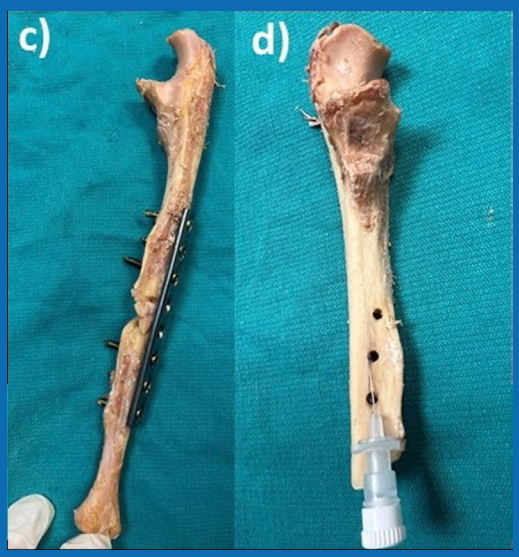
ISSN : 1300-414X
e-ISSN 2645-9027

ULUDAĞ ÜNİVERSİTESİ
TIP FAKÜLTESİ DERGİSİ

Cilt / Volume 51 Sayı / Issue 2 Yıl / Year 2025



Bu Sayıda;

JOURNAL OF ULUDAĞ UNIVERSITY MEDICAL FACULTY

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ISSN 1300-414X
e-ISSN 2645-9027

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Journal of Uludağ University Medical Faculty

Cilt 51 / Sayı 2 / 2025
Volume 51 / Issue 2 / 2025

ULUDAĞ ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ
Journal of Uludağ University Medical Faculty
Cilt / Volume: 51 / Sayı / Issue: 2 / 2025

Yayın Kurulu / *Editorial Board*

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ULUDAĞ ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ
Journal of Uludağ University Medical Faculty
Cilt / Volume: 51 / Sayı / Issue: 2 / 2025

DANIŞMA KURULU / *ADVISORY BOARD*

2023

Bursa Uludağ Üniversitesi / *Bursa Uludağ University* ISSN 1300 - 414X, e-ISSN 2645-9027
Bursa Uludağ Üniversitesi Tıp Fakültesi Yayın Organıdır. / *Published by Bursa Uludağ Uiversity Faculty of Medicine*
Yılda 3 kez Nisan / Ağustos ve Aralık aylarında çevrimiçi olarak yayınlanır. / *Published online 3 times a year in April, August and December.*
Yazışma Adresi / *Mailing Address*: Seyhan Miğal / Bursa Uludağ Üniversitesi Tıp Fakültesi Dekanlığı Yayın Kurulu Sekreteri / 16059 Görükle-BURSA
Tel / *Phone*: (0.224) 4428017 / Faks: (0.224) 4428047 e-posta: uludag.tip.dergisi@gmail.com
Web sayfası / *Web page*: https://dergipark.org.tr/tr/pub/uutfd
Dizgi-Baskı / *Typesetting-Priting*: Bursa Uludağ Üniversitesi Basımevi Bursa – 2025

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ÖZGÜN ARAŞTIRMA / *RESEARCH ARTICLE*

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 165-173, 2025
DOI: https://doi.org/10.32708/uutfd.1672724

ORIGINAL RESEARCH

Ulnar Nutrient Foramen Morphology and Minimizing Screw Damage in Ulnar Fractures

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ABSTRACT

The aim of this study is providing an in-depth investigation of the morphological and morphometric characteristics of the nutrient foramen (NF) to minimize the damaging of the NF during fixation of ulnar fractures. This study was performed by using a sample of 110 dry ulnae (47 right, 63 left). The ulnar length (UL), distance between the NF and the most proximal point of olecranon (NFO), as well the distance between the NF and interosseous border (NFIB) were taken under measurement. The number, location, direction, position, and size of NF was evaluated. Also, the foraminal index (FI) was calculated. In addition, forearm fracture was created experimentally on a cadaver belonging to a 73-year-old Turkish female in our department, and ulnar open reduction internal fixation was performed. After the removal of the plate and screws, the effect of plate fixation on the NF was evaluated and examined for further treatment decisions. The mean UL, NFO, NFIB and FI were measured to be 244.16 ± 17.65 mm, 93.76 ± 18.11 mm, 8.23 ± 3.67 mm, and 38.29 ± 6.45%, respectively. In general, 1 NF was detected on the ulnae, which was located anterior surface, directed upwards, on 2/5 zone and smaller than 22-gauge size. The distances between the most proximal first, second and third screws and interosseous border were measured to be 4.39 mm, 6.20 mm, and 6.56 mm, respectively. The screws were found not to have damaged the NF on fractured ulna of cadaver. According to our dry bone and cadaveric results, being knowledgeable about the morphologic and morphometric characteristics of NF is important in screwing the plate, which should be done to the distal parts of shaft if possible and near the anterior border of ulna for minimizing the damage to the nutrient foramen in order to preserve the blood supply of the bone.

Keywords: Ulna. Nutrient foramen. Fracture. Screw. Cadaver. Anatomy.

Ulna'nın Foramen Nutricium Morfolojisi ve Ulnar Kırıklarda Vida Hasarını En Aza İndirme

ÖZET

Bu çalışmanın amacı, ulnar kırıkların tespiti sırasında foramen nutricium'un (NF) zarar görmesini en aza indirmek için NF'nin morfolojik ve morfometrik özelliklerinin derinlemesine incelenmesidir. Bu çalışma, 110 kuru ulna örneği (47 sağ, 63 sol) kullanılarak gerçekleştirilmiştir. Ulna uzunluğu (UL), NF ile olekranonun en proksimal noktası arasındaki mesafe (NFO) ve NF ile interosseöz kenar arasındaki mesafe (NFIB) ölçüldü. NF'nin sayısı, konumu, yönü, durumu ve boyutu değerlendirildi. Ayrıca, foraminal indeks (FI) hesaplandı. Ayrıca, bölümümüzde 73 yaşındaki bir Türk kadavrası üzerinde deneysel olarak ön kol kırığı oluşturuldu ve ulnar açık redüksiyon internal fiksasyon uygulandı. Plaka ve vidaların çıkarılmasından sonra, plaka fikstürünün NF üzerindeki etkisi değerlendirildi ve ileri tedavi kararları için incelendi. Ortalama UL, NFO, NFIB ve FI sırasıyla 244,16 ± 17,65 mm, 93,76 ± 18,11 mm, 8,23 ± 3,67 mm ve 38,29 ± 6,45% olarak ölçüldü. Genel olarak, ulna kemiklerinde anterior yüzeyde, yukarıya doğru, 2/5 bölgesinde ve 22-gauge boyutundan daha küçük olan 1 NF tespit edildi. En proksimal ilk, ikinci ve üçüncü vidalar ile interosseöz kenar arasındaki mesafeler sırasıyla 4,39 mm, 6,20 mm ve 6,56 mm olarak ölçüldü. Vidaların kadavradaki kırık ulna üzerindeki NF'ye zarar vermediği bulundu. Kuru kemik ve kadaverik sonuçlarımıza göre, NF'nin morfolojik ve morfometrik özellikleri hakkında bilgi sahibi olmak, plakanın vidalanmasında önemlidir; bu vidalama mümkünse şaftın distal kısımlarına ve ulnanın anterior kenarına yakın bir yerde yapılmalı, böylece besleyici foramenin zarar görmesi en aza indirilerek kemiğin kan akışının korunması sağlanmalıdır.

Anahtar Kelimeler: Ulna. Foramen Nutricium. Kırık. Vidalama. Kadavra. Anatomy.

Date Received: 10.April.2025
Date Accepted: 28.May.2025

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Ulnar shaft fractures constitute one of the most common injuries observed in the forearm. Unstable fractures of ulna usually occur on the proximal 1/3 of the ulna. For these fractures, plate fixation with open reduction and internal fixation techniques are used[1]. In the human body, the skeleton is formed by bones, making up the passive component of movement[2]. The arterial supply of long bones are provided by metaphyseal, epiphyseal, periosteal and nutrient arteries[3]. The main blood supply to a long bone derives from the nutrient arteries, which is crucial during the prenatal phase of long bones as well as during the early ossification phase[4,5]. Furthermore, 70–80% of the nutrition of the bones is supplied by nutrient arteries, particularly during puberty. Inadequate vascularization of the epiphyseal plate causes medullary bone ischemia as bone nutrition is diminished[6]. The regional arteries frequently serve as the source for nutrient arteries. The nutrient artery enters the long bones' shafts through a foramen known as the nutrient foramen (NF). The NF is an aperture on the long bone's shaft that allows the passage of peripheral nerves and nutrient arteries into the medullary cavity[2]. The nutrient artery divides into ascending and descending branches in the medullary cavity and supplies the inner two-thirds of the cortex and the bone marrow[7]. The preservation of the nutrient arterial supply is crucial in various surgical procedures and pathologic conditions: microsurgical vascularized bone transplantation, tumor resections, bone grafts, acute osteomyelitis and fracture healing[8,9].

To avoid damage to the NF during fixation of the ulnar fracture, it is important to protect the normal vascular supply of ulna. For this purpose, this study investigated the morphological and morphometric characteristics of the NF in detail in order to minimize damaging of the NF when applying fixation for the ulnar fractures.

Material and Method

This study was performed by using a sample of 110 dry ulnae (47 right, 63 left), which were obtained from the anatomy laboratory of authors. The age and sex of the ulnae were unknown. The bones with cortical deformity, osteoporotic appearance, and fractures were excluded from the study. The ethical approval was obtained from the Ethics Committee of authors' University (date: 09/01/2024, number: 2024/01-56). Initially, the NF was detected macroscopically on the surfaces (anterior, posterior, medial) and borders (anterior, posterior, interosseous) of ulna by using magnifying glass. The presence of the well-defined groove and canal was accepted as NF. 20 (1.1 mm)-22 gauge (0.8 mm) sized hypodermic needles were used to determine the size of foramen, respectively.

The following parameters were evaluated:

1) The ulnar length (UL): distance between the most proximal point of olecranon and the most distal point of styloid process (Fig. 1)
2) Number of NF
3) Location of NF according to surfaces and borders
4) Direction of NF (upward, downward)
5) Position of NF
6) Size of NF
7) Distance between the NF and the most proximal point of olecranon (NFO) (Fig. 1)
8) Distance between the NF and interosseous border (NFIB) (Fig. 1)
9) Foraminal index: (NFO/UL)*100

The ulnar length was measured by use of tape measure and the other measurements were conducted by using 0.001 mm accuracy digital Vernier caliper. The position of NF on ulna was divided under 5 types according to foraminal index:

Zone 1: the foraminal index was lower than 20%
Zone 2: the foraminal index was between the 20% and 40%
Zone 3: the foraminal index was between the 40% and 60%
Zone 4: the foraminal index was between the 60% and 80%
Zone 5: the foraminal index was between the 80% and 100%

Also, localization of NF was divided into 5 types according to interosseous border:

Type 1: NF was located on the interosseous border
Type 2: NF was 0-5 mm away from interosseous border
Type 3: NF was 5-10 mm away from interosseous border
Type 4: NF was 10-15 mm away from interosseous border
Type 5: NF was 15-20 mm away from interosseous border

In addition, a cadaver that belonged to a 73-year-old Turkish female in our department with no forearm fractures and intact subcutaneous muscle integrity, access to the ulnar shaft was achieved via a Subcutaneous Approach between the extensor carpi ulnaris and flexor carpi ulnaris muscles in order to demonstrate the relationship of the NF to the ulnar shaft with anatomical plating on the left sided ulna. An experimental segmental ulnar shaft fracture model was successfully created using a saw and chisel[10,11]. Subsequently, anatomical bridge plate fixation was applied to the ulnar shaft fracture. Following dissection of the muscles and ligaments adhering to the ulna and excision of the ulna for examination of the experimental model, the plate and screws were removed (Fig. 2).

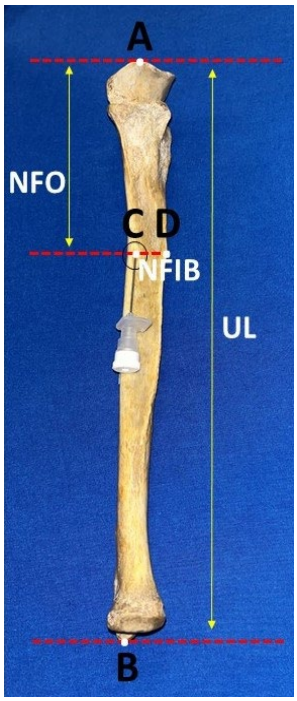

Fig. 1:
Demonstration of morphometric measurements of nutrient foramen. A: the level of the most proximal point of olecranon, B: the level of the most distal point of styloid process, C: the level of entrance of nutrient foramen, D: the interosseous level of nutrient foramen. UL (A-B): the ulnar length, NFO (A-C): distance between the nutrient foramen and the most proximal point of olecranon, NFIB (C-D): distance between the nutrient foramen and interosseous border. Black circle indicates the entrance point of the hypodermic needle into the nutrient foramen.

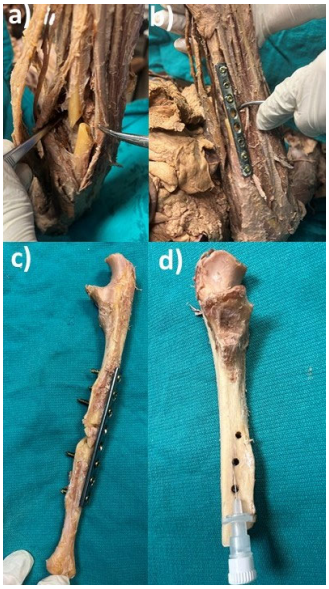

Fig. 2:
Demonstration of fixation of ulnar fracture. a) fractured ulna, b) fixation of ulnar fracture, c) excision of the ulna d) relationship between the screw points and nutrient foramen.

