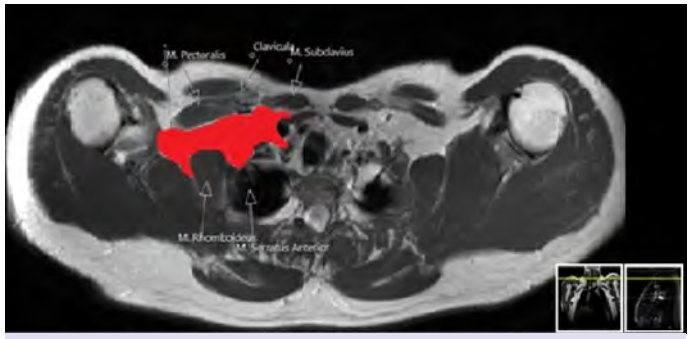

Figure 2. Comparative axial view of area 2 (colored in red). The proximal part starts at the the level of the middle 1/3 of the clavicle and the yellow line indicates the corresponding cross-sectional position of area 2 in both coronal and sagittal planes.

Area measurements were made bilaterally from cranial to caudal, with area 1 at the level of the acromioclavicular joint, area 2 at the mid-part of the clavicle, and area 3 at the most caudal level of the sternoclavicular joint. Measurements were made using axial T1 TSE images. Area 1 was measured by calculating the area bounded anteriorly by the acromioclavicular joint and clavicle, subclavius muscle; posteriorly by the scalenius posterior, rhomboideus, and serratus anterior muscles; laterally by the supraspinatus; and medially by the scalenius anterior muscle. (Fig. 1). Area 2 was measured based on the area bounded by the clavicle, pectoralis major and minor, subclavius muscles anteriorly; serratus posterior and rhomboideus muscles posteriorly; supraspinatus muscle laterally; serratus anterior muscle and trachea medially (Fig. 2). Area 3 was calculated by measuring the area bounded by the sternoclavicular joint, clavicle, and pectoralis major and minor muscles anteriorly, the supraspinatus muscles posteriorly and laterally, the intercostal muscles posteriorly, and the intercostal muscles, ribs, and lung apex medially (Fig. 3).

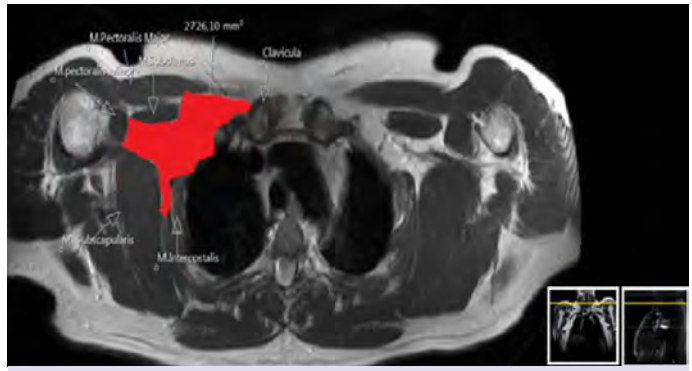

Figure 3. Axial view of area 3 (colored in red). The proximal part starts at the level of the sternoclavicular joint and the yellow line indicates the corresponding cross-sectional position of area 3 in both the coronal and sagittal planes.

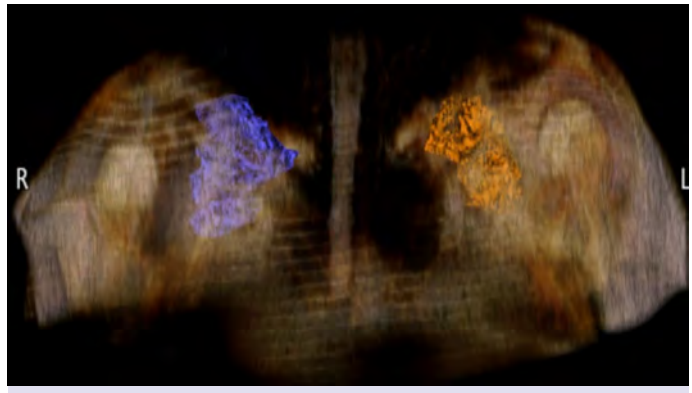

Figure 4. Three-dimensional image of the volume calculation (blue for the unaffected side, orange for the fractured side).

Volume measurements were taken bilaterally from cranial to caudal. This volume was bounded anteriorly by the clavicle, subclavius, pectoralis major and minor muscles; posteriorly by the scalenius posterior, rhomboideus, serratus anterior, supraspinatus muscles; laterally by the supraspinatus muscle; and medially by the scalenius anterior, serratus anterior muscles, ribs, and lung apex. Cranial measurements were taken with the acromioclavicular joint level of the clavicle as the upper limit and the sternoclavicular joint level as the lower limit (Fig. 4).

The Quick DASH score and joint range of motion were measured to assess functional outcomes. Quick DASH is a shortened version of the DASH Outcome Score and uses 11 items from the questionnaire to measure function and symptoms in patients with any upper extremity disorder [16]. At the end of the survey, patients receive a score between 0 and 100 (0 = no disability, 100 = maximum disability).

Statistical Analysis

Statistical analysis was performed using the IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA). The normal distribution of the data was assessed using the Shapiro-Wilk test. Numerical variables with a normal distribution were presented as mean±standard deviation, while those with a non-normal distribution were presented as median (25^{th}–75^{th} percentiles). Categorical variables were expressed as frequencies (percentages). Differences between groups for numerical variables with a normal distribution were determined using the Student's t-test, and for those without a normal distribution, the Mann-Whitney U test was used. A significance level of $p<0.05$ was considered sufficient for testing two-tailed hypotheses.

RESULTS

Complete union was detected at the end of the 3^{rd} month in all patients who participated in the study. Of the patients, 9 had a shortening of 20 mm or more and 7 had a shortening of less than 20 mm. The mean shortening was 14.3±8.2 mm; range, 18 to 69 mm. At the end of treatment, the Quick DASH score was 15.6±6.4 points; range, 21 to 8 points. In one patient, physical examination at month 8 revealed limitations in flexion (30 degrees), extension (20 degrees), and abduction (40 degrees). These findings were considered suboptimal due to an additional rotator cuff rupture in this particular patient. No joint range of motion limitations were noted in the other patients at their last follow-up visits.

In the measurements of area 1, that is, measurements at the level of the acromioclavicular joint, the median value was 1115 (364–3675) mm^2 on the unaffected side and 895.5 (351–3670) mm^2 on the fractured side. The statistical comparison revealed no significant difference (p=0.905).

In the measurements of area 2, that is, at the middle 1/3 level of the clavicle, the median value was 1495 (365–4199) mm^2 on the unaffected side and 1098.5 (340–3191) mm^2 on the fractured site. The comparison revealed no statistically significant difference (p=0.491).

In the measurements made at the level of the sternoclavicular joint, the median value of area 3 was 1201 (197–3812) mm^2 on the unaffected side and 1037.5 (166–3237) mm^2 on the fractured side. There was no significant difference in the statistical comparison (p=0.888) (Table 1).

Table 1. Measurements of the area and volume of the costoclavicular region on the affected and unaffected sides

Paramaters	Affected side	Unaffected side	p
Area 1	895.5	1115	0.905
Area 2	1098.5	1495.5	0.491
Area 3	1201	1037.5	0.888
Volume	34.3	28.9	0.268

P<0.05: Statistically significant.

In volume measurements, the median value on the unaffected side was 34.3 (10.7–69.7) mm^3, while on the fractured side it was 28.9 (8.1–60.9) mm^3, which showed no statistically significant difference (p=0.268).

DISCUSSION

Previous studies have attributed brachial plexus paralysis rarely observed after clavicle fractures to causes such as malunion, hypertrophic callus, or pseudoaneurysm of the subclavian artery or vein [17]. Malunion has been identified as the main cause of the rare vascular and neurogenic thoracic outlet syndrome following these fractures [18–22]. While these studies are mostly case reports and lack biomechanical investigations. When previous biomechanical studies were analysed, changes in the scapulothoracic joint angle after clavicle fracture were measured, and the changes in that angle were observed to be more prominent in the conservative treatment group than in the surgery group [10]. It has been demonstrated how the load on the glenoid and anteversion changes in patients with shortness, affecting the kinetics of the glenohumeral joint and scapula [11–13]. It has been shown that malunion that develops after fracture affects radiological and functional results by causing glenoid malposition [14]. Our study is, to our knowledge, the first to measure area and volume in the costoclavicular region after clavicle shortening and present it along with functional outcomes.

The definition of the costoclavicular region has not been established in previous studies. By measuring the area and volume of this region, we have defined markers that will be informative for future studies. The costoclavicular space is a triangular space bounded anteriorly by the medial portion of the clavicle and the underlying subclavian muscle, its tendon, and the

costocoracoid ligament. It is bounded posteromedially by the first rib and the insertion of the anterior and middle scalene muscles, and posterolaterally by the superior border of the scapula [23]. The area of a triangle is found by dividing the multiplication of the height and sides by 2. Considering the clavicle as one side of the triangle, each 1 cm of shortening at the edge will mathematically result in an average 15–20% reduction in area and volume.

Clavicular fractures are managed based on severity, fracture site, and associated neurovascular injury. The most common fracture site is the middle third of the clavicle (approximately 80%) [24], and this region is prone to displacement. The majority of these fractures are treated conservatively with figure-of-eight bandages or arm slings. In general, most of these fractures heal completely and the rate of nonunion is very low (less than 1%) [25]. An initial shortening of the clavicle by ≥20 mm is considered to be a risk factor for nonunion [26]. The findings of Wick et al. [27] support the notion that fractures with ≥20 mm shortening predispose to nonunion. A 2012 meta-analysis found a nonunion rate of 15% in conservatively treated midshaft clavicular fractures [28]. In our study, complete healing was achieved in all patients, including those with an initial shortening of ≥20 mm.

Hill et al. [29], in their evaluation of post-treatment shortening in clavicle fractures, reported that shortening of ≥20 mm was associated with poor symptomatic and functional outcomes only in healed fractures. Oroko et al. [30], in their study of 41 patients after fracture union, found that three patients with more than 15 mm of shortening had worse functional outcomes. However, they concluded that shoulder function was not affected by shortening. In our study, we found good functional outcomes in all patients, including those with initial shortening of ≥20 mm.

In a study by Mırzatolooeı, the average DASH score for clavicle fractures treated surgically and conservatively was reported to be 8.6 and 21.3, respectively [31]. In another study comparing plate osteosynthesis with an arm sling, the DASH score at 1-year follow-up of 132 patients with clavicular fractures treated surgically and conservatively was 5 versus 15 [32]. In a study by Ozler et al. [33], the DASH score for surgically treated patients was 12.8. In our study, the Quick DASH score yielded results consistent with the literature and did not exceed the scores reported in other studies.

Considering the limiting factors of our study, although we reached 52 patients within 1 year, the exclusion of those, who accounted for the majority of fractures, significantly reduced our sample size. Another aspect of our study that could be criticized is that measurements of the costoclavicular region of the clavicle, in terms of area and volume, were performed after the fractured clavicle had healed. If we could have assessed changes in area and volume immediately after the initial fracture in the early period and performed further examinations for changes in area and volume after union, or if we could have extended the follow-up period to observe ongoing remodeling after fracture healing, the reliability of the results would have been further increased.

Conclusion

In this study, which we conducted to contribute to the literature, despite the geometric reduction observed in the area and volume of the costoclavicular region in patients with displaced midclavicular fractures treated conservatively, no statistically significant difference was found in this study. This suggests that biomechanical factors in this region may tolerate shortening and influence radiologic and functional outcomes.

Ethics Committee Approval: The Kocaeli University Faculty of Medicine Clinical Research Ethics Committee granted approval for this study (date: 11.07.2018, number: 2018/227).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: Not declared.

Authorship Contributions: Concept – KG, MO, NB, EK; Design – KG, MO, NB, EK; Supervision – KG, MO, NB, EK; Fundings – KG, MO; Materials – KG, MO; Data collection and/or processing – KG, MO, EK; Analysis and/or interpretation – KG, MO, NB, EK; Literature review – KG, MO, NB, EK; Writing – KG, MO, EK.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):496–500
doi: 10.14744/nci.2024.98415

Original Article NUCLEAR MEDICINE

Dual-phase images with 18F-FDG PET/CT can exhibit new lesions of colo-rectal cancer

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ABSTRACT

OBJECTIVE: Dual-phase 18F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) has demonstrated superiority over conventional imaging methods in various clinical conditions. However, its efficacy in detecting metastases from colorectal cancer is uncertain. We aim to reveal whether dual-phase FDG-PET/CT can be superior in detecting metastases compared to the standard PET-CT study in patients with an established diagnosis of colorectal cancer.

METHODS: This is a single-center, retrospective case-control study involving 35 patients with colorectal cancer who underwent whole-body FDG PET-CT imaging. Late-phase FDG-PET-CT images were obtained 1–2 hours after the standard technique, emphasizing the identification of new lesions or clarified lesions.

RESULTS: Among the 35 patients evaluated, 5 (14.3%) exhibited new cancer lesions, while 6 (17.1%) demonstrated more evident cancer regions at late-phase FDG-PET-CT. New lesions or more evident cancer regions with the dual-phase technique were described within the liver, in regional lymph nodes, and in peritumoral regions.

CONCLUSION: The study findings suggest that dual-phase FDG-PET-CT can reveal new and more evident metastatic lesions in a subset of colorectal cancer patients. This technique, precious in identifying liver metastases and lymph nodes, enhances the accuracy of colorectal cancer diagnosis and staging.

Keywords: Dual-phase imaging; FDG PET/CT; colorectal cancer.

Cite this article as: *Altun Tuzcu S, Cetin IE, Guzel F, Cetinkaya E, Aday U, Uyar A, Tasdemir B. Dual-phase images with 18F-FDG PET/CT can exhibit new lesions of colo-rectal cancer. North Clin Istanb 2025;12(4):496–500.*

Dual-phase 18F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) involves acquiring PET images at two different time points, allowing for the assessment of dynamic changes in tracer uptake. It is superior to conventional FDG PET/CT and magnetic resonance and computed tomography (CT) imaging, particularly in assessing various clinical conditions such as the evaluation of metastatic lesions in locally advanced or recurrent cervical cancer, the distinction of benign and malignant solitary pulmonary nodules in granuloma-endemic regions with accuracy, and the detection of bone metastasis [1–3].

FDG PET/CT is considered superior to CT in the detection of liver metastases from colorectal cancer, establishing it as the most sensitive noninvasive imaging technique for this purpose [4, 5]. Bipat et al. [4] sug-

Received: January 18, 2024 *Revised:* March 18, 2024 *Accepted:* May 24, 2024 *Online:* August 29, 2025

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gested that FDG PET is the most accurate imaging modality for detecting colorectal liver metastases on both a per-patient and per-lesion basis. Furthermore, PET/CT is a promising technique for evaluating the response to chemotherapy in colorectal and liver metastases [6]. Furthermore, a randomized study has demonstrated that PET/CT can enhance the selection of patients for hepatic surgery in cases with colorectal liver metastases [7].

Studies have demonstrated the utility of dual-phase FDG-PET in the management of colorectal cancer, particularly in the detection of recurrent colorectal carcinoma and colorectal liver metastases [8, 9]. However, the literature is scarce in this regard.

We aim to investigate the detectability of colorectal metastasis with the use of dual-phase FDG-PET/CT in patients with an established diagnosis of colorectal cancer.

Highlight key points

- Dual-phase FDG-PET-CT significantly contributes to cancer detection, showing new cancer regions in 14.3% of patients and more evident cancer regions in 17.1% compared to early-phase FDG-PET-CT.
- Dual-phase PET/CT, incorporating delayed imaging, enhances sensitivity and specificity, particularly in colorectal cancer, by improving lesion detectability and lesion-to-background ratio. This reinforces the potential of dual-phase PET-CT as a robust imaging modality for more accurate colorectal cancer diagnosis and staging.

MATERIALS AND METHODS

This single-center, retrospective case-control study was conducted at Diyarbakir Dicle University Medical Faculty, Department of Nuclear Medicine between 05.2021 and 10.2023. The participants were selected from individuals diagnosed with colorectal cancer who underwent whole-body FDG PET-CT imaging for staging purposes. Dual-phase FDG-PET-CT images were obtained by using the images taken from the abdominal region 1-2 hours after the completion of FDG-PET-CT scans. Special emphasis has been placed on recording new lesions or clarified lesions during this process.

Individuals with a second malignancy or those with a primary liver tumor were excluded from the study.

To obtain FDG PET-CT images, patients were required to fast for more than 6 hours and maintain a blood glucose level of 140 mg/dL. FDG, at a dose of 0.1 mCi/kg, was injected intravenously into the patients. After the injection, patients were kept in a specially lead-coated room for 1 hour to allow the medication to spread throughout the whole body. A CT scan of the total body (from vertex to the knees) was then studied. In the following step, whole-body emission scanning was conducted with PET. The imaging utilized a Siemens Horizon PET/CT device of the 2016 model with 3D-TOF technology. The slice thickness of the device was 3 mm, and the images were generated using PET iterative and CT bp-LOR reconstruction processing methods. The low-dose CT device, employed for anatomical detail and attenuation correction, was set to 80 mA and 120 kV (Siemens Healthcare, GmbH, Henkestrasse 127, 91052 Erlangen, Germany).