Statistical analysis

Descriptive analyzes (percentage, mean, standard deviation, minimum and maximum values) were performed using the SPSS version 23 (Statistical Package for the Social Sciences–SPSS Inc.) software. The normal distribution of the parameters was examined by histogram graphs and Kolmogorov-Smirnov/Shapiro-Wilk tests. The differences between the right and left side were compared using the Student's t test or the Mann-Whitney U test, depending on whether the parameters were normally distributed. Whether there was a statistical difference between the right and left side in categorical parameters was investigated by using the Chi-square test, and Fisher's exact test was used in cases where the Chi-square test assumptions could not be met. p value lower than 0.05 was considered statistically significant.

Results

Number of NF

One hundred and ten ulnae were evaluated in this study. No NF was detected on 3 of 110 (2.73%) ulnae. One NF was detected on 107 of 110 (97.27%) ulnae. On the right side, no NF was detected in 1 of 47 (2.13%) ulnae and 1 NF was detected in 46 of 47 (97.87%) ulnae. On the left side, no NF was detected in 2 of 63 (3.17%) ulnae and 1 NF was detected in 61 of 63 (96.83%) ulnae (Table I).

Table I. Number of NF

Number of NF	Right n (%)	Left n (%)	Total n(%)
0	1 (2.13)	2 (3.17)	3 (2.73)
1	46 (97.87)	61 (96.83)	107 (97.27)

NF: nutrient foramen, n: number

Direction of NF

All of the NF were observed to be directed upward.

Localization of NF

96 of 107 (89.72%) NF were located on anterior surface, 7 of 107 (6.54%) on anterior border, 3 of 107 (2.81%) on interosseus border and 1 of 107 (0.93%) on posterior surface. No NF was observed on medial surface and posterior border. On the right side, 40 of 46 (86.96%) NF were located on the anterior surface, 5 of 46 (10.87%) on the anterior border, 1 of 46 (2.17%) on the interosseous border. On the left side, 56 of 61 (91.80%) NF were located anterior surface, 2 of 61 (3.28%) on the anterior border, 2 of 61 (3.28%) on the interosseous border, and 1 of 61 (1.64%) on the posterior surface. There was no statistically significant difference between right and left sides regarding the location of NF (p=0.401) (Table II).

Table II. Localization of NF

Surface and border	Right n(%)	Left n(%)	Total n(%)	p value
Anterior Surface	40 (86.96)	56 (91.80)	96 (89.72)	0.401
Posterior Surface	-	1 (1.64)	1 (0.93)	
Medial surface	-	-	-	
Anterior Border	5 (10.87)	2 (3.28)	7 (6.54)	
Posterior border	-	-	-	
Interosseous Border	1 (2.17)	2 (3.28)	3 (2.81)	

NF: nutrient foramen, n: number

Size of NF

The sizes of 46 of 107 (42.99%) NF were lower than 22-gauge size, 33 of 107 (30.84%) NF were equal or higher than 20 gauge and 28 of 107 (26.17%) NF were within the 20–22-gauge interval. On the right side, 21 of 46 (45.65%) NF were lower than 22 gauge, 14 of 46 (30.44%) were equal or more than 20 gauge, 11 of 46 (23.91%) NF were between the 20-22 gauge. On the left side, 25 of 61 (40.98%) NF were lower than 22-gauge size, 19 of 61 (31.15%) were equal or higher than 20 gauge, 17 of 61 (27.87%) NF were within the 20–22-gauge interval. There was no statistically significant difference between the right and left sides regarding the size of NF (p=0.863) (Table III).

Table III. Size of NF

Size	Right n(%)	Left n(%)	Total n(%)	p value
≥20 gauge	14 (30.44)	19 (31.15)	33 (30.84)	0.863
Between 20-22 gauge	11 (23.91)	17 (27.87)	28 (26.17)	
<22 gauge	21 (45.65)	25 (40.98)	46 (42.99)	

NF: nutrient foramen, n: number

The ulnar length (UL)

The mean value of UL was found to be 244.77±17.42 mm on the right side, 243.71±17.94 mm on the left side, and 244.16±17.65 mm overall. No statistically significant difference was found between the right and left sides regarding UL (p=0.759) (Table IV).

Table IV. Morphometric properties of NF

Parameter	Right	Left	Total	p value
UL (mm)	244.77±17.42	243.71±17.94	244.16±17.65	0.759
NFO (mm)	91.51±13.91	95.45±20.68	93.76±18.11	0.268
NFIB (mm)	8.75±3.50	7.84±3.77	8.23±3.67	0.278
FI (%)	37.43±5.15	38.95±7.25	38.29±6.45	0.339

NF: nutrient foramen, UL: the ulnar length, NFO: distance between the NF and most proximal point of olecranon, NFIB: distance between the NF and interosseous border, FI: foraminal index

Distance between NF and most proximal point of olecranon (NFO)

The mean value of NFO was found to be 91.51±13.91 mm on the right side, 95.45±20.68 mm on the left side, 93.76±18.11 mm overall. No statistically significant

Distance between the NF and interosseous border (NFIB)

The mean value of NFIB was found 8.75±3.50 mm on the right side, 7.84±3.77 mm on the left side, 8.23±3.67 mm overall. No statistically significant difference was found between the right and left sides for NFIB (p=0.278) (Table IV).

Foraminal index (FI)

The mean value of FI was found to be 37.43±5.15% on the right side, 38.95±7.25% on the left side, 38.29±6.45% overall. No statistically significant difference was found between the right and left sides regarding FI (p=0.339) (Table IV).

Position of NF

72 of 107 (67.29%) NF were detected on Zone 2, 34 of 107 (31.78%) NF on Zone 3 and 1 of 107 (0.93%) NF on Zone 4. No NF was detected on Zone 1 and Zone 5. On the right side, 32 of 46 (69.57%) NF were detected on Zone 2 and 14 of 46 (30.43%) NF on Zone 3. No NF was detected on Zone 1, Zone 4 and Zone 5. On the left side, 40 of 61 (65.57%) NF were detected on Zone 2, 20 of 61 (32.79%) NF on Zone 3 and 1 of 61 (1.64%) NF on Zone 4. No NF was detected on Zone 1 and Zone 5. No statistically significant difference was found between the right and left sides regarding the position of NF (p=0.906) (Table V).

Table V. Position of NF

Position	Right n(%)	Left n(%)	Total n(%)	p value
Zone 1	0 (0)	0 (0)	0 (0)	0.906
Zone 2	32 (69.57)	40 (65.57)	72 (67.29)	
Zone 3	14 (30.43)	20 (32.79)	34 (31.78)	
Zone 4	0 (0)	1 (1.64)	1 (0.93)	
Zone 5	0 (0)	0 (0)	0 (0)	

NF: nutrient foramen, n: number

Distribution of nutrient foramen according to interosseous border

The distance between NF and interosseous border was divided into 5 types, and 52 of 107 (48.60%) NF were observed to be 5-10 mm away (Type 3) from interosseous border, 31 of 107 (28.97%) NF were 10-15 mm away (Type 4), 19 of 107 (17.76%) NF were 0-5 mm away (Type 2), 3 of 107 (2.80%) NF were on the interosseous border (Type 1) and 2 of 107 (1.87%) NF were 15-20 mm away (Type 5). On the right side, 23 of 46 (50.00%) NF were 5-10 mm away (Type 3) from interosseous border, 15 of 46 (32.62%) NF were 10-15 mm away (Type 4), 6 of 46 (13.04%) NF were 0-5 mm away (Type 2), 1 of 46 (2.17%) NF was on

the interosseous border (Type 1) and 1 of 46 (2.17%) NF was 15-20 mm away (Type 5). On the left side, 29 of 61 (47.54%) NF were 5-10 mm away (Type 3) from interosseous border, 16 of 61 (26.23%) NF were 10-15 mm away (Type 4), 13 of 61 (21.31%) NF were 0-5 mm away (Type 2), 2 of 61 (3.28%) NF were on the interosseous border (Type 1) and 1 of 61 (1.64%) NF was 15-20 mm away (Type 5). No statistically significant difference was found between the right and left sides regarding the distribution of NF according to interosseous border (p=0.824) (Table VI).

Table VI. Distribution of NF according to interosseous border

Distance from IB (mm)	Right n(%)	Left n(%)	Total n(%)	p value
On the IB (Type 1)	1 (2.17)	2 (3.28)	3 (2.80)	0.824
0-5 (Type 2)	6 (13.04)	13 (21.31)	19 (17.76)	
5-10 (Type 3)	23 (50.00)	29 (47.54)	52 (48.60)	
10-15 (Type 4)	15 (32.62)	16 (26.23)	31 (28.97)	
15-20 (Type 5)	1 (2.17)	1 (1.64)	2 (1.87)	

NF: nutrient foramen, IB: interosseous border, n: number

Cadaveric results

The length of fractured ulna was observed to be 225 mm. The distance between the fracture line and the most proximal point of olecranon was found to be 126.39 mm. One NF was detected on fractured ulna. The size of NF was between the 20-22 gauge and 96.59 mm away from the most proximal point of ulna. Foraminal index of this NF was 42.93% and located on the anterior surface. The distance between the NF and interosseous border was 8.89 mm. The distance between the most proximal first, second and third screws and the most proximal point of olecranon were 80.07 mm, 92.81 mm, 103.76 mm, respectively. The distances between the most proximal first, second and third screws and interosseous border were 4.39 mm, 6.20 mm, and 6.56 mm, respectively.

Discussion and Conclusion

While ulnar shaft fractures are relatively rare compared to other bones of the upper limb, these fractures result in joint instability, malunion and nonunion. Ulnar shaft fractures may be treated with non-operative and operative approaches. One of the operative approaches is the open reduction and internal fixation[12]. Nutrient arteries provide the main blood supply of long bones which enter the bone through the NF on the shaft of the long bones[13,14]. In a study of Kinose et al., they dissected a total of 67 forearms on cadavers belonging to deceased Japanese persons. As a result, it is found out that the majority of ulnar nutrient arteries originated directly from proximal part of ulnar artery (27 of 68 ulnar nutrient arteries) and anterior interosseous artery (21 of 68 ulnar nutrient arteries)[15]. During fixation of the ulnar shaft fracture, screwing should be done carefully in order to not damage the nutrient foramen. In the event the nutrient foramen is damaged, the nutrition of the bone may be interrupted and delayed fracture healing or non-union may occur. In our experimental ulnar shaft fracture, we did no damage to the nutrient foramen but according to our dry bone results of ulnar NF, screws on the ulna were inside the danger zone according to measurement of distance between NF and interosseous border. Majority of the NF were found near the interosseous border (0-10 mm away from interosseous border) in our study. We should perform screw fixation close to the anterior border in order to minimize damaging of the NF.

The ulnar length was measured as 258.8 ± 37.9 mm by Yılar et al, 249.64 ± 18.96 mm on the right side and 246.80 ± 19.60 mm on the left side by Cihan and Toma, 252.7 ± 20.2 mm on the right side and 248.5 ± 18.2 mm on the left side by Dervisevic et al., 254.5 ± 18.3 mm by Priya et al., 254.3 mm by Pereira et al., 252.5 ± 18.5 mm on the right side and 252.2 ± 18.5 mm on the left side by Desai and Damor, 254.71 ± 21 mm by Özturk et al., 282.8 ± 12.4 mm by Ukoha et al., 259.8 ± 12.3 mm by Veeramuthu et al.[8,13,16-22]. In our study, the ulnar length was measured to be 244.16 ± 17.65 mm, lower than the findings of the aforementioned studies and in compliance with that of Chavda et al. (244.8 mm)[23]. The distance between the NF and most proximal point of olecranon and foraminal index were measured in our study as 93.76 ± 18.11 mm and 38.29 ± 6.45%, which were in accordance to other studies[8,16-23]. Morphometric characteristics of NF were summarized comparatively under Table VII.

Size of NF: Knowing the size of the NF is important as it gives us information about the diameter of the nutrient artery. There are a limited number of studies measuring the size of the NF. In a study by Veeramuthu et al., they evaluated the size of NF by use of 22, 24 and 26 gauge sized hypodermic needles and in the majority of size of NF (42 (71.18%)) were found to be between 0.5 mm and 0.7 mm[21]. Unlike Veeramuthu et al., Rangasubhe and Havaldar, who predominantly revealed size of NF (68 (59.13%)) between 0.71 mm and 1.1 mm[24]. In accordance to the study by Veeramuthu et al., the size of NF was mostly (46 (42.99%)) detected as smaller than 22 gauge (0.8 mm) in this study[21].

Distance between NF and interosseous border: It is described that the NF was localized close to the interosseous border[2]. Unlike this description, Ukoha et al. found the majority of NF (24 (64.9%)) was close to the anterior border[20]. Differing from previous studies the mean value of NFIB was measured in our study as

Table VII. Comparison of morphometric properties of NF

Study (Year)	Population	N	UL (mm)	NFO (mm)	FI (%)
Yılar et al. (2023) [22]	Turkey	155 (70 R, 85 L)	258.8±37.9	94.8±15.7	37.45
Cihan and Toma (2023) [16]	Turkey	89 (38 R, 51 L)	R: 249.64±18.96 L: 246.80±19.60		R: 37.36±5.98 L: 37.17±4.40
Chavda et al. (2018) [23]	India	150 (75 R, 75 L)	244.8	91.0	35.34
Dervisevic et al. (2023) [13]	Bosnia and Herzegovina	50 (27 R, 23 L)	R: 252.7±20.2 L: 248.5±18.2		
Priya et al. (2019) [19]	India	200 (88 R, 112 L)	254.5±18.3		35.83±6.12
Pereira et al. (2011) [8]	Brasil	146	254.3		37.9
Desai and Damor (2022) [17]	India	81 (35 R, 46 L)	R: 252.5±18.5 L: 252.2±18.5	R: 86.2±23.0 L: 88.9±26.5	R: 34.10 L: 30.83
Ozturk et al. (2022) [18]	Turkey	32 (16 R, 16 L)	254.71±21	96.34±18.9	37.75±6.46
Ukoha et al. (2013) [20]	Nigeria	50	282.8±12.4	103.3±13.1	36.70±4.56
Veeramuthu et al. (2017) [21]	India	59	259.8±12.3	97.1±13.7	36.39±5.61
Present study (2024)	Turkey	110 (47 R, 63 L)	244.16±17.65	93.76±18.11	38.29±6.45

N: sample size, R: right, L: left, NF: nutrient foramen, UL: the ulnar length, NFO: distance between the NF and most proximal point of olecranon, FI: foraminal index

Table VIII. Comparison of number of NF

Study (Year)	Population	N	0 NF n(%)	1 NF n(%)	2 NF n(%)	3 NF n(%)
Yılar et al. (2023) [22]	Turkey	155 (70 R, 85 L)	13 (8.39)	139 (89.67)	3 (1.94)	-
Cihan and Toma (2023) [16]	Turkey	89 (38 R, 51 L)	14 (15.91)	65 (73.86)	6 (6.82)	3 (3.41)
Chavda et al. (2018) [23]	India	150 (75 R, 75 L)	3 (2)	145 (96.67)	2 (1.33)	-
Dervisevic et al. (2023) [13]	Bosnia and Herzegovina	50 (27 R, 23 L)	-	R: 19 (70.4), L: 19 (82.6)	R: 7 (25.9), L: 4 (17.4)	R: 1 (3.7), L: -
Pereira et al. (2011) [8]	Brasil	146	-	144 (98.6)	2 (1.4)	-
Challa and Nanna (2019) [7]	India	50	-	49 (98)	1 (2)	-
Desai and Damor (2022) [17]	India	81 (35 R, 46 L)	2 (2.47)	75 (92.59)	4 (4.94)	-
Ozturk et al. (2022) [18]	Turkey	32 (16 R, 16 L)	-	29 (90.63)	3 (9.37)	-
Ukoha et al. (2013) [20]	Nigeria	50	11 (22)	39 (78)		
Rangasubhe and Havaldar (2019) [24]	India	100 (50 R, 50 L)	-	86 (86)	13 (13)	1 (1)
Veeramuthu et al. (2017) [21]	India	59	1 (2)	57 (96)	1 (2)	-
Present study	Turkey	110 (47 R, 63 L)	3 (2.73)	107 (97.27)	-	-

NF: nutrient foramen, R: right, L: left, N: sample size, n: number

8.23 ± 3.67 mm. Furthermore, NFIB was divided into 5 types. In accordance to the general description, NF was found closer to the interosseous border in general and the majority of NF (52 (48.60%)) were detected as Type 3 (5-10 mm away from interosseous border) in our study. No comparable studies were found for NFIB. Knowing the mean value of NFIB gives us information regarding the distance of the nutrient foramen from the interosseous border. This knowledge is important as it gives us reliable information about where we shou ld place the screws relative to the interosseous border in order to avoid damaging the nutrient foramen. We consider that this data is of importance for screw fixation procedures. When screwing, in order to minimize damage to NF, it should be better to apply screw fixation closer to the anterior border.

Number of NF: In the previous studies, 1 NF was detected on ulna in general. In accordance to the previous studies, 1 NF was detected on 107 (97.27%) ulnae in our study[7,8,13,16-18,20-24]. In certain studies, either no NF or more than 1 NF could be detected on the ulnae. No NF was detected on 13 (8.39%) ulnae by Yılar et al., on 14 (15.91%) ulnae by Cihan and Toma, on 3 (2%) ulnae by Chavda et al., on 2 (2.47%) ulnae by Desai and Damor, on 11 (22%) ulnae by Ukoha et al., and on 1 (2%) ulna by Veeramuthu et al.[16,17,20-23]. In our study, no NF was detected on 3 (2.73%) ulnae. Two NF were detected on 3 (1.94%) ulnae by Yılar et al., on 6 (6.82%) ulnae by Cihan and Toma, on 2 (1.33%) ulnae by Chavda et al., on 7 (25.9%) right sided ulnae, on 4 (17.4%) left sided ulnae by Dervisevic et al., on 2 (1.4%) ulnae by Pereira et al., on 1 (2%) ulna by Challa and Nanna, on 4 (4.94%) ulnae by Desai and Damor, on 3 (9.37%) ulnae by Özturk et al., on 13 (13%) ulnae by Rangasubhe and Havaldar, and on 1 (2%) ulna by Veeramuthu et al.[7,8,13,16-18,21-24]. Three NF were detected on 3 (3.41%) ulnae by Cihan and Toma, on 1 (3.7%) right sided ulna by Dervisevic et al., and on 1 (1%) ulna by Rangasubhe and Havaldar[13,16,24]. In our present study, no ulna was detected to possess more than 1 NF (Table VIII).

Direction of NF: During growing period, proximal or distal end of long bones grows up faster than the other zones, and the nutrient canal usually becomes slanted.

On the long bones, direction of nutrient foramen is described as away from knee, towards the elbow[25]. The direction of the NF was found on all of ulnae to be upward (towards the elbow) in the majority of previous studies and, likewise, in our study[7,13,17,18,21-23]. In certain cases, NF could be directed downward or horizontally. Cihan and Toma detected downward directed 1 (1.1%) NF within a sample comprising of Turkish population[16]. Rangasubhe and Havaldar detected downward directed 2 (1.74%) NF and horizontally directed 1 (0.87%) NF among India population[24] (Table IX).

Table IX. Comparison of position and direction of NF

Study (Year)	Population	N	Position of NF n(%)		Direction n(%)
Yılar et al. (2023) [22]	Turkey	155 (70 R, 85 L)	Zone 1	33 (22.76)	U: 145 (100)
			Zone 2	112 (77.24)	
			Zone 3	-	D: -
Cihan and Toma (2023) [16]	Turkey	89 (38 R, 51 L)	Zone 1	20 (32.2)	U: 86 (98.8)
			Zone 2	42 (67.7)	
			Zone 3	-	D: 1 (1.1)
Chavda et al. (2018) [23]	India	150 (75 R, 75 L)	Zone 1	33 (22.15)	U: 149 (100)
			Zone 2	95 (63.76)	
			Zone 3	21 (14.09)	D: -
Dervisevic et al. (2023) [13]	Bosnia and Herzegovina	50 (27 R, 23 L)	Zone1	R: 7 (25.9), L: 5 (21.7)	U: 50 (100)
			Zone 2	R: 20 (74.1), L: 18 (78.3)	
			Zone 3	-	D: -
Priya et al. (2019) [19]	India	200 (88 R, 112 L)	Zone 1	80 (40)	
			Zone 2	120 (60)	
			Zone 3	-	
Challa and Nanna (2019) [7]	India	50	Zone 1	6 (11.8)	U: 51 (100)
			Zone 2	45 (88.2)	
			Zone 3	-	D: -
Desai and Damor (2022) [17]	India	81 (35 R, 46 L)	Zone 1	34 (40.96)	U: 83 (100)
			Zone 2	49 (59.04)	
			Zone 3	-	D: -
Ozturk et al. (2022) [18]	Turkey	32 (16 R, 16 L)	Zone 1	9 (25.71)	U: 35 (100)
			Zone 2	26 (74.29)	
			Zone 3	-	D: -
Ukoha et al. (2013) [20]	Nigeria	50	Zone 1	10 (27)	
			Zone 2	27 (73)	
			Zone 3	-	
Rangasubhe and Havaldar (2019) [24]	India	100 (50 R, 50 L)	Zone 1	98 (85.22)	U: 112 (97.39)
			Zone 2	14 (12.17)	D: 2 (1.74)
			Zone 3	3 (2.61)	H: 1 (0.87)
Veeramuthu et al. (2017) [21]	India	59	Zone 1	19 (32)	U: 59 (100)
			Zone 2	40 (68)	
			Zone 3	-	D: -
Present study	Turkey	110 (47 R, 63 L)	Zone 1	-	U: 107 (100)
			Zone 2	72 (67.29)	
			Zone 3	34 (31.78)	
			Zone 4	1 (0.93)	D: -
			Zone 5	-	

N: sample size, n: number, R: right, L: left, NF: nutrient foramen, U: upward, D: downward, H: horizontal

Table X. Comparison of localization of NF on ulna

Study (year)	Population	N	AS n(%)	PS n(%)	MS n(%)	AB n(%)	PB n(%)	IB n(%)
Yılar et al. (2023) [22]	Turkey	155 (70 R, 85 L)	135 (93.12)	-	-	5 (3.44)	-	5 (3.44)
Cihan and Toma (2023) [16]	Turkey	89 (38 R, 51 L)	64 (73.5)	-	-	-	-	-
Chavda et al. (2018) [23]	India	150 (75 R, 75 L)	134 (89.93)	-	-	8 (5.37)	-	7 (4.7)
Dervisevic et al. (2023) [13]	Bosnia and Herzegovina	50 (27 R, 23 L)	50 (100)	-	-	-	-	-
Priya et al. (2019) [19]	India	200 (88 R, 112 L)	158 (79)	-	2 (1)	33 (16.5)	-	19 (9.5)
Pereira et al. (2011) [8]	Brasil	146	121 (81.76)	18 (12.16)	9 (6.08)	-	-	-
Challa and Nanna (2019) [7]	India	50	40 (78.4)	6 (11.8)	-	2 (3.9)	-	3 (5.9)
Ozturk et al. (2022) [18]	Turkey	32 (16 R, 16 L)	28 (80)	1 (2.86)	-	-	-	6 (17.14)
Rangasubhe and Havaldar (2019) [24]	India	100 (50 R, 50 L)	112 (97.39)	1 (0.87)	2 (1.74)	-	3 (5)	-
Veeramuthu et al. (2017) [21]	India	59	45 (76)	1 (2)	-	9 (15)	-	-
Present study	Turkey	110 (47 R, 63 L)	96 (89.72)	1 (0.93)	-	7 (6.54)	-	3 (2.81)

N: sample size, n: number, R: right, L: left, AS: anterior surface, PS: posterior surface, MS: medial surface, AB: anterior border, PB: posterior border, IB: interosseous border

Position of NF: Position of NF was divided into three parts (at proximal 1/3, at middle 1/3 and at distal 1/3) according the foraminal index in previous studies and NF was located usually on the middle 1/3 of ulna[7,13,16-23]. Several studies revealed that no NF was located at distal 1/3 of ulna[7,13,16-22]. Positioning of NF on distal 1/3 of ulna is observed to be a rare condition. Position of NF on the distal 1/3 of ulna was found on 21 (14.09%) ulnae by Chavda et al., and on 3 (2.61%) ulnae by Rangasubhe and Havaldar[23,24]. In our study, position of NF was examined in detail and divided into five zones. Majority of NF were detected at the 2/5 zone (20%-40%) of ulnae. No NF was detected at the 1/5 zone (0%-20%) and the 5/5 zone (80%-100%) (Table IX).

Localization of NF: In accordance to the former studies, the majority of NF (96 (89.72%)) were observed to be located on the anterior surface of ulna in our study. Former studies and our study reveal that NF may be localized on various surfaces and borders of the ulna, besides the anterior surface[7,8,18,19,21-24] (Table X).

In conclusion, it has been found out that usually there was 1 NF on the ulna, located on the anterior surface and at the 2/5 zone of shaft, directed upwards, smaller than 22-gauge size, 5-10 mm away from interosseous border. According to our dry bone and cadaveric results, screwing the plate should be performed on the distal parts of shaft if possible and close the anterior border in order to minimize damage to the nutrient foramen.

Study Limitation

This study had certain limitations. The age and genders of the ulnae were unknown. Therefore, age and gender differences for morphology of nutrient foramen could not be evaluated. Secondly, although the cadaveric simulation of fracture fixation provided valuable anatomical insights, it was performed on only one specimen. Therefore, the findings from this part of the study particularly regarding the placement of screws to avoid damaging the nutrient foramen of the ulna should be interpreted with caution and cannot be generalized without further support from larger cadaveric series.

Ethics Committee Approval Information:
Ethical Board: Hacettepe University Health Sciences Research Ethical Board
Approval Date: 09.01.2024
Decision No: 2024/01-56
Author Contribution
Idea and design: A.V., B.P., M.Ü., F.B.;
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Support and Acknowledgement Statement:
Non-applicable
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.