This study was carried out in accordance with the Declaration of Helsinki. The consent form is not available since the study is retrospective. The study was carried out with the permission of Dicle University Faculty of MedicineEthic Committee (date:15.03.2023, decision no: 91).

Statıstıcal Analysis

We conducted data analysis employing the statistical software package (SPSS for Windows version 17.0, IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed through the Kolmogorov-Smirnov test and examination of histograms. Descriptive statistics were expressed as mean ± standard deviation for continuous variables and as percentages for categorical variables. Chi-Square test was utilized for comparing categorical variables. Additionally, a logistic regression model was employed to explore the impact of gender on the acquisition of dual-phase PET-CT. Two-tailed p-values less than 0.05 were deemed statistically significant, with a 95% confidence interval.

RESULTS

A total of 35 patients with a diagnosis of colo-rectal cancer were evaluated. Thirteen of those (37.1%) were male, and the remaining 22 (62.9%) participants were female. The mean age was 59.80±16.18. Ten patients had a history of cancer surgery. In 5 patients, new cancer regions were detected at dual phase PET-CT and in 6 patients, the current lessions became more evident. Demogrophical and clinical features of the patients were given in Table 1.

Table 1. The basic clinical features of the patients were given

Age, years	59.80±16.18
Gender, male/female, n (%)	13 (37.1%) / 22 (62.9%)
Cancer surgery, yes/no, n (%)	10 (28.6%) / 25 (71.4%)
New lessions at late phase PET-CT, yes/no, n (%)	5 (14.3%) / 30 (85.7%)
Lessions became more evident at late phase PET-CT, yes/no, n (%)	6 (17.1%) / 29 (82.9)

PET-CT: Positron emission tomography/computed tomography.

Table 2. New cancer regions (cancer regions which was absent at the early phase of PET-CT) and regions which became more evident at the late phase of PET-CT were presented

	Age, gender	Event	Site	Measurements: Suv_{Max}, Suv_{Mean}, MTV, TLG
Case 1	71, female	New lession	Liver segment 3	5.42/1.74/1.47/4.80
Case 2	87, female	New lession	Periportal LN	3.1/1.93/2.69/5.25
Case 3	41, female	New lession	Conglomerate precaval LN	5.6/2.2/2.03/6.9
Case 4	93, female	New lession	Liver segment 7	7.5/3.35/2.54/10.27
Case 5	47, male	New lession	Peritumoral LN	3.6/1.63/0.46/0.81
Case 6	87, female	Evident	Periportal LN activity	1.51/1.11/1.3/2.55 (early) 3.1/1.93/2.69/5.25 (late)
Case 7	56, female	Evident	Liver segment 4	6.65/1.7/2.08/7.28 (early) 8.98/1.54/0.91/4.79 (late)
Case 8	45, female	Evident	Liver segment	13.67/8.04/17.31/147.53 (early) 23.32/10.05/13/176.64 (late)
Case 9	66, female	Evident	Paraaortic LN	3.5/2.2/0.3/0.8 (early) 6.3/4.4/0.3/1.2 (late)
Case 10	45, female	Evident	Peripankreatic LN	5.2/3.06/1.22/3.7 (early) 6.1/2.7/0.81/3 (late)
Case 11	68, male	Evident	Right internal iliac LN	1.5/0.79/1.02/0.97 (early) 2.5/1.47/2.03/2.95 (late)

PET-CT: Positron emission tomography/computed tomography; LN: Lymph node; Suv: Standard uptake value; MTV: Metabolic tumor volume; TLG: Total lesion glycolysis.

Table 2 represents the cases with new cancer regions and more evident cancer regions compared to first images at PET-CT. Female participants demonstrated a higher rate of new cancer lesions at the late phase of PET-CT and more evident cancer regions compared to the images obtained at the early phase of PET-CT (p=0.032 and p=0.019, respectively). A logistic regression model demonstrated female gender has an impact on new lesion determination and more evident cancer region in dual phase PET-CT (p=0.007, OR: 1.54 and CI 95% [0.265–26.861] and p=0.016, OR: 1.214 and CI 95% [0.364–34.185], respectively).

DISCUSSION

The present study demonstrated that dual-phase FDG-PET-CT exhibits new cancer regions in 14.3% of patients and more evident cancer regions in 17.1% of patients compared to the early-phase FDG-PET-CT.

In the management of individuals with cancer, the utilization of positron emission tomography (PET) using 18F-fluorodeoxyglucose (FDG) is an essential diagnostic tool. Its pivotal role extends to tumor staging and restaging, as well as the early and final assessment of treatment response. Furthermore, PET plays a crucial role in discerning between radiotherapy-induced scar tissue and disease relapse, a recognition that has become widespread within the medical community [10, 11]. Consequently, the incorporation of hybrid imaging not only enhances PET accuracy but notably augments PET specificity [11]. This technological synergy underscores the potential for more nuanced and accurate diagnostic information, contributing to enhanced clinical decision-making in the care of cancer patients. While PET/CT is a valuable diagnostic tool in cancer management, its sensitivity has certain limitations. These limitations encompass the biological characteristics of tumors, including low glucose retention or metabolism observed in specific neoplasms such as carcinoid, bronchoalveolar carcinoma, prostate cancer, and hepatocellular carcinoma [9, 12–14]. Furthermore, missed assessments in PET-CT can result from additional factors such as variations in serum blood levels of glucose and insulin, tumor dimensions, and a low lesion-to-background ratio in organs with inherently high background activity levels [9, 15, 16].

There are various techniques that aim to overcome the mentioned false negative assessments. The assessment of the acquisition of FDG in delayed images of PET-CT may be one approach. Kubota et al. [17] reported that primary lung cancer, metastatic mediastinal lymph nodes and lymphoma lesions exhibit a more detectable FDG uptake at 2h that at 1h of PET-CT. A recent study conducted by Boanova et al. [18] revealed that dual-phase PET/CT scan can improves the sensitivity and specificity of colorectal liver metastasis compared to the early images (100% vs 87.7% and 91.0% vs 94.0%, respectively), especially in smaller liver lesions <5 cm^3. Arene at al. reported PET scan of 95 consecutive patients with suspected liver metastases and demonstrated a notable reduction in Standardized Uptake Value mean (SUVmean) values in the background, accompanied by a concomitant increase in SUVmean values of the lesion itself. This dynamic interplay resulted in a significant improvement in the lesion-to-background ratio, highlighting the potential of delayed imaging to uncover pertinent information that may not be apparent in standard PET scans. This approach introduces a valuable adjunct to standard PET protocols, offering an enhanced diagnostic perspective and potentially improving the overall efficacy of PET imaging in clinical settings [9]. The findings of this study are suggestive for previous study; 5 (14.3%) new and 6 (17.1%) more evident metastatic lesion were demonstrated by dual-phase PET/CT. Interestingly, delayed acquisition was more prominent among females. However, in this small cohort, the female gender has dominancy and this result may be related to limited case numbers.

Previous studies have concentrated on evaluating the efficacy of dual-phase PET/CT in detecting delayed acquisition in diverse organs, including the liver, lungs, and regional lymph nodes [19–21]. This current study corroborates these findings, indicating that dual-phase PET-CT can indeed deliver a more precise assessment of colorectal cancer. Specifically, it proves valuable in the identification of liver metastases and lymph nodes, reaffirming the potential of dual-phase PET-CT as a robust imaging modality for enhancing the accuracy of colorectal cancer diagnosis and staging.

Limitations

The study encompasses a relatively small cohort of 35 patients, potentially affecting the generalizability of the findings. The retrospective nature of the study introduces inherent limitations, including the potential for selection bias and the reliance on existing data. The study notes a dominance of female participants, and while logistic regression accounts for this, the imbalanced gender distribution may introduce confounding variables and limit the generalizability of gender-specific findings. Additionally, this study lacks a control group for comparison, making it challenging to delineate the specific contributions of dual-phase FDG-PET-CT in comparison to standard imaging protocols. The study primarily focuses on the detection of new and more evident lesions without extensive follow-up data on the clinical impact of these findings. Long-term outcomes and correlations with patient management would provide a more comprehensive perspective. While emphasizing the benefits of dual-phase FDG-PET-CT, the study does not directly compare its performance with other imaging modalities such as conventional PET/CT or magnetic resonance imaging (MRI), which could provide additional insights into its superiority. The study relies on the interpretation of images for lesion assessment, and subjective interpretations may introduce variability. Objective measures or a blinded review could enhance the objectivity of lesion evaluations.

Conclusion

This study underscores the potential of dual-phase FDG-PET/CT in enhancing the assessment of colorectal cancer, particularly in detecting new lesions and rendering existing lesions more evident. Despite the limitations, including a modest sample size and a retrospective design, the findings align with previous research on the efficacy of dual-phase imaging. The study emphasizes the need for further exploration with larger cohorts, prospective designs, and comparisons with other imaging modalities to validate the clinical impact of dual-phase FDG-PET/CT in colorectal cancer management. Nevertheless, the observed improvements in lesion detection highlight the promising role of this imaging approach in refining diagnostic accuracy and guiding clinical decision-making for patients with colorectal cancer.

Ethics Committee Approval: The Dicle University Non-Interventional Clinical Research Ethics Committee granted approval for this study (date: 15.02.2023, number: 91).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: AI-assisted Technologies (chatGPT) tested the study's clarity and linguistic structures. However, the article's final version was accepted and submitted on the authors' oversight.

Authorship Contributions: Concept – SAT Design – SAT, BT, UA; Supervision – SAT, IEC; Fundings – SAT; Materials – IEC, FG, EC; Data collection and/or processing – IEC, FG, EC; Analysis and/or interpretation – SAT, BT; Literature review – IEC, AUyar; Writing – SAT, BT; Critical review – SAT, UA.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):501–508
doi: 10.14744/nci.2024.93284

Original Article ANESTHESIOLOGY & REANIMATION

Intubation bundle: A prospective observational tertiary cancer centre study of clinical practice and adverse events of tracheal intubation out of operation theatre

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ABSTRACT

OBJECTIVE: Tracheal intubation out-of-operation theatre has a higher risk than intubation inside the theatre, and studies on this topic are sparse. Safety interventions during tracheal intubation can reduce adverse events. This study aims to assess current practices, compliance with tracheal intubation bundle guidelines, and the incidence of adverse events during out-of-operating-theatre intubations in our hospital.

METHODS: A prospective observational study was conducted over a 6-month period on all tracheal intubations occurring outside the operating theatre. Data were collected through discussions with the anaesthesia duty team and review of hospital records, using a standardized proforma based on intubation bundle guidelines and adverse events. The variables were summarized using counts and percentages.

RESULTS: Thirty-two patients required out-of-operating-theatre tracheal intubation, with the most common indication being respiratory failure in 13 (40.6%) cases. Airway assessment was performed in 21 (65.6%) cases, and nil per oral status was confirmed in 26 (81.3%) cases. Role planning by the team leader occurred in 27 (84.4%) cases. Fluid loading was administered in 24 (75%) cases, Ryle's tube aspiration in 29 (90.6%) cases, and pre-oxygenation in 30 (93.8%) cases. Rapid sequence induction was used in 26 (81.3%) cases, with first-attempt endotracheal tube placement in 22 (68.8%) cases, aided by a stylet in 21 (65.6%) cases. Capnography was not used in 29 (90.6%) cases to confirm intubation. Alternative airway securing methods (supraglottic airway) were present in 29 (90.6%) cases. Overall, 13 patients (40.6%) experienced adverse events during tracheal intubation.

CONCLUSION: Adverse airway events can be decreased by adhering to the intubation bundle, and staff training should be provided for effective implementation of guidelines. The use of a stylet as an intubation aid helps achieve successful first-attempt intubation and should be incorporated into the bundle. Capnography should be routinely used to confirm endotracheal tube placement.

Keywords: Airway; checklist; intubation bundle; out-of-operation theatre; tracheal intubation.

Cite this article as: *Gladston DV, Pillai VS, Krishna JKM. Intubation bundle: A prospective observational tertiary cancer centre study of clinical practice and adverse events of tracheal intubation out of operation theatre. North Clin Istanb 2025;12(4):501–508.*

Tracheal intubations out-of-operation theatre are commonly performed to secure the airway during emergencies or patient resuscitations. These situations are unpredictable, occur in suboptimal conditions and are often performed without senior supervision, particularly during odd hours. Such intubations are frequently associated with higher risks compared to those conducted within the operating theatre, due to the lack of immediate access to difficult airway equipment, expert teams, and efficient monitoring post-intubation [1]. Safety in-

Received: February 14, 2024 *Revised:* March 18, 2024 *Accepted:* July 11, 2025 *Online:* August 06, 2025

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terventions and adherence to tracheal intubation bundle guidelines, can reduce the incidence of adverse events. This study aims to assess the current practice and compliance with these guidelines, and the incidence of adverse events during out-of-operating-theatre intubations at our hospital [2].

MATERIALS AND METHODS

A prospective observational study was conducted on out-of-operating-theatre tracheal intubations at a tertiary care cancer centre from July to December 2021. The study followed the Declaration of Helsinki and received Institutional Review Board approval (IRB No: 07/2021/05) on 2nd July 2021. Patients were selected based on inclusion and exclusion criteria, including all out-of-operating-theatre tracheal intubations, excluding pediatric intubations, cardiac arrest intubations, and intubations without the use of drugs (Fig. 1).

All procedures performed in the study are according to the standard practice methods of the institution and treating team. Data were collected through discussions with the anaesthesia duty team and review of hospital records using a standardized proforma based on intubation bundle guidelines, an intubation checklist, and adverse events. The intubation bundle guidelines include 10 components under pre-intubation, during intubation and post-intubation checklist. They are 1) Presence of two operators 2) fluid loading (isotonic saline 500 ml) in absence of cardiogenic pulmonary oedema, 3) Preparation of sedation, 4) Pre-oxygenation 5) Rapid sequence induction: Etomidate 0.2–0.3 mg/kg or ketamine 1.5–3 mg/kg combined with succinylcholine 1–1.5 mg/kg in absence of allergy, hyperkalaemia, severe acidosis, acute or chronic neuromuscular disease, burn patient for more than 48 h and spinal cord trauma 6) Sellick maneuver. 7) Immediate confirmation of tube placement by capnography 8) Norepinephrine if significant hypotension 9) Initiate sedation 10) protective ventilation.

Patient characteristics, location, and indication for intubations were recorded. The checklist covered airway assessment, preoxygenation, Nil Per Oral (NPO) status confirmation, Ryle's tube aspiration, role planning, availability of oxygen source, crash cart, suction apparatus, intravenous access, and fluid preloading. Induction agents, opioids, muscle relaxants, intubation techniques, aids, number of attempts, alternative airways, and tube position confirmation were documented. Adverse events such as death, significant hypotension, significant hypoxemia, dysrhythmia, difficult intubation, dental injury, esophageal intubation, and aspiration of gastric contents were recorded immediately after intubation by the intubating doctor [3].

Highlight key points

- Adverse airway events can be decreased by following the intubation bundle guidelines, with training provided to staff members for effective implementation.
- Stylets should be used as intubating aids to increase first-attempt success rates and should be incorporated into the bundle.
- Capnography should be used routinely to confirm endotracheal tube placement.

Significant hypotension was defined as <20% of baseline systolic arterial pressure; a systolic arterial pressure persistently, 90 mm Hg despite fluid challenge; or a requirement for initiation of vasoactive support [2]. Significant hypoxaemia was defined as a decrease in SpO_2 to lower than 80% [3].

Statistical Analysis

Variables were summarized using counts and percentages. Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Ltd, Chicago, IL).

RESULTS

Thirty-two patients required out-of-operating-theatre tracheal intubation during the 6-month study period with comparable demographic data's (Table 1). The most common indication was respiratory failure in 13 (40.6%) cases (Fig. 2). In accordance with compliance with Intubation bundle; in 21 (65.6%) cases airway assessment was done and NPO status confirmed in 26 (81.3%) prior to intubation. Role planning was assigned by team leader in 27 (84.4%) cases. Fluid loading was given in 24 (75%) cases. Ryle's tube aspiration was done in 29 (90.6%) and pre-oxygenation in 30 (93.8%) cases.

Induction agents, opioids, and neuromuscular blocking agents used were shown in Table 2. Airway was secured in all cases by rapid sequence induction in 26 (81.3%) cases; with endotracheal tube in first attempt 22 (68.8%), with stylet as intubation aid in 21 (65.6%). Capnography was not used in 29 (90.6%) to confirm intubation, instead auscultation was used. Alternative airway securing supraglottic airway was present in 29 (90.6%).

TABLE 1. Patient characteristics and location of intubation

Variable	Frequency (n)	Percent (%)
Age (years)		
0–10	0	0
11–20	0	0
21–30	1	3.1
31–40	4	12.5
41–50	9	28.1
51–60	13	40.6
61–70	4	12.5
71–80	1	3.1
Sex		
Male	15	46.9
Female	17	53.1
Location of intubation		
Surgical ICU	11	34.4
Medical ICU	7	21.9
Surgery ward	5	15.6
Medical ward	4	12.5
Chemo ward	1	3.1
Casualty	3	9.4
Others	1	3.1

ICU: Intensive care unit.