References

1. Sauder DJ, Athwal GS. Management of isolated ulnar shaft fractures. *Hand Clin*. 2007;23(2):179-184. doi:https://doi.org/10.1016/j.hcl.2007.01.004
2. Standring S. *Gray's anatomy: the anatomical basis of clinical practice*. 41th ed. Elsevier Health Sciences; 2016.
3. Brookes M, Revell WJ. *Blood supply of bone: scientific aspects*. Springer Science & Business Media; 2012.
4. Kizilkanat E, Boyan N, Ozsahin ET, Soames R, Oguz O. Location, number and clinical significance of nutrient foramina in human long bones. *Ann Anat*. 2007;189(1):87-95. doi:https://doi.org/10.1016/j.aanat.2006.07.004
5. Zahra SU, Kervancıoğlu P, Bahşi İ. Morphological and Topographical Anatomy of Nutrient Foramen in The Lower Limb Long Bones. *Eur J Ther*. 2018;24(1):36-43. doi:https://doi.org/10.5152/eurjther.2017.147
6. Sharma MD, Mathurm A, Nagar AK, Barjatiya R, Chauhanm P, Shekhawat S. Study of Morphometric Variations in the Nutrient Foramina of Fibula in Central Rajasthan. *Indian J Clin Anat Physiol*. 2016;3(1):65-71.
7. Challa P, Nanna RK. A Study of Nutrient Foramina in Human Upper Limb Long Bones. *J Evol Med Dent Sci*. 2019;8(9):610-613. doi:https://doi.org/10.14260/jemds/2019/135
8. Pereira G, Lopes P, Santos A, Silveira F. Nutrient foramina in the upper and lower limb long bones: morphometric study in bones of Southern Brazilian adults. *Int J Morphol*. 2011;29(2):514-20. doi:http://dx.doi.org/10.4067/S0717-95022011000200035
9. Bonafede M, Espindle D, Bower AG. The direct and indirect costs of long bone fractures in a working age US population. *J Med Econ*. 2013;16(1):169-178. doi:https://doi.org/10.3111/13696998.2012.737391
10. Thompson JE. Anatomical methods of approach in operations on the long bones of the extremities. *Ann Surg*. 1918;68(3):309.
11. Nakamura T, Moy OJ, Peimer CA. Relationship between fracture of the ulnar styloid process and DRUJ instability: a biomechanical study. *J Wrist Surg*. 2020;10(02):111-115. doi:https://doi.org/10.1055/s-0040-1719041
12. Alter TH, Noh KJ, Monica JT. Management of Isolated Ulnar Shaft Fractures. *JBJS reviews*. 2023;11(6):e22. doi:http://dx.doi.org/10.2106/JBJS.RVW.22.00254
13. Dervisevic L, Dervisevic A, Ajanovic Z, et al. Anatomical variations of nutrient foramina on the long bones of the upper extremities-Importance and application in everyday clinical practice. *Acta Marisiensis Seria Med*. 2023;69(1):55-60. doi:https://doi.org/10.2478/amma-2023-0011
14. Ülkir M, Karaçoban L, Günes BE. Morphologic and Morphometric Evaluation of Nutrient Foramina of Tibia. *J Anat Soc India*. 2023;72(3):239-245. doi:https://doi.org/10.4103/jasi.jasi_87_22
15. Kinose S, Kanaya Y, Kawasaki Y, et al. Anatomic characterization of the radial and ulnar nutrient arteries in humans. *Ann Anat*. 2018;216(March):23-28. doi:https://doi.org/10.1016/j.aanat.2017.10.004
16. Cihan ÖF, Toma S. Morphology and Topography of the Nutrient Foramina in the Shoulder Girdle and Long Bones of the Upper Extremity. *Eur J Ther*. 2023;29(3):359-369. doi:https://doi.org/10.58600/eurjther.20232902-395.y
17. Desai U, Damor B. Morphometric Study of Nutrient Foramina in Typical Adult Human Long Bones of Superior Extremity

with its Clinical and Surgical Importance: A Cross-sectional Study. *Int J Anat Radiol Surg*. 07/01 2022;11(3):14-17. doi:https://doi.org/10.7860/IJARS/2022/43270.2796

18. Öztürk K, Dursun A, Ayazoğlu M, Kastamoni Y. An Investigation of the Nutrient Foramen in the Long Bones of the Upper and Lower Limbs in Turkish Population. *Med Rec*. 2022;4(2):179-86. doi:https://doi.org/10.37990/medr.1049282
19. Priya DC, Durga JL, Chandrupatla M. A morphological study of nutrient foramina of human ulna and their clinical importance. *IJRR*. 2019;6(6):75-79.
20. Ukoha UU, Umeasalugo KE, Nzeako HC, Ezejindu DN, Ejimofor OC, Obazie IF. A study of nutrient foramina in long bones of Nigerians. *NJMR*. 2013;3(04):304-308.
21. Veeramuthu M, Elangovan M, Manoranjitham. Nutrient foramina: a study in the long bones of human upper extremities. *Int J Anat Res*. 2017;5(3.3):4394-9. doi:https://dx.doi.org/10.16965/ijar.2017.348
22. Yılar K, Sağlam L, Coşkun O, Ertaş A, Gayretli Ö. Analysis of the nutrient foramen in human dry ulnae of Turkish population: An anatomical study and current literature review. *Eur J Ther*. 2023;29(2):163-167. doi:https://doi.org/10.58600/eurjther.20232902-348.y
23. Chavda SR, Rathwa AJ, Akbari V. A study on variations of nutrient foramen of ulna in Saurashtra region with its clinical relevance. *Int J Anat Res*. 2018;6(4.2):5844-47. doi:https://dx.doi.org/10.16965/ijar.2018.358
24. Rangasubhe P, Havaldar PP. An osteological study on nutrient foramina of human dry adult ulna bones. *Int J Anat Res*. 2019;7(1.2):6149-6153. doi:https://dx.doi.org/10.16965/ijar.2018.425
25. Ashwini S, Kumar P, Shetty S. An osteological study of nutrient foramina in radius and ulna with its embryological and clinical significance. *Int J Anat Res*. 2017;5(2.3):3891-3895. doi:https://doi.org/10.16965/ijar.2017.197

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 175-180, 2025
DOI: https://doi.org/10.32708/uutfd.1673509

ORIGINAL RESEARCH

Analysis of Oxidative Burst Capacity of Neutrophils in Buffy-Coat Concentrates*

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ABSTRACT

Buffy-Coat concentrates (BFCs) can be transfused to provide neutrophil support in patients with febrile neutropenia. In this study, the oxidative burst capacity of neutrophils in BFC was investigated in 12 hours-stored BFCs (n=16). Samples taken from healthy volunteers (n=7) (fresh EDTA; fEDTA) and taken before donation from blood donors who donated BFCs (n=16) (donor EDTA; dEDTA) were included. fEDTA and dEDTA samples were used to compare the effect of time. Nitroblue tetrazolium (NBT) test was performed to evaluate the oxidative burst. Different titrations (1:16, 1:32, 1:64, 1:128) of phorbol myristate acetate (PMA) were used. BFC samples without PMA addition were used as negative control (NC). While similar results were obtained in BFC samples with fEDTA samples in NC, oxidative activity was statistically high in the dEDTA samples. These results suggested that neutrophils in dEDTA may be spontaneously activated. In all PMA titrations except 1:64 PMA titers, the oxidative burst capacity in dEDTA and BFC samples was significantly lower than that in fEDTA samples. Also, oxidative burst significantly decreased in fEDTA-dEDTA-BFC in all PMA titrations. Spontaneous burst was detected in dEDTA samples under PMA-free conditions, but not in BFC samples, indicating that the additive solution in BFC protected neutrophils from spontaneous activation. Despite the statistically significant decrease, neutrophils in BFCs can remain functional due to their high oxidative activity at all titers (above 75%). This indicates that they can function when transfused. This result also shows that we can use BFCs as a source of neutrophils in experiments.

Keywords: Buffy-Coat Concentrates. Nitroblue Tetrazolium Test. Oxidative Burst. Neutrophil.

Buffy-Coat Konsantrelerindeki Nötrofillerin Oksidatif Patlama Kapasitelerinin Analizi

ÖZET

Buffy-Coat konsantreleri (BFC'ler) febril nötropenisi olan hastalarda nötrofil desteği sağlamak için transfüze edilmektedir. Bu çalışmada, BFC'deki nötrofillerin oksidatif patlama kapasitesi araştırıldı. 12 saat depolanmış BFC'ler (n=16), sağlıklı gönüllülerden alınan örnekler (n=7) (taze EDTA; fEDTA) ve BFC bağışlayan kan bağışçılarından bağış öncesi alınan örnekler (n=16) (donör EDTA; dEDTA) çalışmaya dahil edildi. fEDTA ve dEDTA örnekleri zamanın oksidatif patlama üzerindeki etkisini değerlendirmek amacıyla çalışmaya dahil edildi. Oksidatif patlamayı değerlendirmek için Nitroblue tetrazolium (NBT) testi farklı titrasyonlarda phorbol miristat asetat (PMA) ile gerçekleştirildi. PMA'nın 1:1, 1:2, 1:4, 1:8 titrasyonlarında oksidatif patlama %95'ten yüksek bulunduğundan, çalışmaya 1:16, 1:32, 1:64, 1:128 titrasyonları ile devam edildi. Ayrıca PMA eklenmemiş BFC örnekleri negatif kontrol olarak kullanıldı. Negatif kontroldeki fEDTA örnekleri ile BFC örneklerinde benzer oksidatif patlama düzeyleri belirlenirken, dEDTA örneklerinde oksidatif aktivite istatistiksel olarak yüksek bulundu. Bu sonuçlar dEDTA'da bulunan nötrofillerin spontan olarak aktive olabileceğini düşündürdü. dEDTA ve BFC örneklerindeki oksidatif patlama kapasitesi 1:64 PMA titrasyonu hariç tüm PMA titrasyonlarında fEDTA örneklerine göre anlamlı düzeyde düşük bulundu. Ayrıca artan titrasyona bağlı olarak oksidatif kapasitenin tüm gruplarda önemli ölçüde azaldığı tespit edildi. PMA'sız dEDTA örneklerinde spontan patlama tespit edilirken BFC örneklerinde tespit edilmemesi, BFC'deki ek solüsyonun nötrofilleri spontan aktivasyondan koruduğunu göstermektedir. fEDTA örneklerine göre anlamlı azalmaya rağmen, BFC'lerdeki nötrofillerin tüm titrelerde (%75'in üzerinde) yüksek oksidatif aktivite göstermeleri işlevsel kalabildiklerini düşünülmektedir. Elde edilen sonuçlar BFC içindeki nötrofillerin transfüzyon yapıldığında işlev görebileceklerini düşündürmekte, ayrıca deneylerde BFC'leri nötrofil kaynağı olarak kullanabileceğimizi göstermektedir.

Anahtar Kelimeler: Buffy-Coat Konsantreleri. Nitroblue Tetrazolium Testi. Oksidatif Patlama. Nötrofil.

Date Received: 12.April.2025
Date Accepted: 2.June.2025

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* XVIIth National Blood Centers and Transfusion Medicine Congress (03-07 December 2024, Antalya) presented as a poster.

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Neutrophils, also called polymorphonuclear cells (PMNCs), differentiate from granulocyte-monocyte progenitor cells in the bone marrow and are the first cells to arrive at the site of infection[1]. They form the first defence against bacterial and fungal infections[2]. They migrate toward the site of inflammation under the guidance of chemokines and cytokines. They fight pathogens by digesting microbes in phagolysosomes through oxidative burst and reactive oxygen species (ROS) production at the site of inflammation, releasing antimicrobial peptides in their granules and/or using Neutrophil Extracellular Traps that immobilise and kill microorganisms and activate other immune cells[2,3]. One of these pathways, phagocytosis, is a special form of receptor-mediated endocytosis used by neutrophils and macrophages to engulf microbes[4]. In phagocytosis, a pathogen is taken into the cell by neutrophils and transferred to the phagosome[5]. Neutrophils digest microorganisms in phagosomes through the ROS they produce and microbicidal proteins in their granules, such as myeloperoxidase, neutrophil elastase, and matrix metalloproteinases[6]. Therefore, analysing ROS production levels is one of the accepted methods to evaluate the function of neutrophils.

Febrile neutropenia (FN) and neutrophil dysfunction (ND), characterized by a decrease in the absolute number of PMNCs in peripheral blood below 500/mm^3, pose a problem in immunocompromised individuals, especially in the fight against bacterial and fungal infections. Despite the low-grade evidence about the effects of prophylactic granulocyte transfusions[7,8], granulocyte concentrate (GC) transfusions have been used in patients with FN and ND for many years[9,10]. Although apheresis GC (aGC) is beneficial, its obtaining process is expensive and time-consuming. For this reason, buffy coat-derived granulocyte concentrates (BFC) could be an alternative[11–13]. BFCs are a by-product rich in lymphocytes, monocytes, granulocytes, and platelets. They are usually thrown away in situations other than FN and ND. However, if they are to be transfused, they must be used within 24 hours of collection due to the decrease in pH and cell survival[14].

The aim of this study was to investigate the neutrophil oxidative burst level in BFCs. BFCs with a median shelf life of 12 hours were selected for the analysis. The changes in the oxidative burst capacity of these BFCs according to different concentrations of stimuli were analyzed. In this way, it was investigated whether the oxidative burst capacity of neutrophils in BFCs stored for 12 hours would be sufficient for the transfused patient. The BFC results were compared with the oxidative burst capacities of fresh neutrophils to obtain information about product sufficiency. In addition, another aim of the study was to evaluate whether BFCs could be used as a neutrophil source for neutrophil-related experiments.

Material and Method

Preparation of Buffy-Coat Concentrates (BFCs)

Blood components (erythrocyte concentrate, platelet concentrate, and plasma) were separated from whole blood with the Reveos Automated Blood Processing System (Terumo, Lakewood, CO, USA). The BFCs occurring in this process were included in our study (n= 16)

Donor blood samples (Donor EDTA; dEDTA)

EDTA blood samples were taken from the blood donor before blood donation and stored for 12 hours. The samples (BFCs and dEDTA) in our study belongs the same donor and same donation (n=16).

Fresh blood samples (Fresh EDTA; fEDTA)

EDTA blood samples obtained from volunteers immediately before the Nitroblue Tetrazolium Test (NBT) were used. The effects of the 12 hours of storage were evaluated by comparing these fresh samples (n=7) with dEDTAs and BFCs.

Analyses of Leukocyte Counts

Leukocyte counts in all BFC, dEDTA and fEDTA samples were tested with a hematology analyzer (CellDyn 1800, Abbott Diagnostic, Chicago, Illunois, USA).

Nitroblue Tetrazolium Tests

NBT test is the gold standard method to measure oxidative burst and was used also in our study. Tests were performed for 100.000 neutrophils. Phorbol 12-myristate 13-acetate (PMA) (Merck; Sigma-Aldrich;

Darmstadt, Germany; Cat no: P8139) was used to stimulate neutrophils. NBT solutions with and without PMA were used in the analyses. To prepare NBT solution "without PMA; PMA-free", 15 mL NaCl, 18.75 mg NBT, and 1.275 µL human albumin were mixed. After 10 mL of this mixture was separated as PMA-free NBT solution, 25 µL PMA was added to the remaining 5 mL. This NBT solution with PMA was then titrated with the NBT solution PMA-free to obtain NBT solutions containing eight different doses of PMA (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, and 1:128). Since the oxidative burst in PMA titrations at 1:1, 1:2, 1:4, and 1:8 was higher than 95%, the study was continued with titrations at 1:16, 1:32, 1:64, and 1:128. The first six experiments were carried out with 1:16, 1:32, and 1:64 PMA titrations; the next three experiments with 1:32, 1:6,4 and 1:128; and the last seven experiments with all titrations. An equal volume of NBT solution was added to the blood samples for analysis and kept in the incubator for 20 minutes and at room temperature for 10 minutes. The samples were spread on slides as a thin layer and fixed with ethyl alcohol after drying. After the alcohol was dried, the slides were stained with Giemsa stain and kept for 20 minutes, then washed with distilled water and left to dry. After the slides were dried, they were evaluated under the light microscope at 100x magnification.

Statistical Analysis

Descriptive statistics are reported as percentage. Mann-Whitney U and Wilcoxon signed rank tests were used to compare the groups. The data analysis was performed using GraphPad Prism. The significance level was established as $p<0.05$.

Results

Leukocyte Counts

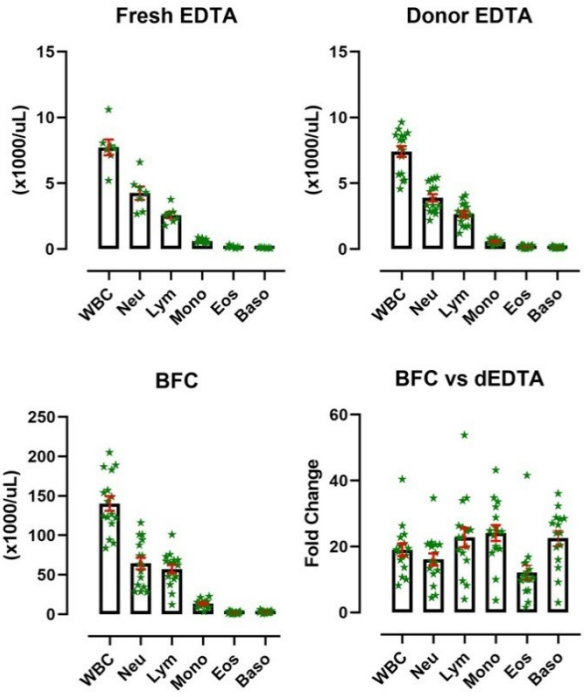
WBCs were 7.68±0.75 K/µL in fEDTA samples and 7.42±1.63 K/µL in dEDTA samples. Approximately 50% of these cells were neutrophils, and 35% were lymphocytes. In BFCs, 140.39±37.1 K/µL WBCs were measured, and approximately 45% were neutrophils and 42% were lymphocytes. It was calculated that the number of WBCs in BFCs increased approximately 19-fold, the number of neutrophils 16-fold, and the number of lymphocytes 23-fold compared to the donor's own dEDTA samples (Figure 1).

Nitroblue Tetrazolium Tests

The oxidative burst capacities of neutrophils in the absence of PMA and 1:16, 1:32, 1:64, and 1:128 titrations of PMA were evaluated. Interestingly, it was determined that dEDTAs led a significantly higher oxidative burst than BFC samples under PMA-free conditions ($p<0.05$) (Figure 2). Oxidative burst was found to decrease in fEDTA, dEDTA, and BFC samples in advanced titrations compared to 1:16 PMA. In all three groups, it was significantly decreased at 1:64 PMA titre compared to 1:16. In addition, statistically significant decreases were detected in PMA titres of 1:32 in fEDTA samples and 1:128 in BFC samples *($p<0.05$)* (Figure 2). Except for the 1:64 dose, oxidative bursts were statistically significantly decreased in dEDTA and BFC samples compared to fEDTA in all other titrations *($p<0.05$)* (Figure 3).

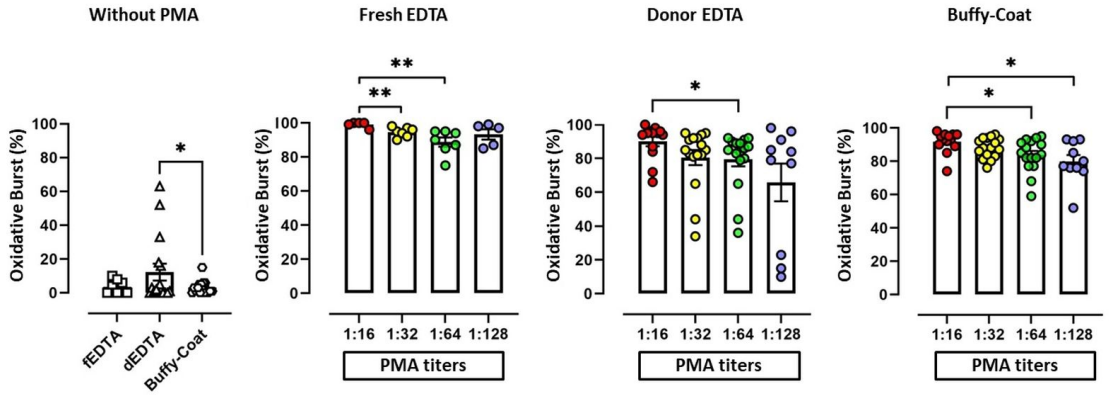
No significant difference was found when BFC samples were compared with dEDTA samples (Figure 4A). While no oxidative burst less than 50% was detected in BFC samples, it was detected in three dEDTA samples (Figure 4B). The levels of one of them were low only at 1:128, while the levels of the other two were low at 1:32, 1:64 and 1:128 titrations.

When the last seven experiments having all PMA titration results were analyzed, there was no significant difference between dETAs and BFSs (Figure 5). Only one dEDTA sample at 1:128 showed a very low oxidative burst.



WBC: White Blood Cell; BFC: Buffy-Coat concentrate; fEDTA: fresh EDTA; dEDTA: donor EDTA

Figure 1:
WBC counts in fEDTA, dEDTA and BFC.



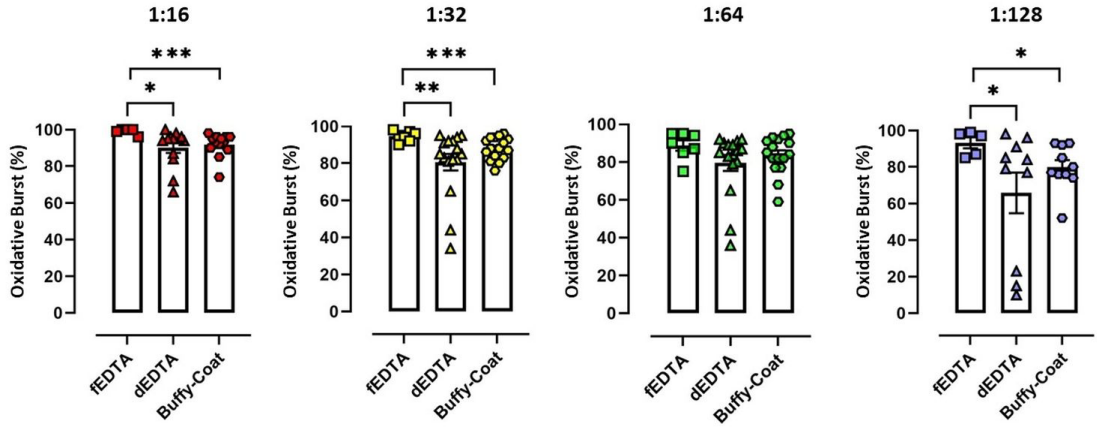
fEDTA samples were tested five times each at 1:16 and 1:128 PMA titres and seven times each at 1:32 and 1:64; dEDTA and BFC samples were tested thirteen times at 1:16, sixteen times each at 1:32 and 1:64, and ten times at 1:128.

BFC: Buffy-Coat concentrate; fEDTA: fresh EDTA; dEDTA: donor EDTA

** 0.05>p≥0.01, ** 0.01>p≥0.005*

Figure 2:

Oxidative bursts without PMA and titrated PMAs in fEDTA, dEDTA and BFC.



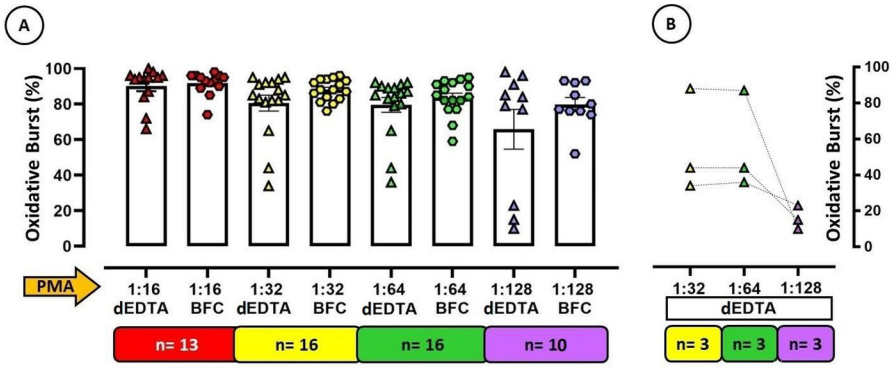
fEDTA samples were tested five times each at 1:16 and 1:128 PMA titres and seven times each at 1:32 and 1:64; dEDTA and BFC samples were tested thirteen times at 1:16, sixteen times each at 1:32 and 1:64, and ten times at 1:128.

BFC: Buffy-Coat concentrate; fEDTA: fresh EDTA; dEDTA: donor EDTA

** 0.05>p≥0.01, ** 0.01>p≥0.005, *** 0.005>p≥0.001*

Figure 3:

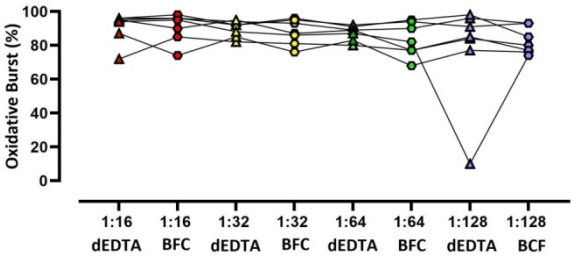
Oxidative bursts in each titer of PMA. Comparing the fEDTA, dEDTA, and BFC.



BFC: Buffy-Coat concentrate; fEDTA: fresh EDTA; dEDTA: donor EDTA

Figure 4:

dEDTA and BFC results A) Comparing the dEDTA and BFC results in all PMA titers B) Three samples of dEDTAs showing low level oxidative burst (< 50%).



BFC: Buffy-Coat concentrate; fEDTA: fresh EDTA; dEDTA: donor EDTA

Figure 5:
The results of seven dEDTA and BFC samples having results in all PMA titers

Discussion and Conclusion

BFCs are considered an alternative to aGCs in acute situations due to some disadvantages of aGCs such as donors' preconditioning process, time-consuming apheresis process, and requirement of the use of some chemicals such as hydroxyethyl starch[11–14]. BFC transfusion offers advantages in terms of time, cost and donor reactions. Also, it was noticed that BFC transfusion was safe but not associated with survival benefits[11]. Many studies driven to detect BFC safety have investigated some properties of neutrophils, such as viability, phagocytosis, oxidative burst, and chemotaxis[14,15]. These studies have shown that BFCs offer a valuable alternative to aGCs. In our study, oxidative burst decreased in relation to both storage (fEDTA versus dEDTA and BFC) time and PMA titer. Although oxidative burst was found to be lower in BFC than in fEDTA, it was seen to be above 75% even at a PMA titer of 1:128. This result indicates that the oxidative burst in BFC is markedly maintained at 12 hours storage, and BFCs can be transfused with functional expectation, as shown in previous studies.

All GCs should be transfused within 24 hours after collection owing to the rapid decrease in pH and cell survival. Increasing lactate levels is one of the main reasons for decreasing cell survival[14]. However, the existance of red blood cells and platelets into the BFC can reduce granulocyte viability, oxidative burst and phagocytic activity[14,15]. The decrease in oxidative burst found in our study may be related to the red blood cells and platelets present in BFCs. Since BFCs are used in our hospital without any purification process, this study was performed with BFCs containing red blood cells and platelets.

Efforts are being made to extend the storage time of GCs. Pooling of BFCs, purification of granulocytes, and use of additive solutions are some of the topics covered in this effort. Storage time can be extended to 72 hours through these topics without decreasing the viability, phagocytosis, or oxidative burst[14–16]. Additive solutions have been shown to participate in this effect[15]. In our study, the fact that oxidative burst was found significantly higher in dEDTA samples than BFC in the "without PMA" group may be an indicator of the additive solutions effect (CPD; Citrate-Phospate-Dextrose) within the BFCs. CPD might have protected the granulocytes from a spontaneous burst in BFC.