TABLE 2. Intubation bundle (n=32)

Variable	Frequency (n)	Percent (%)
Pre-intubation		
Consent	30	93.8
Airway assessment	21	65.6
NPO status	26	81.3
Role planning	27	84.4
Oxygen source available	32	100.0
Crash cart available	32	100.0
Preoxygenation performed	30	93.8
Intravenous access	32	100.0
Fluid pre-loading	24	75.0
Vasopressor available	32	100.0
Suction available	32	100.0
Ryles tube aspiration	29	90.6
Per-intubation		
Induction agent		
Propofol	6	18.8
Ketamine	8	25.0
Etomidate	2	6.3
Propofol and Ketamine	7	21.9
Etomidate and Ketamine	9	28.1
Opioid		
Fentanyl	32	100.0
Neuromuscular blocking agent		
Succinylcholine	28	87.5
Rapid sequence induction	26	81.3
Intubation aids		
Bougie	5	15.6
Stylet	21	65.6
Number of attempts		
1st attempt	22	68.8
2nd attempt	8	25.0
3rd attempt	2	6.3
Alternative airway available		
Supraglottic airway	29	90.6
Airway secured by		
Endotracheal intubation	32	100.0
Post-intubation		
Endotracheal tube confirmation		
Auscultation	29	90.6
Capnography	3	9.4
Debriefing	25	78.1

NPO: Nil per oral.

Thirteen patients (40.6%) experienced adverse events (Fig. 3) during out of hospital tracheal intubations. Hypotension in 2 (6.3%) cases, aspiration in 2 (6.3%) cases, dysrhythmias in 2 (6.3%) cases, esophageal intubation in 2 (6.3%) cases, hypoxemia in 1 (3.1%) case, difficult intubation in 1 (3.1%) case, dental injury in 1 (3.1%) case, deaths due to hemodynamic instability in 2 (6.3%) cases.

DISCUSSION

In this prospective observational study, 32 tracheal intubations performed outside the operating theatre at a tertiary cancer center were analyzed. The primary indication for intubation was respiratory failure, accounting for 40.6% of cases. The study observed varying levels of compliance with the intubation bundle guidelines, with notable high adherence to pre-oxygenation (93.8%) and role planning (84.4%), but low use of capnography (9.4%) for intubation confirmation. Adverse events were documented in 40.6% of patients, including hypoten-

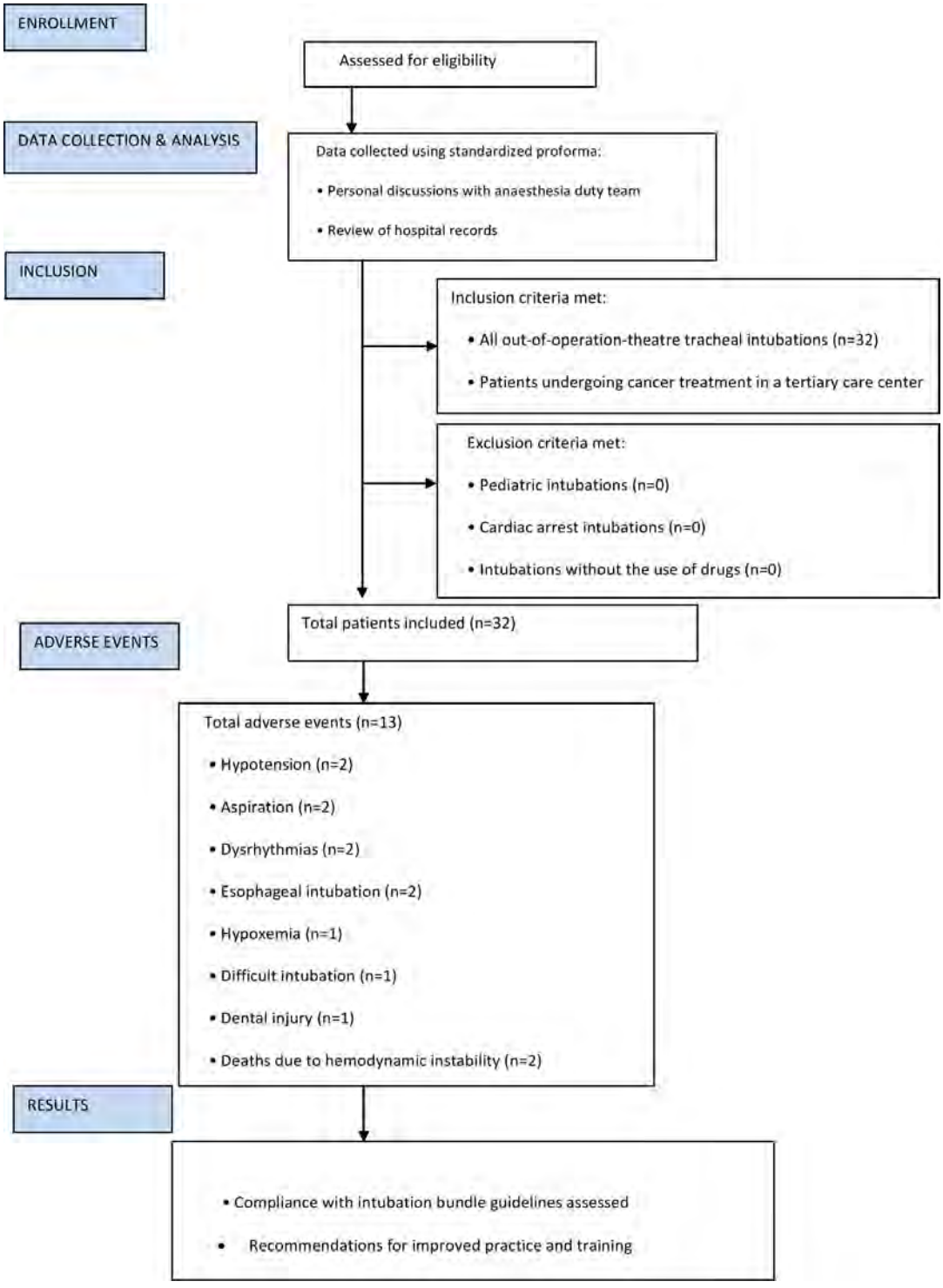

Figure 1. STROBE diagram.

sion, aspiration, dysrhythmias, esophageal intubation, hypoxemia, difficult intubation, and dental injury.

This study confirms that tracheal intubations outside the operating theatre are associated with higher risks due to suboptimal conditions and lack of immediate resources [4]. The high incidence of adverse events (40.6%) underscores the need for strict adherence to intubation bundle guidelines. Most patients in our hospital are undergoing cancer treatment and are immune-compromised. Chemotherapy, radiotherapy, surgery along with other

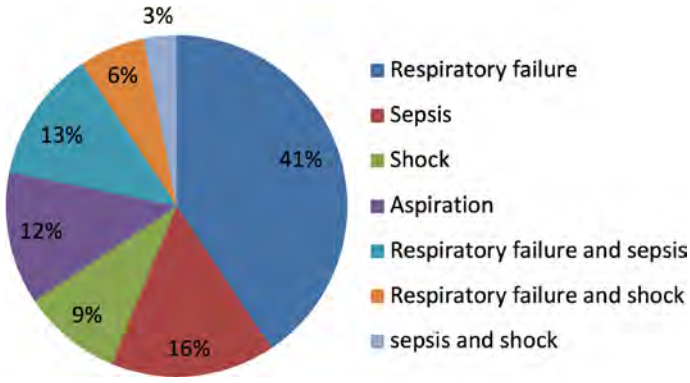

FIGURE 2. Indication for intubation.

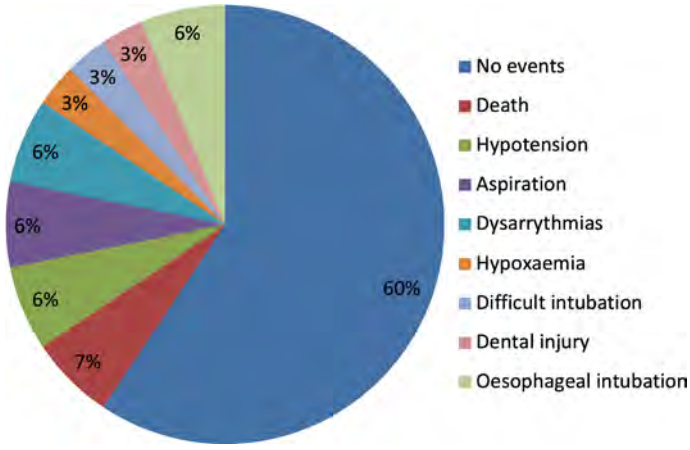

FIGURE 3. Adverse events.

co-morbidities places them at high risk of complications [5]. Head and neck cancers are associated with anticipated difficult airway. Several guidelines were put forward by 4th National Audit Project of the Royal College of Anaesthesiologists [6], Montpellier- ICU intubation algorithm [7, 8], and Difficult Airway Society intubation checklist [9] and it highlight the importance of pre-intubation preparations and role planning [10]. Our findings align with these studies, demonstrating that compliance with bundle guidelines can reduce complications. In this study we assessed the compliance of intubation bundle proposed by Divatia et al. [2, 11].

Pre-intubation variables like airway assessment, NPO status confirmation, role planning by team leader, Ryle's tube aspiration, preloading with fluids, pre-oxygenation and availability of oxygen source, crash cart, suction, and vasopressors can improve the intubation success rate and minimize the adverse effects. Out of 32 cases, 18 intubations were performed in surgical and medical ICUs (34.4% and 21.9%) respectively and during all the intubations there was presence of two operators who are anaesthesiologists and intubation was performed by one of the operators. Respiratory failure, sepsis, shock, aspiration or a combination of these factors were indications for intubation [3].

Preoxygenation aims to increase the duration of the apnea without desaturation, by an increase of the functional residual capacity and the oxygen reserves, thereby reducing the occurrence of hypoxemia [12]. Pre-oxygenation was performed in 30 (93.8%) which has prevented hypoxemia [13]. Role planning was done in 27 cases (84.4%) and rapid sequence induction and sellick maneuvor was performed in 26 (81.3%) since most of the patients are not in NPO status, have abdominal distensions or obstructions, gastroparesis etc. and are at high risk of aspiration. Ryle's tube aspiration was done in 29 cases (90.6%) and rapid sequence induction in 26 (81.3%) which has resulted in no aspiration in 30 cases.

Hemodynamic failure is one of the most severe complications associated with endotracheal intubation in the critically ill patients [14, 15]. The PrePARE study [16] concludes that administration of an intravenous fluid bolus did not decrease the overall incidence of cardiovascular collapse during tracheal intubation of critically ill adults compared with no fluid bolus [16, 17]. Fluid loading with 500 ml crystalloids such as ringer lactate or normal saline was done in 24 cases (75%) in patients without cardiogenic pulmonary oedema. Etomidate and Ketamine are recommended for intubating critically ill patients [10]. In this study; Propofol, Ketamine, Etomidate or a combination of these drugs were used for intubation depending on haemodynamic parameters. But in our study only 2 cases had hypotension, the remaining cases were haemodynamically stable may be due to fluid pre-loading 24 (75%) and the use of ketamine as induction agent in 24 cases. No cases required vasopressors like norepinephrine post-intubation may be due to adequate fluid loading and use of ketamine, etomidate as induction agents. Fentanyl was administered for all cases and neuromuscular blockade with succinylcholine was given for 28 (87.5%) cases [18]. Long term sedation was started in all cases as per guidelines.

The presence of consistent capnography waveforms reinforces tracheal placement of the endotracheal tube [19]. The 2009 Intensive care society recommends [1] the use of capnography for tracheal intubation; however in this audit capnography was used only for 3 (9.4%) cases and is concerning due to immediate non availability outside operation theatre. Auscultation was

done in rest of the cases to confirm endotracheal tube position. For instance, studies conducted in developed countries often report higher compliance with capnography use due to better access to advanced monitoring equipment [2, 20]. In contrast, the low utilization of capnography in this study can be attributed to resource constraints and limited availability of equipment, common challenges in resource-limited settings.

First- intubation attempt success is associated with fewer complications related to intubation [20]. As the number of intubation attempts increases, there is high chance of mucosal injury, bleeding, airway oedema, poor visibility of vocal cords and hence results in difficulty in securing airway leading to catastrophes like hypoxia, hypoxic brain injury or even cardiac arrest [2]. The study done by Russotto et al. [21] found that the incidence of major adverse intubation events was significantly lower with first-pass intubation success. In this study the availability of intubation aids improved successful intubation in first attempt in difficult situations. Intubation with stylet was performed in 21 (65.6%) and bougie in 5 (15.6%) cases respectively. Out of 22 (68.8%) cases which were intubated in first attempt stylet was used in 21 (65.6%) cases which resulted in safe secure of airway. In STYLETO study, a multicentre randomised controlled trial, conducted in 32 intensive care units, among 999 critically ill adults undergoing tracheal intubation, using a stylet improves first-attempt intubation success (78.2%) [22]. The use of stylet as intubating aid during first attempt of intubation can be recommended as it increases the chance of successful intubation. The availability of alternative airway such as supraglottic airway is life saving in difficulty intubation scenarios and unfamiliar locations. This can avoid unnecessary surgical airway manipulations like tracheostomies.

The International Observational Study to Understand the Impact and Best Practices of Airway Management in Critically Ill Patients (INTUBE) study which was an international, multicenter, prospective cohort study involving consecutive critically ill patients from a convenience sample of 197 sites across 29 countries, concluded that cardiovascular instability-were observed frequently peri-intubation, which has lead to mortality and morbidity [21]. In the study conducted by Natesh et al. [23] states that a high incidence of complications (50%), with severe cardiovascular collapse being the commonest during intubation and the importance of vasopressors during intubation. In this audit, 19 (59.4%) patients had no adverse events and were successfully intubated. Hypotension, aspiration, dysarrhythmias and oesophageal intubations were present in 2 patients each (6.3%) respectively and hypoxaemia, difficult intubation and dental injury in rest of patients (3.1%) respectively. Hypotension was managed with fluid boluses and vasopressors; patients who had aspirations were taken care with lung protection ventilator strategies. Dysarrhythmias, especially tachycardia subsided immediately and was managed with sedatives. Oesophageal intubations were reintubated on second attempt with the aid of stylet and airway secured. Hypoxemic episode was transient are saturation was normal immediately after intubation. Patients who had difficult intubation was intubated by the second operator with stylet and airway was secured. Dental injuries were minor and occurred mainly due to loose teeth and misalignment of dentition. All adverse events were adequately managed due to effective team work and role planning. Two death cases were reported as adverse event due to haemodynamic instability, terminally ill and poor general condition of patient. Debriefing was carried out in 25 out of operation theatre scenarios to assess and improve intubation technique and prevent further complications. The incidence of adverse events in this study (40.6%) is higher than the rates reported in some international studies, which typically range from 20–30% [24]. This discrepancy could be due to differences in patient populations, with cancer patients possibly having more complex health issues and higher baseline risks. Additionally, the lack of senior supervision and suboptimal conditions during off-peak hours in this study might contribute to the higher adverse event rate.

The combination of a limited physiologic reserve in the critically ill patients and the potential for difficult mask ventilation and intubation mandates careful planning and justifies the use of an guideline based approach to tracheal intubation [12, 25]. Intubation bundle guidelines when followed in reliable manner, improves patient outcome in difficult scenarios [26, 27].

Conclusion

The study on tracheal intubations conducted outside the operating theatre at a tertiary cancer center highlights several critical insights into clinical practices and the occurrence of adverse events. The findings underscore the significant role of adherence to intubation bundle guidelines in minimizing complications during these high-risk procedures. Adherence to intubation bundle guidelines can significantly reduce adverse airway events.

Recommendations for further improvement are regular training for staff on intubation bundle guidelines; incorporation of stylet on first attempt of intubation; routine use of capnography for verifying tube placement. Policies for the resuscitation of terminally ill patients should be reviewed to avoid unnecessary intubations. This study provides a foundation for future improvements in clinical practice and emphasizes the ongoing need for vigilance and adherence to safety protocols in airway management.

Ethics Committee Approval: The Regional Cancer Centre granted approval for this study (date: 02.07.2021, number: 07/2021/05).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: Not declared.

Authorship Contributions: Concept – DVG, VSP; Design – DVG, VSP, JKKM; Supervision – DVG, VSP; Fundings – DVG, VSP; Materials – DVG, VSP; Data collection and/or processing – DVG, VSP, JKKM; Analysis and/or interpretation – DVG, VSP, JKKM; Literature review – DVG, VSP; Writing – DVG; Critical review – JKKM, VSP.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):509–515
doi: 10.14744/nci.2024.49775

Original Article BIOPHYSICS

Effect of metformin on cell proliferation and apoptosis in steatosis HepG2 cell model

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ABSTRACT

OBJECTIVE: Metformin, which is commonly recommended drug for managing type II diabetes, has been reported to have anti-cancer properties and may improve the prognosis of some malignancies. Epidemiology studies have shown improved survival in cancer patients using metformin. However, the mechanism behind this phenomenon remains incompletely understood. In our study, Our objective was to investigate how metformin influences the proliferation and apoptosis of hepatocellular carcinoma cells induced with steatosis via palmitic acid and oleic acid.

METHODS: We established an in vitro cellular model of non-alcoholic fatty liver disease by inducing lipid accumulation in HepG2 cells through the use of oleic acid and palmitic acid. Oil Red O staining was conducted to observe the distribution of intracellular lipid droplets. Cell proliferation were detected using the BrdU cell proliferation detection kit. Protein expressions were detected by western blot method techniques.

RESULTS: We found that metformin reduced cell proliferation in palmitic acid and oleic acid-induced HepG2 cells compared to the control group. Moreover, our western blot data show that metformin treatment changes apoptosis.