This study showed that granulocytes in BFCs could be stimulated to an acceptable level (above 75 percent) even with low-titre PMA. In addition, the additive solution (CPD) appears to preserve the oxidative burst capacity of granulocyte in BFC. Thus, it is thought that granulocytes in BFC may provide oxidative burst support to FN and ND patients. Also, it has been reported that approximately $1x10^{10}$ granulocytes should be transfused to FN and ND patients[14]. To achieve this target, 15-20 BFCs must transfused. Our results show that our BFCs contain sufficient amount of granulocytes. This result also shows that we can use BFCs that have been stored for at least 12 hours as a neutrophil source in experiments. Additionally, the 1:64 PMA titre appears to be a better option for NBT tests. The reason why the oxidative burst capacity of the dEDTA and BFC samples was significantly lower than that of the fEDTA samples, except for the 1:64 titres, is not understood. However, it was thought that this result was not related to the experimental design. As the values were close for all samples in this titration, the 1:64 PMA titre may be the titre of choice in future studies evaluating the effects of different molecules on neutrophil oxidative burst.

The strengths of our study include systematically examining the oxidative burst capacity at different PMA concentrations and comparing it with fresh samples. However, the study focused on a single storage period (12 hours), and the effects of different storage periods were not analyzed. This constitutes its limitation. Future studies should examine the effects of different storage periods and conditions on neutrophil function more comprehensively.

Ethics Committee Approval Information:
Approving Committee: The ethics committee of Bursa Uludağ University Faculty of Medicine
Approval Date: 11.09.2024
Decision No: 2024-14/4
Author Contribution
Idea and design: S.H.B.;
Data collection and processing: S.H.B., H.A., İ.Ö.;
Analysis and interpretation of data: S.H.B., D.Y.E., Y.H., H.B.O.;
Writing of significant parts of the article: S.H.B., D.Y.E., H.A., İ.Ö., Y.H., H.B.O.
Support and Acknowledgement Statement:
Non-applicable
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.

References

1. Blanter M, Gouwy M, Struyf S. Studying Neutrophil Function in vitro: Cell Models and Environmental Factors. J Inflamm Res. 2021;14:141-162. doi:10.2147/JIR.S284941
2. Gupta S, Chan DW, Zaal KJ, Kaplan MJ. A High-Throughput Real-Time Imaging Technique To Quantify NETosis and Distinguish Mechanisms of Cell Death in Human Neutrophils. J Immunol. 2018;200(2):869-879. doi:10.4049/JIMMUNOL.1700905
3. Zhang N, Aiyasiding X, Li W jing, Liao H han, Tang Q zhu. Neutrophil degranulation and myocardial infarction. Cell Commun Signal. 2022;20(1):1-23. doi:10.1186/S12964-022-00824-4
4. Griffin FM, Griffin JA, Leider JE, Silverstein SC. Studies on the mechanism of phagocytosis. I. Requirements for circumferential attachment of particle-bound ligands to specific receptors on the macrophage plasma membrane. J Exp Med. 1975;142(5):1263-1282. doi:10.1084/JEM.142.5.1263
5. Nordenfelt P, Tapper H. Phagosome dynamics during phagocytosis by neutrophils. J Leukoc Biol. 2011;90(2):271-284. doi:10.1189/JLB.0810457
6. Naish E, Wood AJT, Stewart AP, et al. The formation and function of the neutrophil phagosome. Immunol Rev. 2023;314(1):158-180. doi:10.1111/IMR.13173
7. Estcourt LJ, Stanworth S, Doree C, et al. Granulocyte transfusions for preventing infections in people with neutropenia or neutrophil dysfunction. Cochrane database Syst Rev. 2015;2015(6):1-65. doi:10.1002/14651858.CD005341.PUB3
8. Estcourt LJ, Stanworth SJ, Hopewell S, Doree C, Trivella M, Massey E. Granulocyte transfusions for treating infections in people with neutropenia or neutrophil dysfunction. Cochrane Database Syst Rev. 2016;2016(4):1-76. doi:10.1002/14651858.CD005339.PUB2
9. Cugno C, Deola S, Filippini P, Stroncek DF, Rutella S. Granulocyte transfusions in children and adults with hematological malignancies: Benefits and controversies. J Transl Med. 2015;13(1):1-17. doi:10.1186/S12967-015-0724-5/TABLES/3
10. West KA, Conry-Cantilena C. Granulocyte transfusions: Current science and perspectives. Semin Hematol. 2019;56(4):241-247. doi:10.1053/J.SEMINHEMATOL.2019.11.002
11. Ramachandran M, Gupta AK, Meena JP, et al. A randomized controlled trial to explore the safety and efficacy of irradiated buffy-coat granulocytes in pediatric patients with febrile neutropenia. Am J Blood Res. 2023;13(5):152-161.
12. Gupta AM, Ojha S, Sumathi SH, Poojary M. A Case Report of Apheresis Granulocyte Concentrates and the Whole Blood-Derived Pooled Buffy Coat Transfusions in a Neutropenic Hematopoietic Stem Cell Transplant Recipient. Glob J Transfus Med. 2021;6(1):106-109. doi:10.4103/GJTM.GJTM_97_20
13. Sahlin A, Blomgran R, Berlin G. Granulocyte concentrates prepared from residual leukocyte units produced by the Reveos automated blood processing system. Transfus Apher Sci. 2020;59(2):102682. doi:10.1016/J.TRANSCI.2019.102682
14. Klinkmann G, Doss F, Doss S, et al. Purified Granulocyte Concentrates from Buffy Coats with Extended Storage Time. Transfus Med Hemother. 2024;51(6):383-392. doi:10.1159/000537698
15. Bashir S, Stanworth S, Massey E, Goddard F, Cardigan R. Neutrophil function is preserved in a pooled granulocyte component prepared from whole blood donations. Br J Haematol. 2008;140(6):701-711. doi:10.1111/J.1365-2141.2008.06996.X
16. Klinkmann G, Doss F, Goudeva L, et al. Prolonged storage of purified granulocyte concentrates: Introduction of a new purification method. Transfusion. 2022;62(1):194-204. doi:10.1111/TRF.16732

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 181-186, 2025
DOI: https://doi.org/10.32708/uutfd.1668101

ORIGINAL RESEARCH

Evaluation of Blood and Urine Culture Results Obtained in a University Hospital Emergency Department

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ABSTRACT

The identification of emergency department patients at risk of bacteremia is of critical importance, and the culture method is considered the gold standard for diagnosis. Our study aimed to investigate adult patients admitted to the emergency department whose blood and urine cultures were taken with suspicion of bacteremia. In our study, we retrospectively analyzed the data of adult patients who were admitted to Balikesir University, Faculty of Medicine, Department of Emergency Medicine, between February 2020 and October 2024 and whose blood and urine cultures were taken by emergency physicians to test for bacteremia. The mean age of the 991 patients included in the study was 60.9 years. The most common diagnosis was urinary tract infection (36.8%). Bacterial growth was detected in 398 of the 1296 samples included in the study. While growth was observed in 302 (35.4%) of the 853 urine samples, growth was detected in 96 of 443 blood cultures. Ciprofloxacin was the most resistant antibiotic, and amikacin was the most sensitive to the *E. coli* and *K. pneumoniae* isolates taken from the urine cultures. *K. pneumoniae* isolates were more resistant to all antibiotics than *E. coli* isolates. We suggest that guidelines for empirical antibiotic treatment be developed by evaluating aggregated data on bacteremia from healthcare institutions, and that limitations in antibiotic selection by emergency physicians be implemented, as these may enable patients to access appropriate antibiotics more quickly.

Keywords: Emergency department. Antibiogram. Blood culture.

Bir Üniversite Hastanesi Acil Servisinde Alınan Kan ve İdrar Kültür Sonuçlarının Değerlendirilmesi

ÖZET

Acil serviste bakteriyemi riski taşıyan hastaların tespit edilmesi hayati öneme sahiptir. Teşhis için altın standart yöntem olarak kültür incelemesi kabul edilmektedir. Çalışmamızın amacı acil servise başvuran ve bakteriyemi şüphesi ile kültür alınan hastaları incelemektir. Bu doğrultuda, kültür alınan hastaların demografik özellikleri, şikayetleri, izole edilen mikroorganizmaların sonuçları ve antibiyogram sonuçları değerlendirilmiştir. Çalışmamız, Şubat 2020- Ekim 2024 arasında Balıkesir Üniversitesi Tıp Fakültesi Acil Tıp Anabilim Dalındaki hastalardan ve acil hekimleri tarafından bakteriyemi düşünülerek kan ve idrar kültürü alınan 991 erişkin verilerinin retrospektif olarak incelenmesiyle gerçekleştirilmiştir. Çalışmadaki 991 hastanın yaş ortalaması 60,9 dur. Hastalara en sık konulan tanı üriner sistem enfeksiyonu (%36,8) idi. Çalışmaya dahil edilen 1296 örneğin 398'inde bakteriyel üreme olduğu saptandı. Gönderilen 853 idrar örneğinin 302'sinde (%35,4) üreme gözlenirken, gönderilen 443 kan kültürünün 96'sında (%21,6) üreme saptandı. İdrar kültürlerinde gözlenen *K. pneumoniae* ve *E. coli* ve izolatlarının en dirençli olduğu antibiyotik siprofloksasin, en duyarlı olduğu ise amikasin olarak saptanmıştır. *K.pneumoniae* izolatlarının, *E. coli*'ye kıyasla tüm antibiyotiklere daha yüksek oranda dirençli olduğu tespit edilmiştir. Sağlık kuruluşlarının bakteriyemi ile ilgili kayıtları düzenli aralıklarla bakılarak ampirik antibiyotik tedavisi için güncellemeler yapılabilir. Kültürlerin daha hızlı elde edilmesini sağlayacak yöntemlerin geliştirilmesi hayat kurtarıcı bir adım olabilir.

Anahtar Kelimeler: Acil servis. Antibiyogram. Kan kültürü.

Date Received: 29.March.2025
Date Accepted: 10.June.2025

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Many patients go to the emergency department with symptoms suggestive of infectious diseases, such as fever, cough, shortness of breath, burning urine, abdominal pain, diarrhea, and sore throat. Some of these patients have serious infections that can lead to complications, such as sepsis and septic shock, which are life-threatening and require urgent medical intervention. Early diagnosis of these infections and the rapid initiation of appropriate treatment are very important for patient management[1]. The evaluation of patients in the emergency department in terms of initial history and physical examination, identification of those at risk of bacteremia, and early initiation of

empirical antibiotic treatment for possible pathogens are necessary to prevent morbidity and mortality[2]. However, it is widely known that resistance to empirically initiated antibiotics is developing at increasing rates.

Culture methods are the gold standard in the diagnosis of infectious diseases. Appropriate culture samples should be taken and sent to the microbiology laboratory as soon as possible, and under the right conditions, before starting antibiotic treatment in patients. Such a practice serves as a model for the correct identification of microorganisms and the determination of antibiotic susceptibilities. If this protocol is followed, it is possible to change the empirical treatment started in the emergency department to an antibiotic treatment directed at the causative agent[3].

Gram-positive cocci (especially *Staphylococcus* spp. and *Enterococcus* spp.) and gram-negative bacilli (especially *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, and *Klebsiella* spp.) are frequently isolated microorganisms in bacteremia[4.] Gram-negative bacteria are the most common microorganisms, causing urinary tract infections in all age groups and both sexes. Among these, *Escherichia coli* is the most frequently isolated agent (50-90%), followed by *Klebsiella pneumoniae*[5]. However, isolated agents and antibiotic resistance rates vary from hospital to hospital[6]. Furthermore, these rates may vary among different units of the same hospital. Considering that treatment is usually initiated empirically, it would make the most sense for individual centers to follow their own epidemiological surveillance protocols, treatment choices, and patient management approaches[7].

In this study, we aimed to evaluate the demographic characteristics and diagnoses of adult patients admitted to the Department of Emergency Medicine of the hospital involved in the current study, and whose blood and urine cultures were suspected of bacteremia. This was done to determine the distribution and antibiotic resistance rates of the isolated microorganisms and appropriate treatments.

Material and Method

Research Model

This study was designed as a retrospective descriptive observational study. Observational studies are concerned with investigating relationships among characteristics of human populations, after the manner of an experiment, but comparing groups among which the 'treatments' are not randomly assigned[8].

Data Collection

Between February 2020 and October 2024, 1,296 blood and urine culture samples of 991 adult patients with suspected bacteremia, who applied to the emergency department within this time frame, were evaluated. These samples had all been sent to the microbiology laboratory of the hospital. Demographic data, emergency department diagnoses, bacteria isolated from blood and urine culture specimens, and antibiotic susceptibility results were obtained retrospectively from the hospital's electronic information system and laboratory information system. Additionally, surveys, interviews, and/or additional data were not used.

Blood and Urine Culture Analysis

Blood culture samples were monitored in the BD BACTEC FX (Becton Dickinson, USA) automated blood culture system. All samples with a positive growth signal were examined by gram staining and simultaneously inoculated on blood agar, Eosin Methylene Blue (EMB) agar, and chocolate agar media. All plates were incubated at 37°C for 24–48 hours.

All urine samples were inoculated on 5% sheep blood agar and EMB agar with a 0.01 ml capacity sterile plastic ring extract and incubated at 37°C for 24–48 hours. Samples with single/two types of uropathogens and $\geq 10^4$ cfu/ml growth were considered to exhibit significant growth and were then evaluated[9].