CONCLUSION: Our results show that metformin inhibits cell viability of steatosis HepG2 cells. These findings may be preliminary for new studies in steatosis HepG2 cells and may provide new therapeutic targets or treatment strategies against hepatocellular carcinoma.

Keywords: Bax; Bcl-2; cancer; Metformin; oleic acid; palmitic acid.

Cite this article as: *Tanriverdi Bademci AM, Aydin B, Cabadak H. Effect of metformin on cell proliferation and apoptosis in steatosis HepG2 cell model. North Clin Istanb 2025;12(4):509–515.*

Hepatic carcinoma ranks sixth in terms of frequency and fourth in terms of fatality among malignant diseases globally and remains a significant threat to human health [1]. Hepatocellular carcinoma (HCC) constitutes approximately 90% of all liver malignancies. HCC is a predominant histological liver cancer. Important factors of the complex etiology of HCC also include heavy alcohol consumption and non-alcoholic fatty liver disease (NAFLD) [1]. Today, the treatment options used in the treatment of liver cancer are conventional surgical excision, radiofrequency ablation, transcatheter arterial chemoembolization and alternative modalities [2]. Studies on elucidating the molecular mechanism underlying HCC progression are important to identify effective therapeutic targets and indicators that predict relapse.

The liver, which is an important organ in terms of lipid biosynthesis and fatty acid oxidation, contributes to lipid metabolism with these features [3]. Hepatic steatosis is known to have a crucial role in the initiation and progression stages of NAFLD [4]. It is known that triglycerides accumulate in the cytoplasm of hepatocytes in hepatic steatosis, and studies have

Received: March 14, 2024 *Revised:* May 14, 2024 *Accepted:* July 21, 2024 *Online:* August 11, 2025

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found that free fatty acids are found at high levels in the blood serum of patients with NAFLD. Two of these triglycerides, oleic acid (OA) and palmitic acid (PA), have been used to create in vitro cellular models of hepatic steatosis (a cell model of non-alcoholic fatty liver disease). These models are frequently used in studies of lipid uptake, triglyceride synthesis and fatty acid oxidation in hepatic steatosis [5–7].

Apoptosis activation involves the participation of pro-apoptotic factors like Bax and anti-apoptotic factors such as Bcl-2 [8]. Bcl-2 can bind to Bax and inhibit apoptosis. When Bax induces the release of cytochrome c, caspase-3 is activated and facilitates the process of apoptosis [9]. Upregulation of Bax with simultaneous downregulation of Bcl-2 leading to an increase in the Bax/Bcl-2 ratio is an extensively studied parameter in the apoptotic death of cancer cells [10]. Apoptotic signalling pathways are obvious drug targets for targeted different cancer therapies.

Metformin (1,1-dimethylbiguanide hydrochloride) is now mostly used in the treatment of diabetes and is prescribed for type 2 diabetic patients [11]. Metformin, which research has shown to have anti-cancer properties, is an antidiabetic biguanide agent derived from Galega officinalis [12, 13]. Metformin exerts a suppressive impact on cellular proliferation, angiogenesis, epithelial-mesenchymal transition and tumor growth in various cancers [14]. With these properties, metformin can be thought to improve the prognosis of cancer patients and prevent tumor formation.

There are studies showing that metformin suppresses cell growth and triggers programmed cell death in cancer cells through elevated AMP/ATP ratio and activation of AMP-activated protein kinase (AMPK) [15]. Metformin functions as an agonist of AMPK, a serine/threonine kinase [16]. Metformin has been discovered to inhibit complex 1in the mitochondrial electron transport chain [16], which serves as a significant generator of ROS (reactive oxygen species) [17]. Metformin inhibits the respiratory complex I of the electron transport chain (ETC) in the mitochondria and causes a slight electron transport leak, leading to ROS production and a slight decrease in ATP production. Decrease in ATP causes activation of AMPK, which inhibits the mammalian target of rapamycin (mTOR) pathway and leads to a decrease in cell proliferation. It is known that excessive inhibition of the mTOR pathway can induce apoptosis and cell cycle arrest in the cell [18, 19].

Highlight key points

- Metformin reduces the proliferation of steatosis HepG2 cells.
- Metformin has a stimulatory effect on the apoptosis pathway in steatosis HepG2 cells.
- Metformin treatments in steatosis HepG2 cells may contribute to preventing NAFLD progression to HCC.

The model we created using oleic acid and palmitic acid is an NAFLD steatosis model. HCC and NAFLD are known to be interrelated. Obesity, NAFLD increases the risk of HCC [20]. Studies have shown that NAFLD may act synergistically with other HCC risk factors to accelerate cancer development [21]. NAFLD is recognized as an inducible cause of HCC [22], and nonalcoholic steatohepatitis, a manifestation of NAFLD, can progress to HCC [23]. To study hepatic steatosis in vitro, a cellular hepatic model was established by treating human HepG2 cells with free fatty acids [24].

In this study, we aimed to investigate the effect of metformin on the proliferation of steatosis HepG2 cells, and to identify the possible apoptosis mechanism of the effect of metformin on steatosis HepG2 cells, we measured the expression of cytochrome C, Bax, Bcl-2 and caspase 3 proteins in steatosis HepG2 cells. This research was purposed to explore the impact of metformin on cellular proliferation and apoptosis in steatosis-induced hepatocellular carcinoma cells.

MATERIALS AND METHODS

Agents, Chemicals and Assay Kits

Oleic acid, palmitic acid and actin antibodies were supplied from Santa Cruz Biotechnology (CA, USA). Metformin was supplied from Cayman Chemicals (Ann Arbor, MI, USA). Oil Red-O was supplied from Sigma-Aldrich Biotechnology (Steinheim, Germany). BrdU Cell Proliferation ELISA Assay kit and protease cocktail inhibitor were obtained from Roche Molecular Biochemicals (Mannheim, Germany). Lowry Smart BCA Protein kit was obtained from Intron Biotechnology (Seoul, Korea). RPMI-1640 was obtained from Pan Biotechnology (Aidenbach, Germany). Fetal Bovine Serum (FBS) was supplied from Gibco (Grand Island, NY, USA).

Cell Lines and Cell Culture Conditions

HepG2 cells were supplied from American Type Culture Collection (Manassas, VA, USA). HepG2 cells were

maintained in RPMI-1640 culture medium (Pan Biotech.). Each medium was supplemented with 10% FBS (Gibco) and contained 100 units/ml penicillin and 100 µg/ml streptomycin. All cell cultures were maintained at a temperature of 37°C under a 5% CO_2 atmosphere.

Stock solutions of palmitic acid (66 mM) and oleic acid (132 mM) were prepared in DMSO. The final concentration of metformin stock solution in PBS is 1 mg/ml (pH 7.2). All solutions underwent sterile filtration using a 0.22 µm pore membrane filter and were subsequently preserved at -20°C.

PA and OA-induced Steatosis Model

Cells were plated in six-well plates at a density of 1×10^6 cells per well, followed by incubation of HepG2 cells in RPMI-1640 medium with palmitic acid at a concentration of 0.33 mM and oleic acid at a concentration of 0.66 mM for 24 h [25].

Oil Red O Staining

The cells were washed with phosphate-buffered saline (PBS), followed by fixation with 4% paraformaldehyde for 30 minutes and subsequent staining using a freshly prepared working solution of Oil Red O for 30 minutes. Afterward, the cells underwent washing with deionized water. Stained Oil Red O was further dissolved using 100% isopropanol at room temperature for 15 minutes. The absorbance of the sample was measured using a spectrophotometer (Synergy H1, BioTek, Winooski, VT) at 510 nm.

Cell Proliferation Assay

Cells were plated onto a 96-well plate at a density of $1x10^4$ cells per well and allowed to grow overnight. The cells were then incubated with metformin (2 mM) [26] for 24 hours. Cell proliferation was measured with a quantitative colorimetric BrdU (Roche Biochemicals) assay kit. It was determined by measuring optical density at 370 nm with a microplate scanning spectrophotometer (Synergy H1, BioTek, Winooski, VT).

Preparation of Cell Lysates and Western Blot Analysis

Cells were washed with cold PBS and centrifuged at 300x g for 15 min at 4 °C. The supernatant was collected and homogenized in lysis buffer included protease inhibitor cocktail. Protein concentration was determined by Lowry's Assay (Smart BCA protein kit- iNtRON) used to normalized protein level [27]. The protein sample (50 µg) was prepared and subjected to boiling at 95°C for 3 min. The proteins were loaded on SDS-PAGE. Subsequently, the gels were transferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH). The membrane was blocked in 10% BSA in TBST buffer for 1 h at room temperature. After blocking, membranes were incubated overnight at 4°C with primary antibodies; caspase 3 (Invitrogen, Carlsbad, CA, USA), cytochrome c (Invitrogen, Carlsbad, CA, USA), Bcl-2 (Invitrogen, Carlsbad, CA, USA), Bax (Invitrogen, Carlsbad, CA, USA). Actin (Santa Cruz Tech., CA, USA) antibody was used for control. The membrane underwent three washes with TBST buffer before being exposed to the secondary antibody for 1 hour. The membranes were washed and subjected to incubation with secondary antibodies (Thermo Fisher Sci., Waltham, USA) for 1 hour at room temperature. The specific bands labeled by the antibody were visualized using NBT/BCIP or chemiluminescent substrate containing ECL substrate (Pierce ECL Western Blotting Substrate luminol-based activator- Thermofisher Scientific, Waltham, USA). The relative expression levels of proteins were determined by analyzing band intensities through computerized densitometry, employing the freely available version of ImageJ software (NIH, USA). The molecular weights of cytochrome c, caspase 3, Bcl-2, Bax and actin are 15 kDa, 32 kDa, 23 kDa, 21 kDA and 43 kDa, respectively.

Statistical Analysis

The experimental data were demonstrated as means±-standard error of the mean (SEM). All data reflect a minimum of three experiments performed in triplicate. The analysis of all data was conducted using Prism software (ver. 8; GraphPad Software Inc., San Diego, CA, USA). The Mann Whitney U test was employed to assess statistical differences between means. A significance level of $p<0.05$ was considered statistically significant.

RESULTS

Creating a Steatosis Model in HepG2 Cells Using Palmitic Acid and Oleic Acid

Steatosis was determined by Oil Red-O staining in cells incubated with palmitic acid at a concentration of 0.33 mM and oleic acid at a concentration of 0.66 mM for 24 hours. The findings indicate notable accumulation of lipid droplets within the cytoplasm of HepG2 cells in comparison to the control group ($p=0.0159$; Fig. 1).

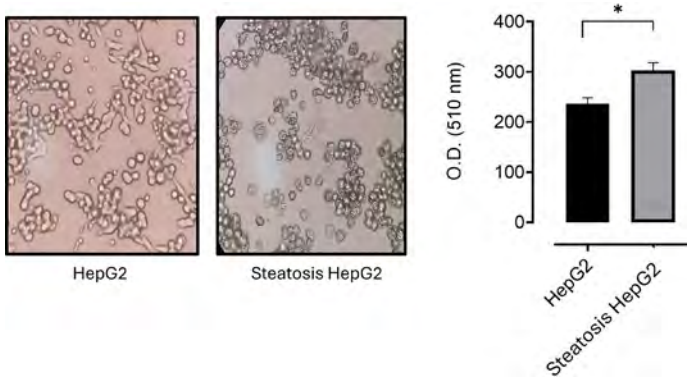

Figure 1. HepG2 and steatosis HepG2 cells Oil Red O staining (magnification x400), Comparison of the degree of steatosis with Oil red O staining in the steatosis model created in HepG2 cells incubated for 24 hours using palmitic acid (0.33 mM) and oleic acid (0.66 mM). Results are given as ±SEM. Experiments were performed in triplicate (*p<0.05).

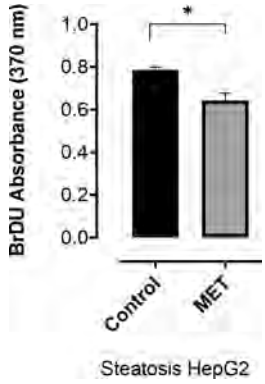

Figure 2. Effect of metformin on cell proliferation in steatosis HepG2 cells. Cells were treated with metformin (2mM) for 24 hours and cell counting was performed using the BrDU kit. Results are given as ±SEM. Experiments were performed in triplicate. (*p<0.05) Control: No drug; MET: Metformin.

In this study, we developed a steatosis cell model with HepG2 cells using OA and PA. We were able to quickly and accurately measure the degree of steatosis caused by PA and OA. The oil red-O-based colorimetric quantitative assay used in this study is not only convenient and measurable but also sensitive and reproducible.

Effect of Metformin on Cell Proliferation In Steatosis Hepg2 Cells

It is known that metformin can protect hepatocytes from death caused by saturated fatty acids. In our study, steatosis HepG2 cells were treated with metformin (2 mM) for 24 hours and its effects on cell proliferation was investigated. Metformin inhibited cell proliferation in steatosis HepG2 cells compared to the control group (p=0.0286; Fig. 2).

Effect of Metformin on Apoptosis in Steatosis HepG2 Cells

The effect of metformin on apoptosis in HepG2 cells with steatosis was assessed through western blot analysis of cytochrome C, caspase 3, Bcl-2, and Bax protein expression levels.

In steatosis HepG2 cells, cytochrome c expression and Bax/Bcl-2 expression levels rate increased in the metformin group compared to the control group (p=0.0286; Fig. 3). The change in caspase 3 expression level compared to the control was not found to be statistically significant (p=0.0571).

DISCUSSION

Metformin, an oral biguanide drug that has been prescribed for almost 60 years, has some features that stand out for its use in the prevention and treatment of cancer. In this case, it becomes important to determine whether metformin can directly affect the metabolism in tumor cells. The most important reason why the liver is one of the targets of the antidiabetic effect of metformin is that it plays a role in the management of glucose metabolism [28]. In vitro studies have shown that metformin used at concentrations greater than 1 mM causes mitochondrial complex 1 inhibition, decreases in aerobic metabolism, decreases in energy production and therefore eventually causes cell death [29]. Indirect effects of metformin in cancer: reducing glucose level, reducing hyperinsulinemia, reducing IGF-1 level, reducing NF-κB, reducing pro-inflammatory cytokines and increasing immune response in cancer cells. AMP-activated protein kinase (AMPK) dependent effects of metformin: It reduces mTOR, reduces myelocytomatosis oncogene (c-MYC) and increases p53 phosphorylation. AMPK-independent effects of metformin: It reduces ROS, increases mammalian target of rapamycin complex 1 (mTORC1), decreases cyclin D1, decreases autophagy and increases cancer cell apoptosis [30].

Current studies show that cytochrome c is first released from the intramitochondrial membrane following depolarization of the mitochondrial membrane, which then activates cytosolic caspases [31, 32]. The ratio of

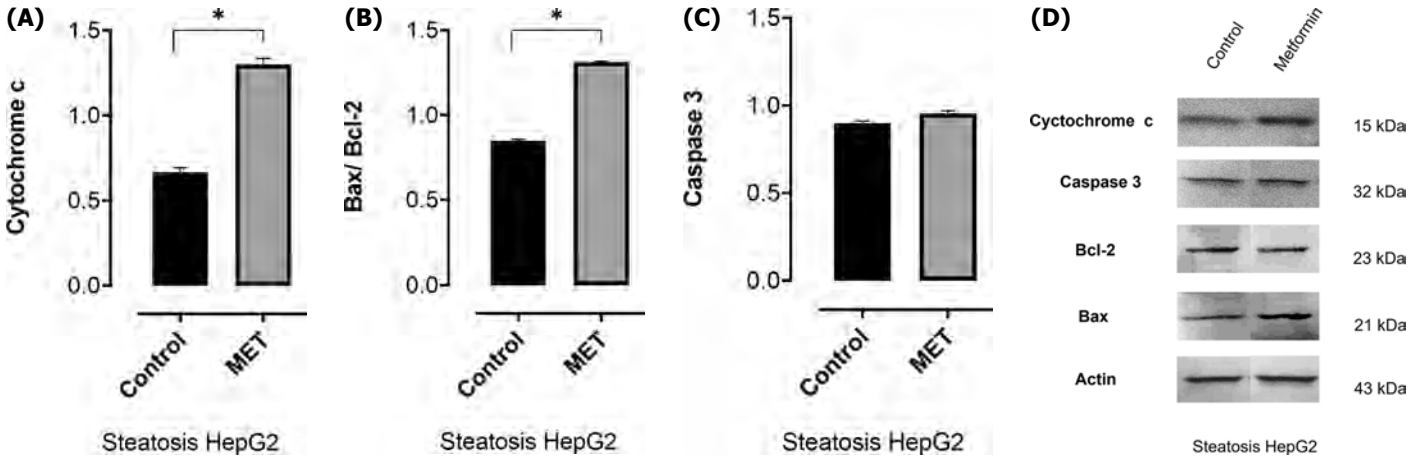

FIGURE 3. Changes in signaling proteins during apoptosis. Western blot analysis of apoptotic proteins in steatosis HepG2 cells was performed to elucidate the underlying mechanism(s) of apoptosis. Steatosis HepG2 cells were cultured for 24 hour. Protein extracts were loaded from cultured steatosis HepG2 cells. Samples were electrophoresed on an SDS-PAGE gel before being blotted onto a nitrocellulose membrane. Specific antibodies were used to probe the presence of **(A)** cytochrome c, (*p<0.05) **(B)** Bax/ Bcl-2 (*p<0.05) and **(C)** caspase-3 (p<0.05) on the blots. **(D)** The representative membrane images of Cytochrom c, Bax, Bcl-2, caspase 3. The positions relative to the molecular weight standards are represented on the right. Results are given as ±SEM. Experiments were performed in triplicate. Control: No drug; MET: Metformin.

Bax/ Bcl-2 proteins is crucial in dictating cellular fate, either survival or death [33].

Metformin has been demonstrated to decrease hepatic triacylglycerol and free fatty acid concentrations, while enhancing lipoprotein lipase activity in fructose-fed rats exhibiting hyperinsulinemia and hyperglycemia [34]. Zang et al. [35] found that metformin stimulated AMPK alpha phosphorylation in HepG2 cells and reduced intracellular lipid content in a dose (0.5–2 mM) and time-dependent (0–24 hours) manner. In that investigation aimed at assessing the impact of metformin-induced AMPK activation on the modulation of hepatocellular lipids, it was demonstrated that metformin prevented lipid accumulation induced by elevated glucose levels through an AMPK-dependent mechanism. Their research provided compelling biochemical proof that metformin's influence on the lipid composition of HepG2 cells dependent on AMPK activation. In our study, metformin was used to examine its anti-cancer effects on fatty liver cancer cells by creating a steatosis model, taking into account its effect on glucose metabolism in the liver. There are studies in the literature investigating the anti-cancer effects of metformin. The difference of our study is to investigate these effects in fatty liver. In our study, free fatty acids PA and OA were used to create an in vitro model of NAFLD. Our Oil red-O staining results confirmed that the model is functional.

Fendt et al. [36] found that metformin decreased proliferation in prostate carcinoma (LNCaP and DU145) and prostate adenocarcinoma (PC3) cells in a dose-dependent manner in the concentration range of 0.5–2.5 mmol/L. Li et al. [37] found that metformin reduced cell proliferation in osteosarcoma cells both dose-dependently (5–40 mM) and time-dependently (24–72 hours). Additionally, they showed in their study that metformin significantly reduced colony formation. Their results suggest that metformin inhibits cell viability of osteosarcoma cells. They suggested that metformin causes cell cycle arrest in the G2/M phase by upregulating cell cycle-related proteins. Mogavero et al. [38] observed that treatment with metformin at a concentration of 5 mM resulted in diminished proliferation, migration, and invasion of colorectal cancer cell lines. They noted an elevation in the proportion of cells in the G0/G1 phase and a reduction in the expression of various cell cycle regulatory proteins in CRC cells following metformin treatment. Their findings suggested an augmentation in ROS production in cells, proposing that metformin's mechanism of action may involve elevating ROS levels and inhibiting mTOR in these cell lines. Similar findings are found in ovarian and luminal breast cancer. They showed that metformin reversibly inhibited colony formation. They predicted that the anti-proliferative effect of

metformin is caused by the suppression of the mTOR pathway, which plays a critical role in cell growth. As a main result of our study, metformin (2mM) was able to the decreases of cell proliferation in PA and OA-induced HepG2 cells compared to the control group in the steatosis HepG2 cell. We previously showed that colony formation was reduced in steatosis HepG2 cells compared to control HepG2 cells.

Li et al. [37] showed that the percentage of cell apoptosis increased significantly after treatment with metformin. From all their results, they suggested that apoptosis may occur by activating extrinsic and intrinsic pathways after metformin treatment. Shen et al. [39] found that metformin (10–20 μM) inhibited the migration and colony formation of HCC cells (HepG2 and Huh7) and induced apoptosis. They found that metformin inhibited HCC cell growth in vivo and had anti-tumor and pyroptosis-inducing effects on HCC cells. Wu et al. [40] observed a notable increase in apoptosis rates in HepG2 cells treated with oleic acid compared to the control group, which significantly decreased following treatment with metformin. They proposed that metformin effectively ameliorated steatosis and could enhance HepG2 functionality in a cellular model of NAFLD. Based on these findings, they suggested that metformin's mechanism of action might involve mitigating oxidative stress damage, modulating the expression of proteins associated with the mitochondrial apoptosis pathway, and suppressing cellular apoptosis. Our western blot results show that metformin treatment induces apoptosis. Metformin increase in Bax/Bcl-2 protein expression ratio and also cytochrome c protein expression in steatosis HepG2 cells. Our results show that metformin inhibits cell viability of steatosis HepG2 cells.

Conclusion

Based on all of our results, we suggest that the mechanism by which metformin operates might involve the inhibition of cell proliferation and the modulation of protein expression associated with apoptosis pathways. In summary, in this study, upregulation of cyt c and bax play an important role in the mechanism of metformin-induced apoptosis in steatosis HepG2 cells. However, the effect of metformin on caspase 3 needs to be further clarified. These findings may provide insight into finding new therapeutic targets or treatment strategies against HCC.

Ethics Committee Approval: Ethics committee approval is not required for this study.

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

Financial Disclosure: This work was supported by Marmara University Scientific Research Projects Coordination Unit (Istanbul/Turkiye) under grant number SAG-C-DRP-TDK-2021-10286 to Hulya Cabadak. This work was supported by the Scientific and Technical Research Council of Turkiye (grant number: SBAG-221S561) to Ayse Melek Tanriverdi Bademci.

Use of AI for Writing Assistance: The study did not use artificial intelligence (AI)-supported technologies (such as Large Language Models [LLMs], chatbots or image generators, ChatGPT).

Authorship Contributions: Concept – HC, AMTB; Design – HC, AYTB; Supervision – HC; Analysis and/or interpretation – DM, SPK; Literature review – HC, AMTB, BA; Writing – AMTB, HC; Critical review – HC, AMTB, BA.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):516–526
doi: 10.14744/nci.2024.78642

Original Article EMERGENCY AID & DISASTER MANAGEMENT

Bibliometric analysis of global research findings on refugee mental health (1992–2022)

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ABSTRACT

OBJECTIVE: In this study, we aimed to examine the thirty-year effectiveness and trend of research on refugee mental health.

METHODS: A bibliometric analysis methodology was used. Web of Science (WOS) database was used to obtain the necessary data. In particular, a review and analysis encompassed the quantity of publications featuring articles on refugee mental health, the most prolific countries and institutions, the highly cited articles, citation patterns, international collaboration, and the relevant journals. The study's timeframe was defined from 1992 to 2022.

RESULTS: The number of documents obtained is 3912. The majority of the documents obtained were in the field of psychiatry. The quantity of publications and citations experienced a notable upsurge, particularly following the year 2016. The United States emerged as the leading country in terms of both the highest number of publications and citations on this subject. The institutions with the highest publication rates are, in order, the University of New South Wales in Australia, the University of Melbourne in Australia, and McGill University in Canada. This bibliometric study shows that publications on refugee mental health have been observed since 1992 and are gaining momentum, especially after 2016. In addition to the terms "refugees" and " mental health," the keywords "depression," " Post-traumatic stress disorder (PTSD)," and "children" were most commonly used.

CONCLUSION: Refugee communities also appear to have similar mental illnesses and experiences regardless of where and when they settled in the world. Research collaboration and networks should be encouraged to prioritize research in refugee mental health.

Keywords: Depression; bibliometric analysis; mental health; refugees.

Cite this article as: *Kinik K, Dag N, Caliskan C, Celebi I, Dogan M, Bek Yagmur O. Bibliometric analysis of global research findings on refugee mental health (1992–2022). North Clin Istanb 2025;12(4):516–526.*

In recent years, the displacement of people due to political and religious conflicts, climate change, human rights violations, pandemics, and economic factors has become increasingly common. In 2021, the United Nations Refugee Agency (UNHCR) documented an unprecedented number of 84 million people being forci-

Received: February 01, 2024 *Revised:* February 21, 2024 *Accepted:* July 24, 2024 *Online:* August 28, 2025

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bly displaced, marking the highest figure in history. The majority of cross-border refugees originated from Syria, Venezuela, Afghanistan, South Sudan, and Myanmar. Notably, Turkiye, Jordan, Uganda, Pakistan, and Lebanon were among the countries that hosted the largest numbers of refugees [1].

The rising number of displaced individuals can exert considerable strain on mental health services within the host countries. Primarily, the influx of such a substantial population in a brief timeframe necessitates a prompt and adequate response to ensure the fulfillment of their right to basic healthcare. Furthermore, individuals undergoing migration are susceptible to mental health risk factors before, during, and after the process, often encountering obstacles in accessing suitable care post-resettlement [2]. Consequently, mental health issues are disproportionately prevalent among refugee populations when compared to non-refugee populations [3]. A systematic review of psychiatric research on approximately 7,000 refugees living in Western countries found that the prevalence of PTSD among refugees was almost ten times higher than in the non-refugee population [4]. Exposure to trauma, which often first occurs during the pre-migration phase, is the most important determinant of PTSD among resettled refugee populations [5]. However, the origin of depression may be due to post-resettlement factors such as language difficulties, social isolation, and unemployment problems [6]. This situation is prompting researchers to look at refugee mental health.

Bibliometrics is a tool used to assess the state of the science in a given field [7]. Therefore, employing bibliometric analysis to identify and scrutinize the scientific contributions of authors, articles, journals, institutions, and countries through the examination of keywords and citation counts is a crucial component. This approach enables researchers to pinpoint potential avenues and novel directions within a scientific research topic [8]. Bibliometric methodologies are considered useful as tools to support decision-making in, among others, setting research priorities, tracking the development of science and technology, allocating funds and rewarding scientific excellence. Given their versatility, these methods quickly spread beyond the field of information and library science from where they began. Part of this expansion is due to the abundance and ease of accessibility of data [9]. Scholars use bibliometric analysis for a variety of reasons, including uncovering emerging trends in article and journal performance, patterns of collaboration, and research components, and exploring the intellectual structure of a particular field in existing literature [10].

Highlight key points

- Psychiatry emerges as the leading field, constituting the highest percentage of articles at 30.88%.
- During the period from 1992 to 2022, the number of citations exhibited a consistent upward trend, culminating in its zenith at 13.383 in 2021.
- The top three contributing countries, based on the number of publications, are the United States with 1455 publications, Australia with 620 publications, and England with 465 publications.

As far as we reviewed the literature, many bibliometric studies were conducted on refugees [11–13]. However, we have not encountered a worldwide bibliometric study on the mental health of refugees. Hence, the objective of this study is to assess the effectiveness and trends in research on the mental health of refugees over the span of thirty years.

MATERIALS AND METHODS

Data Collection

This research paper employs descriptive bibliometric analysis as its methodology. Through the application of mathematics and statistical methods, bibliometric analysis elucidates and visualizes the academic research landscape [8]. For this investigation, data were sourced from the Web of Science Core Collection (WOS) database (Thomson Reuters, New York, USA) spanning the years 1992 to 2022 [data access date: 5 January 2023]. The first article on this subject was published in 1982. However, there are approximately 50 articles in the 10-year period (1982–1991). For this reason, the first 10-year period was not included in the interpretation. The keywords "mental health" and "refugee" were used by selecting the "Title" section. No restrictions were applied regarding the selection of journals and authors in this study. All research articles, reviews, systematic reviews, short reports are included. A total of 3912 publications were evaluated. The bibliometric analyses and the creation of bibliometric network visualization maps were conducted using VOSviewer software (version 1.6.17).

Cluster Analysis with Network and Density Visualization Maps

Network visualization maps encompass various elements such as colors, labels, lines, and circles. Cluster analysis is conducted as an integral part of network analysis. The

Table 1. The 10 most frequently appearing research fields in the period of 1992–2022

Research fields*	Frequency	%
Psychiatry	1208	30.88
Public environmental occupational health	885	22.62
Psychology clinical	379	9.69
Social work	285	7.29
Demography	235	6.01
Psychology multidisciplinary	213	5.45
Psychology developmental	204	5.22
Ethnic studies	196	5.01
Pediatrics	187	4.78
Medicine general internal	175	4.73

*: Categories of research areas were taken from the WoS database.

colors within the maps signify the clusters to which the elements belong, and rings of the same color within a cluster denote a stronger relationship among them. The size of the rings corresponds to the magnitude of the citation counts, indicating that larger sizes represent a higher number of citations or articles. The closeness or distance between the rings signifies their relationship; typically, closer proximity implies a stronger connection between the respective elements. Moreover, the thickness of the lines denotes the strength of the relationship. In density visualization maps, each point assumes a distinct color corresponding to the density of elements present at that specific location. By default, this color changes from red to blue. The color of a point shifts towards yellow when there is a greater number of adjacent elements and higher weights associated with those elements. Conversely, the color of a point tends towards blue when there is a smaller number of adjacent elements and lower weights.

RESULTS

Publication Types and Research Fields

3,912 of the publications including the keywords "refugee" and "mental health" were found in the WOS database from 1992 to 2022.

Of the publications, 3,286 were in the "article" category, 403 in the "Review article" and 104 in the "editorial material" category. 3,826 of the publications were in English, 54 in German and 9 in French. Table 1 illustrates the top 10 most prevalent research fields in publications concerning the mental health of refugees from 2012 to 2021. Psychiatry emerges as the leading field, constituting the highest percentage of articles at 30.88%.

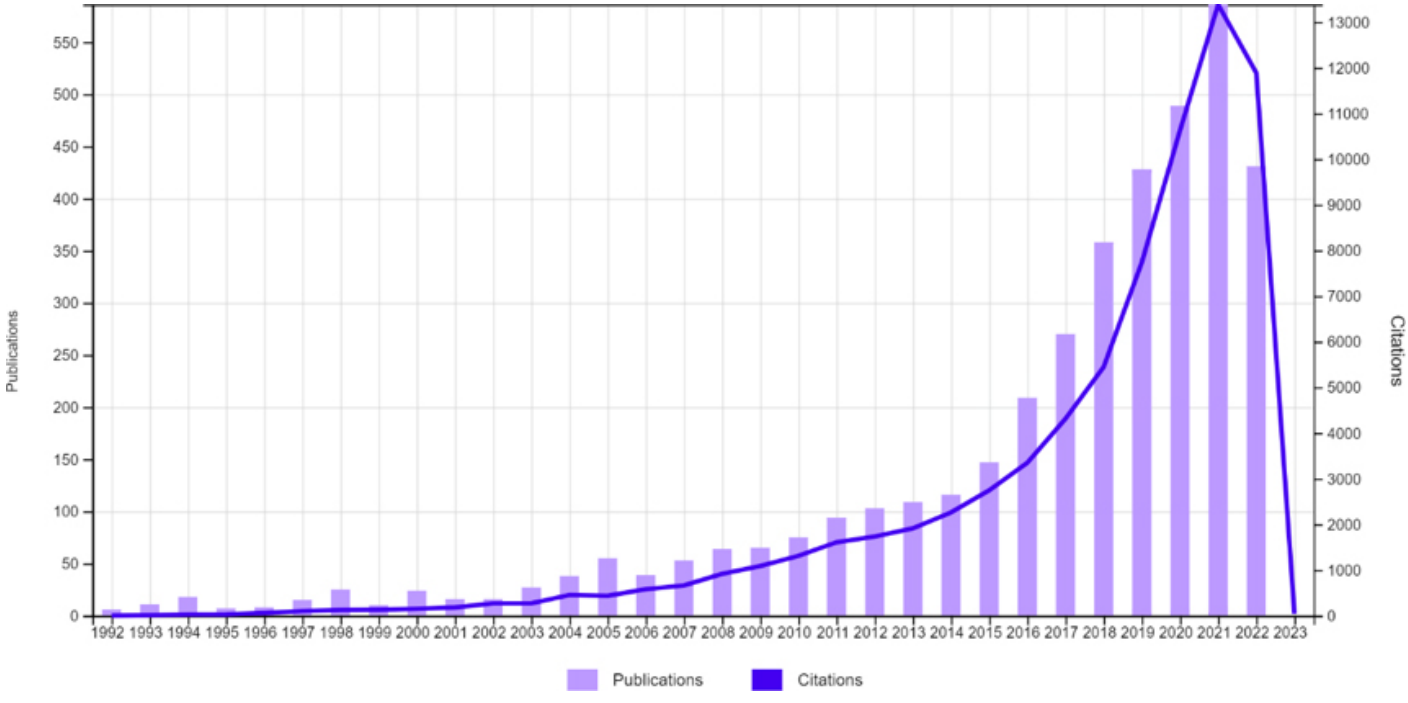

Figure 1. Number of publications and citations indexed by Web of Science Core Collection in this field.