Isolates were identified using conventional methods and the BD Phoenix 100 automated identification system (BD Phoenix System, Becton Dickinson, USA). In-vitro antibiotic susceptibilities of the isolates were determined using the Phoenix TM 100 automated identification system (BD Phoenix System, Becton Dickinson, USA) and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria[10].

Ethical Approval

Ethical approval for this study was obtained from Balikesir University Health Sciences Non-Interventional Research Ethics Committee, with decision number 2024/245 dated 17.12.2024.

Statistical Analysis

The collected data were analyzed using SPSS 26.0 software; descriptive statistics (frequency, percentage) and the chi-square test were used for significance testing. Bivariate correlations (Pearson's R and Spearman's tests) were used to evaluate the correlations between the data.

Results

The ages of the 991 patients whose blood and urine culture samples were evaluated ranged between 18–99 years. The mean age was 60.9 years. Of these patients, 438 (44.1%) were male, and 553 (55.9%) were female. Urinary tract infection (36.8%), gastrointestinal tract infection (15.2%), septicemia (14.2%), pulmonary infection (10.9%), and septicemia (14.2%) were the most common causes of bacteremia in these 991 patients. Orthopedic infection (4.7%), neurological infection (4.3%), cardiac infection (4.3%), viral/parasitic infection (3.8%), dermatological infection (2%), gynecological infection (2.3%), and surgical infection (1.1%) were other notable causes (Table I).

Table I. Diagnoses of patients whose blood and urine cultures were sent for microbiologic examination

Diagnoses	%
Urinary tract infections	36.8
Gynecological infections	2.3
Dermatologic infections	2
Cardiac infections	4.3
Septicemias	14.2
Neurological infections	4.3
Surgical infections	1.1
Pulmonary infections	10.9
Orthopedic infections	4.7
Gastrointestinal infections	15.2
Viral/parasitic infections	3.8
Total	100

Of the 1296 samples sent to the microbiology laboratory, 443 (34.2%) were blood culture samples, and 853 (65.8%) were urine culture samples. In total, 398 of the 1,296 samples showed growth, of which 302 (75.9%) were urine cultures and 96 (24.1%) were blood cultures**.** While growth was observed in 302 (35.4%) of the 853 urine samples, growth was detected in 96 (21.6%) of the 443 blood cultures. Of the 362 patients with culture growth, 46% were male, and 54% were female. The rate of growth in the urine cultures was higher in both men and women than in the blood cultures (Figure 1).

The most frequently isolated microorganism from the urine cultures was *E. coli* (n = 198), followed by *K. pneumoniae* (n = 34), *Enterococcus* spp. (n = 16), and other *Enterobacterales* species (n = 16) (Table II).

In the present study, the most frequently isolated microorganism from the blood cultures was coagulase-negative *Staphylococcus* spp. (n = 29), followed by *E. coli* (n = 25) and *Staphylococcus aureus* (n = 13), respectively (Table III).

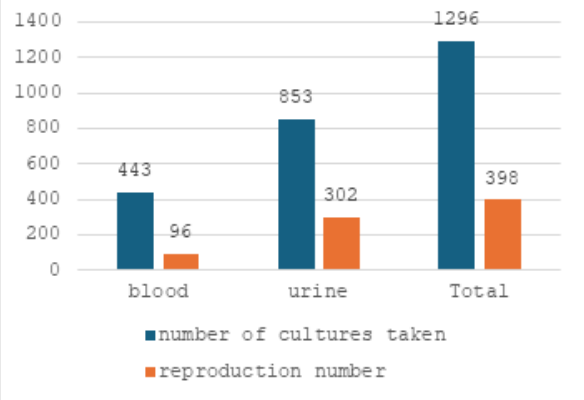

Figure 1.
Culture and growth numbers

Table II. Distribution of microorganisms isolated from urine cultures

Microorganism	Reproduction number	%
Escherichia coli	198	65.5
Klebsiella pneumoniae	34	11.2
Enterococcus spp.	16	5.2
Other *Enterobacterales* species	16	5.2
Candida spp	11	3.6
Pseudomonas spp.	10	3.3
Coagulase-negative *Staphylococcus* spp.	8	2.6
Staphylococcus aureus	4	1.3
Streptococcus agalactiae	3	0.9
Acinetobacter baumannii	2	0.6
Total	302	100

Table III. Distribution of microorganisms isolated from blood cultures

Microorganism	Reproduction number	%
Coagulase-negative *Staphylococcus* spp.	29	30.2
Escherichia coli	25	26
Staphylococcus aureus	13	13.5
Klebsiella pneumoniae	8	8.3
Enterococcus spp.	5	5.2
Pseudomonas aeruginosa	4	4.1
Salmonella spp.	3	3.1
Brucella spp.	2	2
Corynebacterium striatum	1	1
Streptococcus mitis group	1	1
Other	5	5.2
Total	96	100

The antibiotic resistance rates of the *E. coli* and *K. pneumoniae* isolates are shown in Table IV. Among the 198 *E. coli* isolates taken from the urine cultures, the most resistant antibiotic was ciprofloxacin

(41.9%), and the most sensitive was amikacin (1.5%). Other antibiotics with high resistance were levofloxacin (38.3%), trimethoprim/sulfamethoxazole (36.8%), ceftriaxone (35.3%), and ceftazidime (32.8%). Among the 34 *K. pneumoniae* isolates taken from the urine cultures, the most resistant antibiotic was ciprofloxacin (52.9%), and the most sensitive was amikacin (8.8%). Other antibiotics with high resistance were ceftriaxone (50%), ceftazidime (50%), piperacillin/tazobactam (47.1%), and amoxicillin/clavulanic acid (47.1%). When both isolates were evaluated among themselves, it was determined that *K. pneumoniae* isolates were more resistant to all antibiotics than those of *E. coli* (Table IV).

Table IV. Antibiotic resistance rates of *E. coli* and *K. pneumoniae* isolates

Antibiotic	*E. coli* (percentage)	*K. pneumoniae* (percentage)
Levofloxacin	38.3	44.1
Ciprofloxacin	41.9	52.9
Trimethoprim/sulfamethoxazole	36.8	44.1
Amoxicillin/clavulanic acid	29.7	47.1
Gentamicin	23.7	29.4
Imipenem	3.1	17.6
Amikacin	1.5	8.8
Meropenem	3.5	17.6
Piperacillin/tazobactam	14.1	47.1
Ceftazidime	32.8	50.0
Ceftriaxone	35.3	50.0

Discussion and Conclusion

Patients with acute infections mostly apply to the emergency department. In addition, patients with skin, respiratory, and urinary tract infections with moderate symptoms usually cannot wait for treatment in outpatient clinics and present to emergency departments[3]. Physicians working in the emergency department should distinguish serious and life-threatening infections from minor infections and identify them quickly[11.] Rapid history taking, physical examination, targeted imaging methods, and laboratory tests can help to find the likely source of infection. At the same time, appropriate cultures should be obtained before initiating antibiotic therapy[12]. Current guidelines recommend obtaining cultures during or after the identification of sepsis before rapid empirical antimicrobial therapy is initiated[13].

Determining the types of microorganisms grown in blood and urine cultures in a clinic is very important for physicians when choosing the right antibiotic empirically until the culture results are available. Many hospitals throughout the world and in our country are conducting scientific research to determine their own culture flora[14]. Knowing the most common pathogen detected in possible bacterial infections in units with rapid circulation and high patient density, such as the emergency department, and rapidly starting treatment for the pathogen will be most beneficial in the patient's recovery process.

The ages of the patients who underwent blood and urine cultures in the present study were between 18–99 years, and growth in the culture results was observed more often in females. In another study, in cultural growth was observed more frequently in males, which is not compatible with our study[15].

The most common reason for admission in this study was urinary tract infection (36.1%), followed by gastrointestinal infection (15.28%) and septicemia (14.2%). Urinary tract infections are the most commonly diagnosed infections in emergency departments[16]. Other studies have also identified the most common diagnosis as urinary tract infection[17]. This distribution indicates that urinary tract infections are among the most common complaints in emergency departments. Septicemia carries an elevated risk of death, with serious clinical pictures. Due to complications such as chronic renal failure and hypertension, urinary tract infections continue to be a major health problem worldwide, generating high economic costs. Urinary tract infections may show different epidemiologic and etiologic characteristics depending on gender, age, and region. Therefore, regional studies conducted at different times are of great importance for a better understanding of the disease, effective treatment of complications, and prevention of complications.

In a study by Downey et al., urine and blood cultures were compared in the neonatal period, and the concordance in the results was evaluated. The frequency of microorganism growth, according to the culture results, was expressed as *E. coli* 18%, *Candida* 15%, *CNS* 14%, and *Enterococci* 13%. In another study, *CNS* was the most frequently grown microorganism; *Klebsiella, E. coli*, and *Acinetobacter* were grown in that order; *Candida* was not grown; and *Enterococci* rates were found below 5%[15]. In our study, the most frequently grown microorganisms were *E. coli*, *K. pneumoniae*, and *Staphylococcus epidermidis*. While *E. coli* and *K. pneumoniae* were the most frequently grown bacteria in the urine cultures, other *Staphylococcus* spp. bacteria and *E. coli* were the most frequently grown in the blood cultures. *E. coli* was the most frequently isolated microorganism in cultures with growth (55.3%). This was followed by *K. pneumoniae* (9.3%) and *Staphylococcus epidermidis* (5.3%). It is an expected finding that *E. coli* was commonly detected, especially in urinary infections, and that the *Klebsiella* species may play an important role in nosocomial infections.

Çetin et al. found that blood culture results were evaluated, and 67.3% of the microorganisms isolated from the cultures were gram (+) bacteria, 29.4% were gram (-) bacteria, and 3.3% were fungi. The gram (-) bacteria grown were *E. coli*, *Acinetobacter* spp., *Klebsiella* spp., *Pseudomonas* spp., and others, according to their frequency, and the gram (+) bacteria were *S. aureus*, *Enterococcus* spp., and *Streptococcus* spp., according to their frequency[18]. In another study, gram (-) bacteria growth was found more frequently in cultures; the most common gram (-) bacteria was *E. coli*, and gram (+) bacteria were observed most frequently[14].

Complicated urinary tract infections occur in patients with structural anomalies, prostatic hypertrophy, neurogenic bladder, and diabetic patients. Patients presenting with existing urinary symptoms often have complaints such as high fever, burning urine, hematuria, or anuria. In a previous study, the majority (35%) of the patients who applied to the emergency department and had samples tested were patients who presented due to urinary tract infection, and the most frequently isolated bacteria from these patients was *E. coli* (70%)[3]. When the presenting complaints and isolated bacteria were compared, the results were found to be compatible with those of our study.

It should be noted that empirical antibiotic therapy and antibiotic treatment were initiated earlier in patients with positive culture results. These findings suggest that the early acquisition of culture results in patients with suspected infections appearing to be critical in shaping treatment strategies. While antibiotic resistance was previously considered an important problem for nosocomial infections, it has now become a key issue for community-acquired infections[19]. Today, trimethoprim/sulfamethoxazole, ciprofloxacin, and beta-lactams are the most commonly used agents, especially in empirical treatment, and their sensitivity to them is decreasing[20,21].

Similar to the findings in the existing literature, the antibiotics to which *E. coli* and *K. pneumoniae* isolates were most resistant in our study were ciprofloxacin, levofloxacin, trimethoprim/ sulfamethoxazole, ceftriaxone, ceftazidime, piperacillin/tazobactam, and amoxicillin/clavulanic acid. *K. pneumoniae* isolates were found to be more resistant to all antibiotics than those of *E. coli*.

In conclusion, as seen in our study and similar research, culture results may vary from community to community, from hospital to hospital, and even between clinics. Therefore, screening the community flora, the frequency of microorganism growth according to age and special conditions, and the distribution of hospital flora according to the clinic at certain intervals will enable the prediction of possible causative agents of diseases and the early initiation of effective treatment. This will reduce morbidity and mortality, hospitalization time, and treatment costs. Performing such microorganism scans from time to time will also give an idea of the changing flora and the reasons for these fluctuations. At the same time, antimicrobial agent resistance would be detected and could thus form the basis for studies to prevent the development of resistance.

It should be kept in mind that contaminated blood cultures will cause additional increases in patient length of stay, unnecessary antibiotic use, and financial costs. To increase the accuracy of the culture results, aseptic culturing techniques and personal training should be increased. Contamination rates in blood cultures taken in emergency departments are high, and we maintain that the busy emergency department environment is the cause. In our view, improvements should be made to reduce the contamination rate, necessary protocols for blood culture collection should be developed, and employees should be regularly trained.

We believe that local guidelines for empirical antibiotic treatment can be developed by evaluating the aggregated data of healthcare institutions on bacteremia, and re-evaluating the limitations in antibiotic selection by emergency physicians may enable patients to access appropriate antibiotics more quickly. Furthermore, the high incidence of urinary tract infections requires a review of infection management and screening protocols.

Researcher Contribution Statement:
Idea and design: R.K.
Data collection and processing: R.K.
Analysis and interpretation of data: T.K.A.
Writing of significant parts of the article: T.K.A.
Support and Acknowledgement Statement:
Non-applicable
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Balikesir University Health Sciences Non-Interventional Research Ethics Committee
Approval Date: 17/12/2024.
Decision No: 2024/245

References

1. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):801-810.
2. Cevizci M. Acil Servise Başvuran Hastalarda Kan Kültür Sonucu İle Mortalite Arasındaki İlişkinin Retrospektif İncelenmesi (Doktara/Uzmanlık Tezi). Necmettin Erbakan Üniversitesi; 2024.
3. Yücel N, Kuzucu Ç, Yetkin F, Tunç E. Enfeksiyon Bulguları İle Acil Servise Başvuran Hastaların Kültür Sonuçlarının Değerlendirilmesi. J Turgut Özal Med Cent. 2010;17(4):359-364.
4. Kula Atik T, Uzun B. Kan Kültürlerinden İzole Edilen Enterobacteriaceae Türlerinin Antibiyotik Duyarlılıklarının Araştırılması. ANKEM Derg. 2020;34(2):33-40.