TABLE 2. Top ten most cited articles on the mental health at refugee in the period from 1992 to 2022

Article	Author	Journal	Total citation	Year	Average citations per year
Association of torture and other potentially traumatic events with mental health outcomes among populations exposed to mass conflict and displacement a systematic review and meta-analysis	Steel, Z; Chey, T; Silove, D et al. [14]	Journal of the American Medical Association	1187	2009	79.13
The Harvard Trauma Questionnaire - Validating a cross-cultural instrument for measuring torture, trauma, and posttraumatic-stress-disorder in indo-chinese refugees	Mollica, RF; Caspiyavin, Y; Bollini, P et al. [15]	Journal of Nervous and Mental Disease	1165	1992	36.41
Predisplacement and postdisplacement factors associated with mental health of refugees and internally displaced persons - A meta-analysis	Porter, M; Haslam, N [16]	Journal of the American Medical Association	965	2005	50.79
Mental health of displaced and refugee children resettled in high-income countries: Risk and protective factors	Fazel, M; Reed, RV; Panter-Brick, C et al. [17]	The Lancet	713	2012	59.42
War exposure, daily stressors, and mental health in conflict and post-conflict settings: Bridging the divide between trauma-focused and psychosocial frameworks	Miller, KE; Rasmussen, A. [18]	Social Science&Medicine	692	2010	49.43
Common mental health problems in immigrants and refugees: General approach in primary care	Kirmayer, LJ; Narasiah, L; Munoz, M et al. [19]	Canadian Medical Association Journal	621	2011	47.77
Measuring trauma and health status in refugees - A critical review	Hollifield, M; Warner, TD; Lian, N et al. [20]	Journal of the American Medical Association	438	2002	19.91
Multicultural assessment of child and adolescent psychopathology with Achenbach System of Empirically Based Assessment and Strengths and Difficulties Questionnaire Instruments: Research findings, applications, and future directions	Achenbach, TM; Becker, A; Dopfner, M et al. [21]	Journal of Child Psychology and Psychiatry	414	2008	25.88
Anxiety, depression and post-traumatic stress disorder in asylum-seekers: Associations with pre-migration trauma and post-migration stressors	Silove, D; Sinnerbrink, I; Field, A et al. [22]	British Journal of Psychiatry	411	1997	15.22
Review of child and adolescent refugee mental health	Lustig, SL; Kia-Keating, M; Knight, WG et al. [23]	Journal of the American Academy of Child and Adolescent Psychiatry	394	2004	19.7

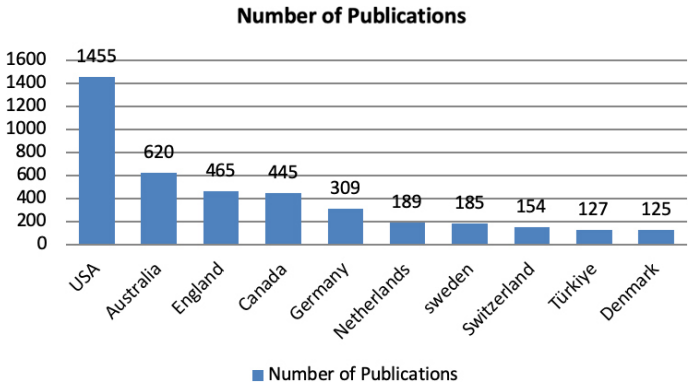

FIGURE 2. Top 10 countries according to total number of publications.

Development of Publication and Citation Numbers

It was found that the 3,912 manuscripts included in the evaluation were cited a total of 73,833 times and had an H-index of 102 (These results were calculated automatically by the WoS database). Figure 1 illustrates the trend in the number of publications and citations per year. Figure 1 shows that the number of publications has tended to increase over the years, and this increase continued exponentially, especially after 2016. However, in 2022, there is a decrease in the number of publications. The number of citations exhibited an upward trajectory over the years, culminating in its peak at 13,383 in 2021. In 2022, however, it decreased (citations: 11,886).

Active Country, Institution and Collaboration Analysis

The outcomes of the active country analysis were derived from 32 countries, each having a minimum of 25 publications, out of a total of 118 countries. The top three contributors, based on the number of publications, were United States of America (USA) with 1,455 publications, Australia with 620 publications, and England with 465 publications. The top 10 countries are shown in Figure 2. When Figure 3, which includes the citation links of the countries, is examined, it is seen that there are a total of six clusters and 720 links. The size of each circle

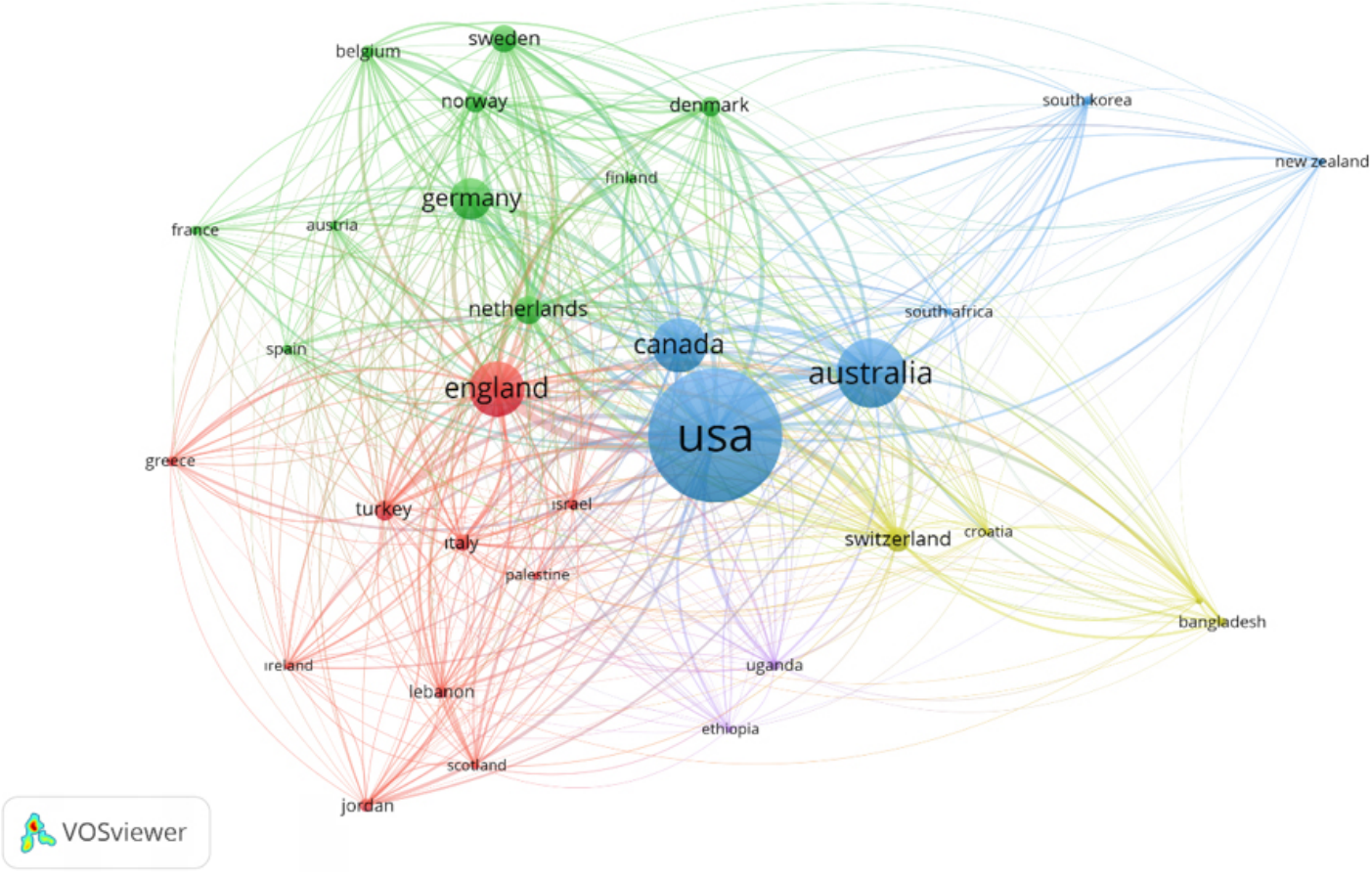

FIGURE 3. Citation link of countries.

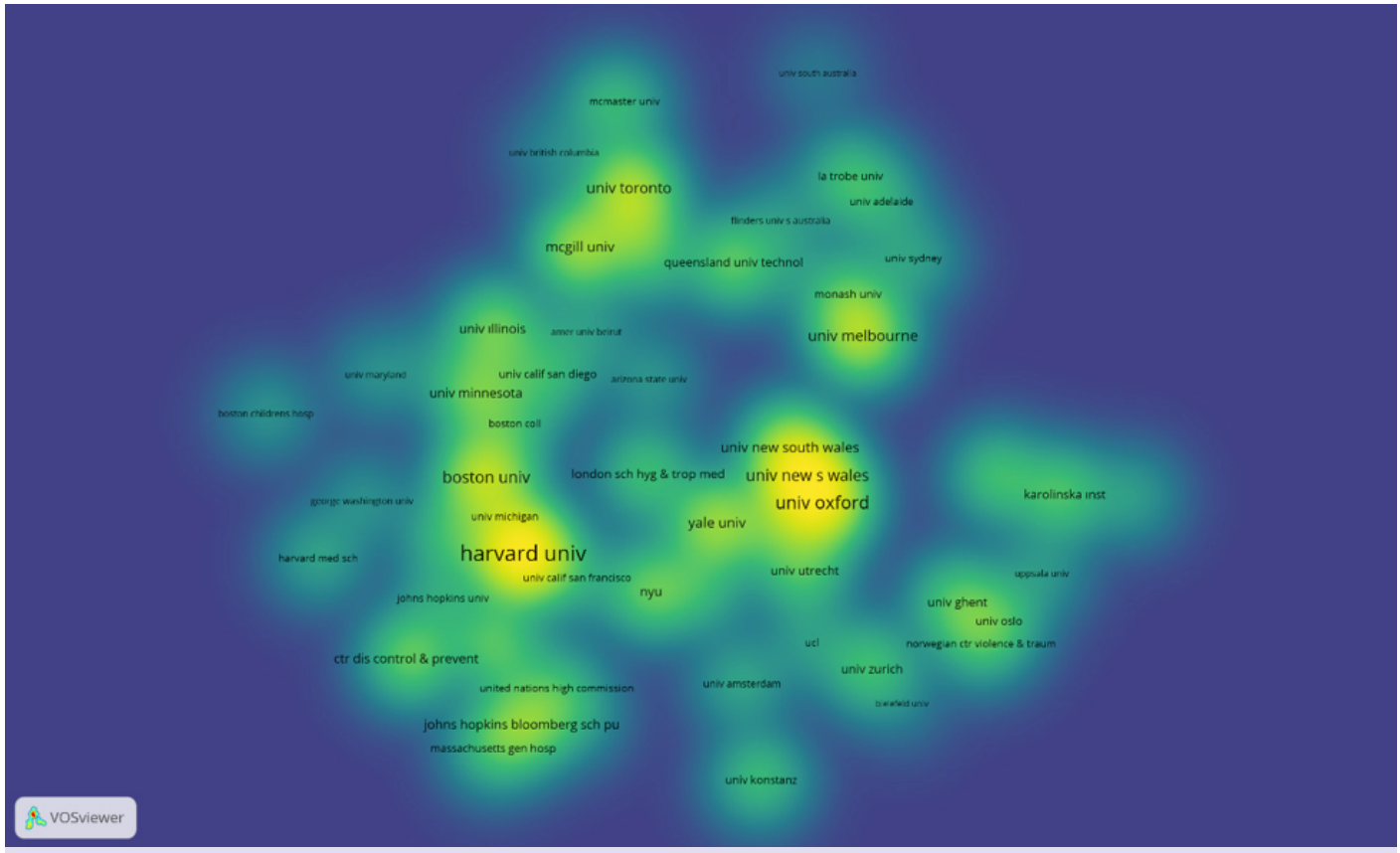

FIGURE 4. Density visualization map of citation analysis of active institutes.

corresponds to the number of publications, with larger circles indicating a higher quantity.

Density analyses of institutes were conducted, involving 60 institutions that published a minimum of 25 articles out of a total of 3,463 institutes. In Figure 4, clusters of the four hotspots have been grouped together due to their correlation with publishing articles. In Figure 4, institutions with the highest citation density are depicted in yellow, with the color transitioning to turquoise as the citation density decreases. The institutions that publish the most are University of New South Wales-Australia (108), the University of Melbourne-Australia (100), and McGill University-Canada (96), respectively. The most cited institutions are Harvard University-USA (4,961), Oxford University-England (3,287) and New South Wales University-Australia (2,782), respectively.

TABLE 3. First 10 authors by record count and citation on the mental health of refugee

Authors	Record count	%	Citation
Silove D	57	1.45	2613
Nickerson A	51	1.29	1470
Bryant RA	46	1.17	2337
Ventevogel P	41	1.04	820
Rousseau C	39	0.99	1194
Derluyn I	34	0.86	1105
Bolton P	30	0.76	893
Steel Z	30	0.76	2017
Betancourt TS	29	0.73	864
Mollica RF	27	0.63	348

Most Cited Articles

Table 2 presents the top ten most cited articles on the mental health of refugees from 1992 to 2022. The most cited publication with 1,187 citations was published in "Journal of the American Medical Association (JAMA)" with the title of "Association of Torture and Other Potentially Traumatic Events With Mental Health Outcomes Among Populations Exposed to Mass Conflict and Displacement A Systematic Review and Meta-anal-

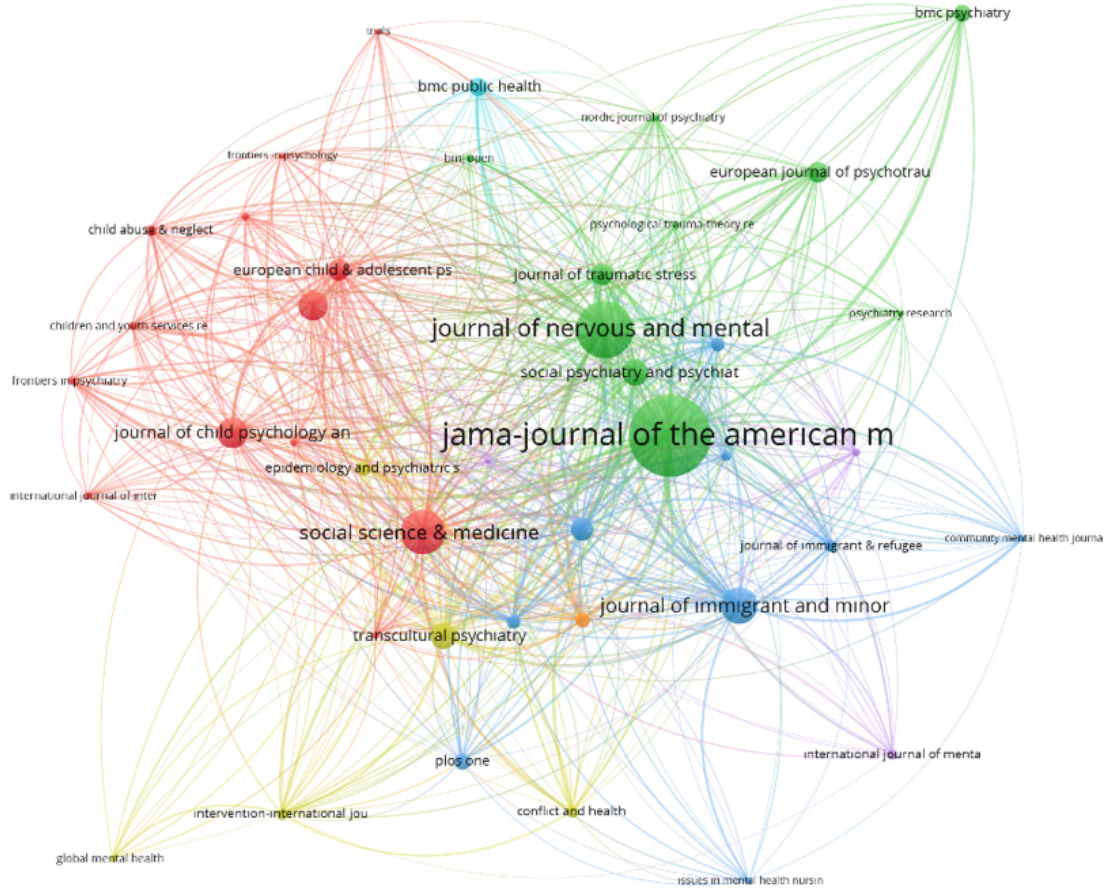

FIGURE 5. Network visualization maps of citation analysis of active journals.

ysis" by a team of researchers led by Steel [14]. The second most cited paper (1,165) was "The Harvard Trauma Questionnaire - Validating A Cross-Cultural Instrument For Measuring Torture, Trauma, and Posttraumatic-Stress-Disorder in Indo-Chinese Refugees" [15]. The third most cited paper (965) predisplacement and postdisplacement factors associated with mental health of refugees and internally displaced persons - A meta-analysis [16] (Table 2).

Most Active Authors

Table 3 enumerates the ten most prolific authors who have contributed the highest number of articles in the field of "mental health of refugees." The authors being in the top three with respect to the number of publications were Silove D (1.45%), Nickerson A (1.29%), and Bryant RA (1.17%). The authors being in the top three with respect to the number of citiations were Silove D (2,613), Bryant RA (2,337), and Steel Z (2017) (Table 3).

Most Active Journals, Citation and Co-citation Analysis

Citation analysis of the most active journals was conducted, encompassing 42 journals with a minimum of 15 articles on the subject, out of a total of 1,013 journals and 3,912 publications (Fig. 5, 6). Figure 6 presents the network visualization map of the citation analysis of active journals, categorized into seven distinct clusters denoted by different colors. The size of each circle represents the number of citations received by the journals. Co-citation analysis of active journals was conducted among 102 journals, each with a minimum of 250 citations, out of a total of 1.013 journals and 3.912 publications (Fig. 6). Once again, the circle size indicates the number of citations received by the journals.

Table 4 outlines the first ten most active journals and their respective citation counts in the field. The most active journals were International Journal of Environmental Research and Public Health (112; 2.84%), Journal of Immigrant and Minority Health

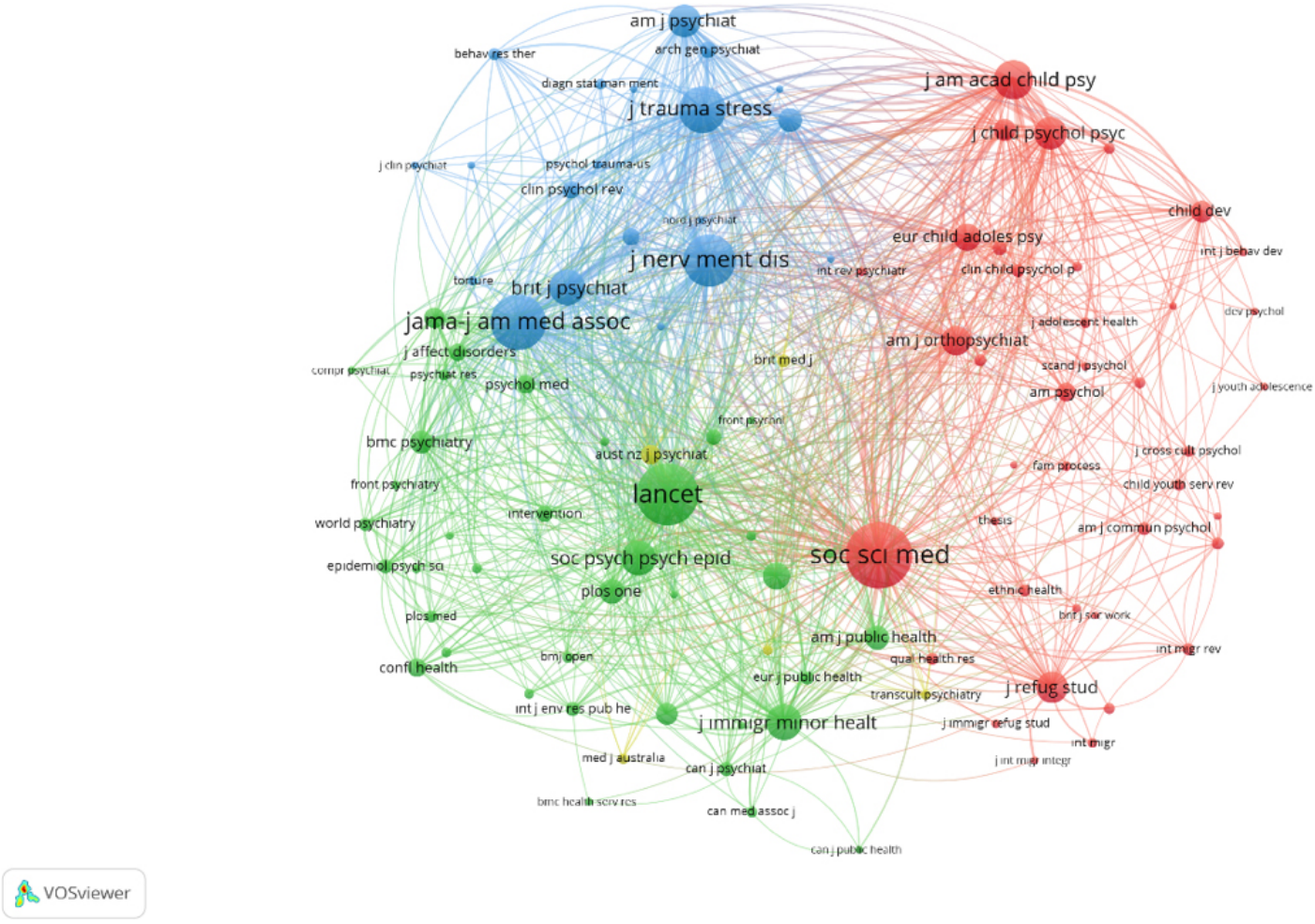

FIGURE 6. Network visualization maps of co-citation analysis of active journals.

(107; 2.72%), and Intervention International Journal of Mental Health Psychosocial Work and Counselling in Areas of Armed Conflict (104; 2.03). The top three most cited journals were Journal of Nervous and Mental Disease (3.588), Journal of Immigrant and Minority Health (2066) and Transcultural Psychiatry (1.392), respectively.

Commonly Used Keywords Analysis

Table 5 presents the top 10 most frequently utilized keywords that appeared in publications on the mental health of refugees from 1992 to 2022. The three most frequently used keywords were "Refugees", "Mental health", and "Refugee". A total of 5606 distinct keywords were identified across 3912 publications. Bibliometric analyses of frequently used keywords were conducted, revealing 35 keywords that were employed at least 50 times.

DISCUSSION

This study was conducted to identify the bibliometric characteristics of 3912 research articles on mental health of refugee using WOS from 1992 to 2022, as listed in the supplementary material. This study represents the inaugural bibliometric analysis in this particular field. The bibliometric analysis reveals a consistent rise in the number of publications and citations over the years, particularly notable after 2016. This increase demonstrates the importance of bibliometric analysis in facilitating further research on refugee mental health by researchers. The increase in refugee mental health studies in the mid-2010s can be linked to the Syrian civil war. In fact, on a global scale, the largest irregular migration wave since World War II was experienced [24]. We predict that this increase will continue steadily in the coming years. We think that scientists will especially examine the changes in the mental health of refugees in depth.

Table 4. First ten journals sources by number of publications and citations in the mental health of refugee

Journal Name	Number of publications	%	Citations	Citations/article
International Journal of Environmental Research and Public Health	112	2.84	744	6.64
Journal of Immigrant and Minority Health	107	2.72	2066	19.31
Intervention International Journal of Mental Health Psychosocial Work and Counselling in Areas of Armed Conflict	104	2.64	552	5.31
Journal of Refugee Studies	80	2.03	656	8.20
Journal of Immigrant Refugee Studies	69	1.75	573	8.30
Transcultural Psychiatry	63	1.60	1392	22.10
European Journal of Psychotraumatology	59	1.50	1074	18.20
Journal of Nervous and Mental Disease	53	1.34	3588	67.70
Conflict and Health	51	1.29	544	10.67
BMC Public Health	49	1.24	896	18.29

Table 5. Most frequently used 10 words and centrality

Words	Frequency	Total link strength
Refugees	887	1209
Mental helath	843	1312
Refugee	726	1038
Trauma	348	695
Depression	217	501
Post traumatic stress disorders	197	403
Children	160	323
Asylum seekers	148	265
Migration	122	207
Resilience	122	206

The results of this study show that refugee mental health is an important issue, especially for researchers working in the fields of psychiatry, clinical psychology, and public health. In terms of active countries and collaboration, the United States is surprisingly the largest contributor to publications on refugee mental health. The United States also ranks first in article citations, indicating its central role in scientific collaboration (Fig. 3). The United States is followed by Australia and England. These findings suggest that the aforementioned countries are attempting to address refugee health issues through their integration. In this regard, the study was consistent with previous bibliometric studies on various issues related to refugees [6, 8, 20, 25]. It is interesting to note that Turkiye [1], the country that hosts the most refugees in the world, ranks ninth among the countries that publish the most on this topic. We think that Turkiye follows the literature on this topic due to lack of resources and indifference. We think that encouraging measures should be taken for researchers in Turkiye to focus on this issue.

Furthermore, a majority of the most productive collaborations between institutions and institutes in this field have been identified in Australia and Canada. Harvard University (USA) is the institution that has the most references in this field. Strong citation collaboration was also observed between the University of Oxford and the University of New South Wales. The number of citations received is indicative of scientific productivity and quality, serving as a measure of the robust relationship within an active, motivated, and productive research team [26]. In this study, Steel et al. [14], Mollica et al. [15], and Porter and Haslam [16] were the articles with the three highest citation counts in this area. The extensively cited articles served as primary references in this domain, while the most frequently cited authors are recognized figures in their respective field [27]. In this study, Silove D.'s [14] research team was the most active with the most articles and the most citations. Silove is also the author of the highest rated publication.

In this study, the analyses of publication efficiency, citations and co-citations of active journals, International Journal of Environmental Research and Public Health, Journal of Immigrant and Minority Health and Intervention International Journal of Mental Health Psychosocial

Work And Counselling in Areas of Armed Conf. obtained from the bibliometric analysis showed that it was the journals that published the first 10 articles most frequently. Most of the research papers were published in the most prominent journals in the field. The most important journals in terms of citation and co-citation analysis were Journal of Nervous and Mental Dissease, Journal of Immigrant and Minority Health, and Transcultural Psychiatry.

Keywords provide a logical description of a journal topic and can profile an author's research preferences. They are also crucial for emphasizing the focal points of research and exploring trends within scholarly studies [24]. According to the results of the network analysis, the most frequently used keywords from 1992 to 2022 were refugees, mental health, refugee, trauma, depression, Post-traumatic stress disorder (PTSD), children. Not surprisingly, the terms refugee and mental health were central. However, it was interesting that children were on the list. This shows that researchers focus on studies related to children.

This study has several limitations that should be highlighted. The first limitation is that although the title search minimised false positives, it may also yield false negatives. In the study, a search was made at the title level using only two words. This may cause deviations from focus by keeping the scope searched too broad, or may cause some articles to be missed because it is based only on title. Another limitation is that the keywords utilized in our study might not be exhaustive, and certain authors may have employed different terminology, leading to the exclusion of relevant publications. Finally, bibliometric data have limitations, such as differences in spelling, errors and inconsistencies related to indexing of topics, changes in journal titles, different ways of presenting authors' last names and initials, etc.

Conclusions

In summary, this bibliometric study shows that publications on refugee mental health have been observed since 1992 and are gaining momentum, especially after 2016. Most of these publications come from three countries: the United States, Australia, and the United Kingdom. In addition to the terms "refugees" and " mental health," the keywords "depression," "PTSD," and "children" were most commonly used. Refugee communities also appear to have similar mental illnesses and experiences regardless of where and when they settled in the world. Research collaboration and networks should be encouraged to prioritize research in refugee mental health.

Ethics Committee Approval: This study is based on an open-access dataset, therefore, an ethical board review was not sought.

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: The authors declared none.

Authorship Contributions: Concept – IC, KK; Design – ND, IC, MD, OBY; Supervision – KK; Materials – IC, KK, CC, ND; Data collection and/or processing – IC, KK, MD; Analysis and/or interpretation – IC, CC; Literature review – ND, CC, IC, OBY; Writing – IC, ND; Critical review – KK, CC, MD, OBY.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2025;12(4):527–530
doi: 10.14744/nci.2024.58730

REVIEW RHEUMATOLOGY

Toxic metals in rheumatological diseases: A systematic review

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ABSTRACT

Heavy metals exposure might be linked to rheumatic diseases and has been studied in a few articles. The aim of this article is to review the studies that evaluated metal toxicity in rheumatological diseases. A systematic search of PubMed, Embase, and Scielo databases was performed, looking for articles on toxic metals and rheumatic diseases published between 1966 and March 2023. A total of 31 studies (1,559 RA and 4,308 patients with other rheumatic diseases) were included. Most patients were females, ranging from 4 to 62 years old. Although most studies showed higher concentrations of toxic metals in rheumatic diseases, a few showed a positive association with disease activity or severity of the conditions. This systematic review reveals the presence of toxic metals in patients with rheumatic disease. Screening for toxic metals may be elucidative in selected cases.

Keywords: Metal toxicity; metals; rheumatic diseases; rheumatoid arthritis.

Cite this article as: *de Carvalho JF, Skare TL. Toxic metals in rheumatological diseases: A systematic review. North Clin Istanb 2025;12(4):527–530.*

The frequency of rheumatic diseases ranges from rare conditions such as primary vasculitis to very prevalent disorders, including fibromyalgia, which affects 5% of the population, and osteoarthritis, which involves 60% of the old population at 70 years old [1].

The causes of rheumatic disorders are not entirely known, but genetic and environmental factors are considered critical factors in these disorders. Concerning genetic factors, HLA-DR4, PTPN22, and HLA-B27 are widely known to predispose patients to autoimmunity [1]. On the other hand, environmental factors are related to hormones, ultraviolet radiation, medication use (e.g., estrogen, procainamide, hydralazine, etc.), vaccines, and metals. In this context, some studies reported that mercury and gold may induce autoimmunity in animal models [1]; there is evidence of the interplay of toxic metals in the pathophysiology of rheumatic diseases [2].

The purpose of this study is to provide a systematic review of studies assessing metal toxicity in rheumatic diseases.

METHODS

Literature Review

We systematically searched the papers published from 1966 to March 2023 in PubMed/MEDLINE, EMBASE, and Scielo utilizing these MeSH entry terms: "metal toxicity" OR " metal poisoning" OR "metal intoxication" OR "trace element" AND "rheumatic" OR "rheumatologic" OR "fibromyalgia" OR "rheumatoid arthritis" OR "spondyloarthritis" OR "Sjögren's syndrome" OR "myositis" OR "systemic sclerosis" OR "osteoarthritis" OR "gout" OR "antiphospholipid syndrome" OR "gout" OR "osteoarthritis" OR "spondyloarthritis" OR "ankylos-

Received: March 11, 2024 *Revised:* March 27, 2024 *Accepted:* May 24, 2024 *Online:* August 25, 2025

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ing spondylitis" OR "psoriatic arthritis" OR "vasculitis" OR "Behçet's disease" OR "familial Mediterranean fever". In other datasets, we used similar techniques. No language restrictions were present in any associated articles. The chosen articles' reference lists were examined to find other works. Initially, the literature search and research abstract selection were carried out independently by JFC and TLS. The full-text articles chosen by abstracts were reviewed separately by the same reviewers in the second step. At consensus meetings, conflicts were settled by a third reviewer. The writers adhered to PRISMA standards [3]. The authors, year of publication, number of patients investigated, demographic information, duration of the ailment, toxic metal specification, results, and effects were all extracted from the studies using a standardized manner. Evaluations of hazardous metals, rheumatic disorders, and clinical trials were required for inclusion. Articles involving animal experiments, review papers, and in vitro research were excluded.

RESULTS

Appendix 1 summarizes the studies in which toxic metals were evaluated in RA [4–11]. These articles came from Pakistan (n=2), Denmark (n=1), Iraq (n=1), Italy (n=1), Korea (n=1), Taiwan (n=1), and the United States (n=1). Study design, most of them were cross-sectional (n=6) [4–9], case-control (n=1) [10], and case report (n=1) [11]. A total of 8 studies with 1,559 RA patients were evaluated. Age varied from 20 to 62 years old, and female gender from 44% [9] to 100% [5]. In summary, all studies demonstrated increased levels of toxic metals in R.A. subjects, including Cd (n=5), Cu (n=1), Ni (n=2), Pb (n=3), As (n=1), and Hb (n=1).

Appendix 2 shows 22 articles that evaluated toxic metals in other rheumatic diseases [12–34], including SLE (n=5) [12–16], gout or hyperuricemia (n=4) [17–20], systemic sclerosis (n=3) [21–23], miners (n=2) [24, 25], fibromyalgia (n=1) [26], rheumatic fever (n=1) [27], polyarteritis nodosa (n=1) [28], more than one disease studied (n=4) [29–32], unspecified arthritis (n=1) [33] and granulomatosis with polyangiitis (n=1) [34]. Brazil (n=3), Czechia (n=1), Canada (n=1), Italy (n=1), Norway (n=2), France (n=1), Turkiye (n=1), the UK (n=1), the USA (n=5), New Zealand (n=1), and Switzerland (n=1) were the nations that generated the articles. Concerning study design, most of them were cross-sectional (n=14) [12, 13, 17, 19–21, 23–25, 27, 29–31, 33], followed by case report (n=3) [14, 16, 28], prospective (n=2) [15, 26], case series (n=1) [32], case-control (n=2) [22, 34]. A total of 7,883 participants, including 4,259 patients with gout [17–20], 174 lupus [12–14], 179 systemic sclerosis [21–23], 617 miners [24, 25], 33 rheumatic fever [27], 1 polyarteritis nodosa [28], 1 granulomatosis with polyangiitis [34], 39 pediatric rheumatic diseases [14, 27, 32], and 2,546 with two or more conditions [29–32]. Age varied from 4 [32] to 64.7 [19] years old, and female gender from 0 [18] to 96.2% [13]. Disease duration ranged from 2 weeks [32] to 33 years [28].

Regarding lupus studies, low levels of lead were observed in one study, although high lead levels were associated with lupus diagnosis [12]. In the other study, mercury levels were negatively linked to disease activity measured by BILAG and SLICC [13]. The other 2 studies were case reports showing lupus-like induced by toxic metals [13, 14]. The gout studies showed an association of this metabolic disorder with mercury and lead. SSc was associated with increased levels of several toxic metals [21–23]. The authors reported that mercury exposure was linked to positive ANA, anti-nucleolar, and other autoantibodies directed against GSTA1, TNF ligand superfamily member 13, and others [24, 25]. The authors of the fibromyalgia paper discovered that all patients had at least one positive metal test; the most frequently positive were lead, nickel, inorganic mercury, and cadmium. No difference with healthy controls was detected in patients with acute rheumatic fever [27]. In one pediatrician study [32], the authors described 5 young relatives with clinical pictures of polyarteritis nodosa-like and erythromelalgia and were successfully treated with metal chelation. One study evaluated late hypersensitivity to heavy metals, not serum or hair metal levels [30].

Highlight key points

- Toxic metals are frequently detected at higher levels in patients with rheumatic diseases compared to healthy controls.
- Only a minority of studies link metal exposure directly to disease activity or severity.
- Mercury, lead, cadmium, and nickel are the most recurrent metals associated with autoimmune and inflammatory conditions.
- Metal exposure may mimic rheumatic symptoms, complicating diagnosis and clinical management.
- Screening for toxic metals could provide valuable insights in selected rheumatology cases.

DISCUSSION

This is the first study to systematically review all articles published on toxic metals in rheumatic diseases.

The human body requires certain trace elements (Cu, Zn, Mn among others) essential for human health. They are important because they form an essential part of enzymes involved in metabolic or biochemical processes. However, at high levels, these metals may become toxic, causing severe symptoms and even death. Harmful effects in most people result from years of accumulated exposure and storage in the body [29].

Heavy metal toxicity results from increasing pollution levels, industrial utilization, dental treatment, and exposure to jewelry use. They are present in soil, air, drinking water, cosmetics, medicines, fuel, etc. Dental restorations were connected to mercury, palladium, and gold exposure, while the patient's surroundings were linked to nickel and titanium exposure. The metal's distribution in the various target organs depends on the dose, route, and length of exposure [35–37].

Toxic metals alter the metabolism of metallothioneins and promote the synthesis of free radicals, increasing oxidative stress and causing depletion of the body's primary antioxidants. According to Lawrence et al. [38], toxic metals can damage mitochondrial DNA, resulting in mitochondrial dysfunction and oxidative stress. The depletion of glutathione and the amino acid cysteine, which is essential for glutathione synthesis, reduces the body's defenses against free radicals. Some of them, like Pb, negatively affect the metabolism of cytokines, including the interleukins IL-2, IL-1b, IL-8, IL-6, IL-4, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN), as well as the expression and functioning of inflammatory enzymes such as cyclooxygenases [39]. It has also been proposed that exposure to certain toxic metals could disrupt the delicate balance between the immune and central nervous system [33]. It is interesting to note that the symptoms of metal toxicity, such as Pb and Al, include poor sleep, diffuse muscle pain, brain fog, chronic malaise, headaches, numbness, dizziness, and anxiety, which are the same as those of fibromyalgia [29].

Pathways that are toxic or allergic are crucial in metal pathology. Genetics may determine a person's susceptibility to metals and ability for detoxification. Additionally, it has been shown that smoking, which is linked to nickel sensitivity, is connected to SLE and R.A [7, 8]. Alcohol consumption may reduce the risk of arthritis at higher concentrations of Hg and Se [29]. Workplace co-exposure to mineral lubricants, silica, traffic pollution, and certain metals, including nickel, mercury, and palladium, are additional risk factors for connective tissue diseases. SLE has been observed to occur often in a population exposed to petroleum products and mercury [40]. A significant frequency of delayed-type metal hypersensitivity, notably to gold, titanium, nickel, palladium, mercury, or chromium, has also been shown to occur in SLE, Sjögren's syndrome, and R.A. patients. In people with metal sensitivities, reducing metal exposure may reduce inflammation and improve the effectiveness of conventional treatment [29].

Further research is needed on the role of metals in developing rheumatic illnesses and on potential immunotoxic processes; it is also unknown if metals interact with shielding micronutrients like vitamins and selenium.

The study's strengths include research with people who meet the worldwide criteria for rheumatic disorders and various study designs on toxic metals in rheumatic diseases. The authors assert that all documented instances of hazardous metal exposure in rheumatoid arthritis patients were gathered this way. However, some limitations were also observed. For example, no comparison between supplementation of micronutrients and classical treatments used in rheumatic diseases was available. Moreover, the number of participants in each study was small. In addition, a few rheumatic diseases were studied. Therefore, future studies, including larger patient samples, long-term follow-up, and diverse kinds of rheumatic disorders, are needed to understand the actual value of toxic metals in rheumatic diseases.

Conclusion

There are few studies in the literature evaluating the effects of toxic metals in rheumatological diseases, and only nine such conditions were addressed in this review.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: AI-assisted Technologies (chatGPT) tested the study's clarity and linguistic structures. However, the article's final version was accepted and submitted on the authors' oversight.

Authorship Contributions: Concept – JFDC, TLS; Design – JFDC, TLS; Supervision – JFDC, TLS; Fundings – JFDC, TLS; Materials – JFDC, TLS; Data collection and/or processing – JFDC, TLS; Analysis and/or interpretation – JFDC, TLS; Literature review – JFDC, TLS; Writing – JFDC, TLS; Critical review – JFDC, TLS.

Peer-review: Externally peer-reviewed.

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Appendix 1. Summary of the studies that evaluated toxic metals in rheumatoid arthritis

Author, reference	Study design	Country	n/gender	Age, years	Disease	Disease duration	Metal intoxication	Clinical and laboratory features
Khadim et al., 2023 [4]	Case-control	Iraq	120 R.A. and 60 HC 100% female.	20–60	R.A.	ND	Zn, Cu, Mg, Mn, Fe, Ni, Cr, K, Na, Ca, Pb, and Cd	In R.A. →Cu, Ni, Na, Pb, and Cd were high.
Joo et al., 2018. [5]	Cross-sectional study using Korean National Health and Nutrition Examination Survey (KNHANES) data	Korea	1,5559 R.A. from 53,829 subjects, 77% females	55.7	R.A.	ND	Cadmium, lead, mercury, zinc, and arsenicum were evaluated.	Cadmium was elevated in R.A. vs. control: 1.30±0.07 μg/L vs. 1.17±0.01 μg/L, p<0.01. No significant differences in urine levels of As or serum levels of Pb, Hg, Mn, or Zn
Yang et al., 2016 [6]	Cross-sectional	Taiwan	122 72% females	55.37± _ 13.51	R.A.	ND	Copper, Cr, As, Pb, Hg, Zn, Se.	R.A. patients living where farm soils contained high levels of copper had increased white blood cell counts, erythrocyte sedimentation rate, and disease activity score 28, compared with patients living where copper levels were low.
Afridi et al., 2015 [7]	Cross-sectional	Pakistan	53 49% females	42–56	R.A.	ND	Arsenic, cadmium mercury, and lead in blood and hair.	As Cd, Hg, and Pb were significantly higher in scalp hair and blood samples of R.A. than controls.
Afridi et al., 2011 [8]	Cross-sectional	Pakistan	53 49% females	42–56	R.A.	ND	Zn, Cu, Mn, Pb, and Cd were measured in scalp hair	Cd and Pb were significantly higher in scalp hair samples of R.A.
Bamonti et al., 2011. [9]	Case report	Italy	1 Female	63	R.A.	10 years	Aluminum, cadmium, and lead.	Lack of metacarpophalangeal joint range of motion and alignment. Increased CRP and ESR. She was treated with EDTA once a week for 1 year. the patient did not show any signs of mental intoxication. RA symptoms and oxidative status improved. After 6 months, methotrexate was reduced to 10 mg once every 10 days, and prednisolone to 5 mg/day.

Appendix 1 (cont). Summary of the studies that evaluated toxic metals in rheumatoid arthritis

Author, reference	Study design	Country	n/gender	Age, years	Disease	Disease duration	Metal intoxication	Clinical and laboratory features
Christensen et al., 1986 [10]	Cross-sectional	Denmark	10 vs. 10 HC	ND	R.A.	ND	Cr. Ni and Cd in blood, serum, urine, and sweat	Ni was higher in R.A. in the 3 fluids. Cr is higher in urine and lower in blood in R.A.
Niedermeyer et al., 1971 [11]	Cross-sectional	United States	44	45	R.A.	ND	Copper, iron, manganese, strontium, chromium, zinc, and molybdenum, Aluminum. barium, nickel, cesium, tin, lead and cadmium	In serum copper, barium, cesium, manganese, tin, and molybdenum were high; iron, zinc, and lead were low, and aluminum, nickel, strontium, chromium, and cadmium were normal. In joint fluid, the levels were normal.

ANA: Antinuclear antibodies; Cd: Cadmium; Cu: Copper; HC: Healthy controls; Mn: Manganese; RA: Rheumatoid arthritis; OA: Osteoarthritis; Pb: Lead; Zn: Zinc; ND: Not described; HC: Healthy controls.

Appendix 2. Summary of the studies that evaluated toxic metals in several rheumatic diseases

Author, reference	Study design	Country	n/gender	Age (years)	Disease	Disease duration	Metal intoxication	Clinical features
Pedro et al., 2017 [12]	Cross-sectional	Brazil	105 SLE and 120 HC 96% females	39.7 years	SLE	8 (3-15)	Li, V, Cu, Zn, Mo, Cd, and Pb in serum.	Lower V, Zn, and Pb, Mo, and Li in SLE. SLE diagnosis is associated with higher Li and lower V, Zn, and Pb. No association with disease activity.
Crowe et al., 2016 [13]	Cross-sectional	United Kingdom	52 96.2% females	48± 13.19	SLE	ND	Mercury in hair	Hair Hg, BILAG, and SLICC/ACR had a negative connection (r=_0.323, p=0.029 and r=_0.377, p=0.038, respectively). Hg in the urine did not correlate with disease activity or damage.
Kisaoglu et al., 2006 [14]	Case report	Turkiye	1 Female	13	SLE	1 month	Mercury	Myalgia, weight loss, hypertension, and proteinúria. Positive ANA, anti-dsDNA, and hypocomplementemia. SLE cured after treatment.
Prochazkova et al., 2004 [15]	Prospective	Czech Republic	15 66.7% females	36.5	SLE	13.3 months	Amalgama (inorganic mercury, silver, organic mercury, and lead)	10/15 improved, 4/15 unchanged, and 1 worsed objectively in the long-term.
Federmann et al., 1994 [16]	Case report	Switzerland	1 Female	24	SLE	ND	Lymphocyte transformation test (during prednisone at 10 mg/d) indicated a hypersensitivity to molybdenum	Clinical picture; fever, arthritis, oral ulcers, alopecia, pancytopenia, Coombs, anti-Ro/SS-A, anti-Sm, anti-RNP. and low complement levels.
Xu et al., 2022 [17]	Cross-sectional	United States	736 gout and 2,766 hyperuricemia from 14,871 50.7% females	50.02± 17.57	Gout and hyperuricemia	ND	Mercury	Mercury (quartile 2 and 4), lead (quartiles 2, 3, and 4), and selenium (quartiles 2 and 4) were found to be positively correlated with SUA and hyperuricemia. Higher levels of mercury and lead was associated with gout,
Zhang et al., 2021 [18]	Cohort	China	408 100% males	42.5 (33–53)	Gout	ND	Cadmium and lead	C and Pb were higher in gout than H.C. Cd levels were significantly associated with gout flare.

Appendix 2 (cont). Summary of the studies that evaluated toxic metals in several rheumatic diseases

Author, reference	Study design	Country	n/gender	Age (years)	Disease	Disease duration	Metal intoxication	Clinical features
Krishnan et al., 2012 [19]	Population-based cross-sectional	United States	290 gout from 6,153 individuals	64.7	Gout	ND	Lead	Gout was 6.05% (95% CI, 4.49% to 7.62%) among patients with the highest lead levels. Each doubling of lead levels was associated with an odds ratio of 1.74 (CI, 1.47 to 2.05) for gout and 1.25 (CI, 1.12 to 1.40) for hyperuricemia.
Halla et al., 1982 [20]	Cross-sectional	United States	42	ND.	Gout	ND	Lead	In 62% of patients, gout and plumbism were diagnosed simultaneously; in 26%, the plumbism preceded the gout; and in 12%, gout preceded the plumbis. EDTA was used with a good response.
Forte et al., 2018 [21]	Cross-sectional	Italy	27 vs. 30 HC 66.7% females	55.7± 14.3	SSc	8.11± 8.01	Al, Cd, Hg, and Pb in blood and urine	Al was lower in blood and increased in urine. Pb was increased in the blood. Hg in urine was associated with a higher severity of the disease.
Marie et al., 2017 [22]	Case-control	France	100 vs. 300 HC 78% females	52	SSc	2 years	Antimony, cadmium Lead, mercury, molybdenum, palladium, and zinc	The median values of the following metals were greater in SSc patients: antimony, cadmium, lead, mercury, and palladium.
Arnett et al., 2000 [23]	Cross-sectional	Canada	13 (anti-fibrillarin 11 SSc), 39 SSc, and 32 controls 92% females	46	SSc, Anti-fibrillarin	6 years	Urinary mercury	Mean urinary mercury was higher in positive anti-fibrillarin patients. Although, no difference was seen when patients with low creatine were excluded.
Motts et al., 2014 [24]	Cross-sectional	Brazil	371 30.7% females	ND.	Miners	ND	Mercury	Exposure to mercury and increased blood levels of 3760 autoantibodies. The following are the most significant proteins: interferon-induced transmembrane protein, signal peptide peptidase like 2B, triggered by retinoic acid 13, tumor necrosis factor ligand superfamily member 13, and antibodies to GSTA1.

Appendix 2. Summary of the studies that evaluated toxic metals in several rheumatic diseases

Author, reference	Study design	Country	n/gender	Age (years)	Disease	Disease duration	Metal intoxication	Clinical features
Gardner et al., 2010 [25]	Cross-sectional	Brazil	246 30% females	ND.	Miners	ND	Mercury	As compared to diamond and emerald miners who had no occupational exposure to mercury, mercury-exposed gold miners exhibited greater frequencies of detectable ANA and ANoA, higher titers of ANA and ANoA, and higher levels of IL-1 β, TNF α, and IFN.-y.
Stejskal et al., 2013 [26]	Transversal and prospective	Norway	15 Females	47.6 (34–66)	Fibromyalgia	11 (2–29) years	Nickel, inorganic mercury, cadmium and lead	All FM patients had positive test results for at least one of the metals examined. Nickel-related reactions were most common, followed by inorganic mercury, cadmium, and lead. Dental metal restorations should be replaced, and known sources of metal exposure should be avoided. After five years, 30% of patients still had FM, 20% had improved, and 50% no longer met the criteria for the F.M. diagnosis. All patients reported an improvement in their subjective health. This was connected with the in vitro normalization of reactions to metals.
Cemek et al., 2010 [27]	Cross-sectional	Turkiye	33 48.5% females	11.4± 3.82	Acute rheumatic fever	ND.	aluminum (Al) and barium (Ba) were lower, whereas the copper (Cu), beryllium (Be), cadmium (Cd), chromium (Cr), gallium (Ga), and strontium	Metals were similar to controls. Metals did not change after R.F. treatment.
Wronski et al., 1977 [28]	Case report	Germany	1 Female	50	PAN	33	Mercury	A dentist assistant chronically exposed to mercury had erethism, tremors, and mercurial psellism. Peripheral arterial circulatory disorders occurred in the course of the disease, as well as abdominal colic and polyneuropathy

Appendix 2. Summary of the studies that evaluated toxic metals in several rheumatic diseases

Author, reference	Study design	Country	n/gender	Age (years)	Disease	Disease duration	Metal intoxication	Clinical features
Fang et al., 2023 [29]	Cross-sectional	United States	1,375 OA and 468 RA from 9,735 51.1% females	50	OA RA	ND	Cd, Pb, and mercury	Cadmium (Cd) and lead (Pb) statistically grew the risk of total arthritis, O.A., and R.A.
Stejskal et al., 2015 [30]	Cross-sectional	Norway	38 92% females	51 (27–77)	SLE (n=0), R.A. (n=16), and S.S. (n=13).	ND.	The test menu for late hypersensitivity included inorganic and organic mercury, tin, copper, silver, gold, níquel, Pd, cadmium, lead, and titanium.	87% had a positive lymphocyte reaction to at least 1 metal and 63% to ≥2 metals. Control 43% (1 metal) and 18% (≥2 metals). The most frequent allergens were nickel, mercury, gold, and palladium.
Albert et al., 2004 [31]	Questionnaire	United States	53 (W.G.), 50 (O.A.), and 53 Gout; 50.9% females	61.8	WG, OA, and Gout	ND		Lead exposure was positively associated with W.G. as compared to OA and gout.
Yildiz et al., 2020 [32]	Case series	Turkiye	5 pediatric patients; 60% females	4, 6, 7, 9, 14	Pt. 1 and 2: PAN-like Pt. 3: asymptomatic Pt. 4 and 5: Erythromelalgia	2 (n=1) and 3 (n=2) weeks; 5 months (n=2)	Mercury	After 3 weeks of DMSA treatment, all symptoms improved. In addition, antihypertensive drugs were gradually reduced.
Guan et al., 2023 [33]	Cross-sectional	United States	514 arthritis from 2,174 individuals. 53.5% females	59.26± 13.2	Arthritis	ND	Cadmium (Cd), lead (Pb), mercury (Hg)	Increased risk of arthritis: Pb [OR (95% CI): 2.96 (2.18, 4.03), the p-value for trend (P-t) <0.001], Cd [OR (95% CI): 2.28 (1.68, 3.11), P-t <0.001]. Subgroup analysis showed that Pb and Cd ions significantly correlated with osteoarthritis and rheumatoid arthritis.
Stamp et al. 2015 [34]	Case-control	New Zealand	49 cases vs. 196 controls 53% males	64.9± 12.4	Granulomatosis with polyangiitis	11.3 years	Occupation exposure: brick/foundry worker, sand blaster, dental technician, mine/quarry worker	No association with metal exposure in the year prior.

ANA: Antinuclear antibodies; ANoA: Anti-nucleolar antibodies; SUA: Serum uric acid; Cd: Cadmium; Cu: Copper; HC: Healthy controls; Mn: Manganese; RA: Rheumatoid arthritis; SSc: Systemic sclerosis; OA: Osteoarthritis; PAN: Polyarteritis nodosa; Pb: Lead; WG: Wegener's granulomatosis; DMSA: Meso-2,3-dimercaptosuccinic acid; Zn: Zinc; ND: Not described.