5. Karamanlıoğlu D, Aysert Yıldız P, Kaya M, Sarı N. Extended-Spectrum B-Lactamase Production Rates And Antibiotic Susceptibilities Among Enterobacteriaceae İsolated From Urine Cultures. Klimik Derg. 2019;32(3):233–239.
6. Aytaç Ö. İdrar kültürlerinden izole edilen Klebsiella pneumoniae suşlarının antibiyotik direnç oranları. Northwestern Med J. 2024;4(2):64-69.
7. Şerife Y, Asgin N. Distribution of Antibiotic Susceptibility Profiles of Bacteria Frequently Isolated in Blood Cultures by Years. ANKEM Dergisi. 2019; 33:95-101.
8. Kitsantas A, Ware HW, Kitsantas P. Observational Studies. In: Kimberly Kempf-Leonard (eds). Encyclopedia of Social Measurement. Elsevier, 2005: 913-918.
9. Çaycı T, Bıyık İ, Çınar C, Birinci A. Karbapeneme dirençli Enterobacteriaceae izolatlarının 2015-2018 yılları arasındaki antibiyotik direnci. Turk Mikrobiyol Cemiy Derg. 2020;*50*(3):134-40.
10. Kahlmeter G, Brown J, Goldstein W, et al. European Committee on Antimicrobial Susceptibility Testing (EUCAST) technical notes on antimicrobial susceptibility testing. Clinical Microbiology and Infection. 2006;*12*(6):501-503.
11. LEMAN P. Acil serviste hedefi yolunu tespit etmede toplam tahlili ve mikroskopinin kullanıma sunulması. Avrupa Acil Tıp Dergisi. 2002; 9(2):141-147.
12. Catenacci MH, King K. Severe sepsis and septic shock: improving outcomes in the emergency department. Emerg Med Clin North Am. 2008;26(3):603-7.
13. Guarino M, Perna B, Cesaro AE, et al. 2023 Update on Sepsis and Septic Shock in Adult Patients: Management in the Emergency Department. J Clin Med. 2023;12(9):3188.
14. Asena M. Bir Araştırma Hastanesinde Kan ve İdrar Kültür Sonuçlarının Değerlendirilmesi. Dicle Med J. 2020;47(1):208-215.
15. Doi Y, Iovleva A, Bonomo RA. The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world. J Travel Med. 2017;24(suppl 1):44-51.
16. Hans D, Kelly E, Wilhelmson K, Katz ED. Rapidly fatal infections. Emerg Med Clin North Am. 2008;26(2):259-67.
17. Kaur R. İdrar yolu enfeksiyonlarının belirtileri, risk faktörleri, tanısı ve tedavisi. Lisansüstü Tıp Dergisi. 2021; *97* (1154): 803-812.
18. Çetin ES, Kaya S, Pakbaş İ, Demirci M. Yoğun Bakım Ünitelerinde Yatan Hastalardan İzole Edilen Mikroorganizmalar ve Antibiyotik Duyarlılıkları. J Turgut Ozal Med Cent. 2007;14(2):69-73.
19. Karabay O, Bastug A, Ozturk R, et al. Antibiotic consumption, resistance data, and prevention strategies. Mediterr J Infect Microb Antimicrob. 2018;*7*(35):1-39.
20. Teker B, Sever N, Garashova D. Yaş ve Cinsiyetin Üriner Sistem Enfeksiyonu Etkeni Escherichia coli Kökenlerindeki Antibiyotik Direncine Etkisi. OTSBD. 2021;6(2):300-309.
21. Kömürlüoğlu A, Aykaç K, Özsürekçi Y, et al. Gram-Negatif İdrar Yolu Enfeksiyon Ajanlarının Antibiyotik Direnci Dağılımı: Tek Merkez Deneyimi. Türkçe J Pediatr Dis. 2018;12(1):10-17.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 187-192, 2025
DOI: https://doi.org/10.32708/uutfd.1668151

ORIGINAL RESEARCH

Evaluation of Patients Hospitalized Due to Bleeding Complications Associated with Warfarin Treatment*

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ABSTRACT

The most significant complication of warfarin therapy is bleeding. The disadvantages of warfarin include its narrow therapeutic range and drug-drug interactions. This study aimed to determine the severity of bleeding events associated with warfarin treatment, mortality, and factors that may influence these outcomes. A total of 119 patients hospitalized due to bleeding complications associated with warfarin use between January 2007 and December 2010 were included in the study. Demographic and clinical data—including age, gender, comorbidities, International Normalized Ratio (INR), and mortality—were analyzed. Bleeding severity was classified according to the Fihn criteria as minor bleeding and major bleeding (severe, life-threatening and fatal bleeding). Fifty-six patients (51.4%) were female,53 (48.6%) were male, and 53.2% of the patients were aged 65 years and older. Hypertension (50.5%) and diabetes mellitus (22.0%) were the most common comorbid conditions. The primary indications for warfarin use were atrial fibrillation (35.8%), followed by cardiovascular diseases (32.1%), history of stroke (26.6%), and prosthetic heart valve (22.0%). The most common presenting symptoms were ecchymosis and upper gastrointestinal bleeding. Major bleeding was significantly more common in males (p=0.046). Ninety-eight percent (n=107) of patients experiencing bleeding had supratherapeutic INR values. Mortality was significantly higher in patients aged 65 years and older (p=0.029). There was no significant difference between genders in terms of overall bleeding event frequency and mortality. In conclusion, advanced age and labile INR are significant factors in warfarin-related bleeding events. Mortality due to warfarin-related bleeding is significantly higher in older patients.

Keywords: Oral anticoagulant therapy. Warfarin. Bleeding. Haemorrhage.

Varfarin Tedavisine Bağlı Kanama Komplikasyonları Nedeniyle Hastaneye Yatırılan Hastaların Değerlendirilmesi

ÖZET

Varfarin tedavisinin en önemli komplikasyonu kanamadır. Varfarinin, terapötik aralığının dar olması ve ilaç-ilaç etkileşimleri dezavantajları arasında yer almaktadır. Çalışmada varfarin tedavisine bağlı kanama olaylarının şiddeti, mortalite ve etki edebilecek faktörlerin belirlenmesi amaçlandı. Ocak 2007-Aralık 2010 tarihleri arasında varfarin kullanımına bağlı kanama nedeniyle hastaneye yatırılan 109 hasta dahil edildi. Demografik ve klinik özellikler -yaş, cinsiyet, eşlik eden hastalıklar, INR düzeyi ve mortalite- analiz edildi. Kanama şiddeti Fihn kriterlerine göre minör kanama ve major kanama (ciddi, hayatı tehdit edici ve fatal kanama) olarak sınıflandırıldı. Hastaların; 56'sı kadın (%51,4), 53'ü (%48,6) erkek, %53,2'si 65 yaş ve üzerindeydi. Komorbid hastalıklar içinde birinci sıklıkta hipertansiyon (%50,5), ikinci sıklıkta diabetes mellitus (%22,0) saptandı. Varfarin kullanım endikasyonları arasında en sık atriyal fibrilasyon (%35,8), sonrasında kardiyovasküler hastalıklar (%32,1), geçirilmiş stroke (%26,6) ve prostetik kalp kapağı (%22,0) gelmekte idi. En sık geliş semptomu ekimoz ve üst gastrointestinal kanama idi. Majör kanama erkek cinsiyette anlamlı şekilde yüksekti (p=0,046). Kanama geçiren hastaların %98'i (n=107) supraterapötik INR değerine sahipti. Mortalite ileri yaş (≥65 yaş) hastalarda anlamlı şekilde yüksekti (p=0,029). Genel kanama olaylarının sıklığı ve mortalite açısından her iki cinsiyet arasında fark yoktu. Sonuç olarak ileri yaş ve labil INR varfarin ilişkili kanama olaylarında etkilidir. Varfarine bağlı kanama olaylarında mortalite ileri yaş hastalarda anlamlı şekilde daha yüksektir.

Anahtar Kelimeler: Oral antikoagulan tedavi. Varfarin. Kanama. Hemoraji.

Date Received: 7.April.2025
Date Accepted: 10.June.2025

* Presented as an oral presentation at the "15. Uludağ İç Hastalıkları Kış Kongresi" (March 2019, Bursa).

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Warfarin was introduced in 1954 and has since become widely used in anticoagulant therapy worldwide[1]. Its disadvantages include a narrow therapeutic range, variability of dosage between patients, frequent drug-drug and food interactions, and the need for dose monitoring using the International Normalized Ratio (INR)[2]. In 2010, direct oral anticoagulants (DOACs) were introduced, replacing warfarin in the prevention of venous thromboembolism (VTE) recurrence and in preventing complications such as stroke and systemic embolism secondary to atrial fibrillation (AF). However, in patients with prosthetic heart valves, antiphospholipid antibody syndrome, and a history of gastrointestinal (GI) bleeding, warfarin remains the treatment of choice[3].

Bleeding is the major complication of anticoagulant therapy. Bleeding that leads directly to death or requires symptomatic treatment or transfusion of two or more units of blood is classified as major bleeding[4]. The risk of major bleeding with warfarin is higher in the geriatric population and in patients with cancer[5,6]. Particularly, advanced age and polypharmacy contribute to over-anticoagulation and an increased risk of bleeding with warfarin treatment. Numerous drugs, including antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), central nervous system agents, and platelet-inhibiting medications, have been reported to interact with warfarin[7,8]. Bleeding events associated with warfarin and the resulting hospitalizations lead to increased treatment costs[9].

Although bleeding is a major complication of anticoagulant therapies, the benefits of these drugs in reducing thromboembolic events remain significant. Therefore, the aim of the present study was to determine the severity of bleeding complications associated with warfarin therapy and to identify factors affecting morbidity and mortality.

Material and Method

Study Population

A total of 109 patients admitted to the Emergency Internal Medicine Service of Ankara Numune Education and Research Hospital between January 01, 2007 and December 31, 2010 due to complications associated with warfarin use were retrospectively evaluated. Data reviewed including the indication for drug use, comorbid conditions, reason for hospital admission, daily medication dose, INR at the time of admission, prothrombin time, hematocrit level, treatment for bleeding, length of hospital stay, bleeding severity, and mortality. The upper limit of INR was accepted as 4.5 for patients with prosthetic heart valves and 4.0 for other patients. Values above these limits were defined as supratherapeutic. Because laboratory results did not provide a numeric value for INR levels >10, these patients were categorized as having an INR >10. The average daily warfarin dose was calculated by dividing the total dose by the number of days according to the dosing regimen and was recorded.

Bleeding severity was classified according to the Fihn criteria[10] and divided into two main groups as minor and major bleeding events:

1. Minor bleeding: reported but not requiring additional testing, follow-up or visits,
2. Major bleeding: divided into three subgroups as; (i) severe bleeding: requiring treatment, medical evaluation, or ≤2 units of blood transfusion, (ii) life-threatening bleeding: causes irreversible end-organ damage; or requires surgical or angiographic intervention; or two of the following: loss of ≥3 units of blood, systolic hypotension (<90 mmHg), critical anemia (hematocrit ≤20%), (iii) fatal bleeding: bleeding directly leading to the patient's death.

Statistical Analysis

Data were analyzed using SPSS for Windows 11.5 software package. Descriptive statistics for continuous variables were expressed as mean ± standard deviation or median (minimum-maximum), and categorical variables were expressed as number of cases and (%). The data were analyzed using Pearson's Chi-Square or Fisher's Exact Chi-Square tests. A p-value of <0.05 was considered statistically significant in all analyses.

Results

Demographic Data

We analyzed a total of 109 patients, 56 (51.4%) females and 53 (48.6%) males in the study. Fifty-three percent of the patients were 65 years of age or older. Among patients aged 65 and older, 79% had at least one comorbid condition, and among patients aged 80 and older, 88% had at least one comorbidity. Regarding comorbidities, hypertension was the most common (55 patients, 50.5%), followed by diabetes mellitus (24 patients, 22.0%) and heart diseases (15 patients, 13.8%). In terms of the duration of warfarin use, most patients (66 patients, 60.6%) had been on the medication for over one year, followed by those who had used it for between 3 months and 1 year (32 patients, 29.4%). The mean length of hospitalization was 2 (range: 1-45) days. The average daily warfarin dose was 4.95 mg/day in 58 patients aged 65 and over, and 4.82 mg/day in 51 patients under 65 years of age. The demographic and clinical characteristics of the cases are presented in Table I.

Table I. Demographic and clinical characteristics of patients

Variable	n=109 (%)
Age	
<65 years	51 (46.8%)
≥65 years	58 (53.2%)
Gender	
Female	56 (51.4%)
Male	53 (48.6%)
Comorbidities	
Hypertension	55 (50.5%)
Diabetes mellitus	24 (22.0%)
Pulmonary disorder	3 (2.8%)
Malignancy	7 (6.4%)
Other	27 (24.8%)
Concomitant medications	
Aspirin (acetylsalicylic acid)	24 (22.0%)
Digoxin	10 (9.2%)
Antihypertensives	46 (42.2%)
Antibiotics	12 (11.0%)
PPIs	45 (41.3%)
NSAIDs	22 (20.2%)
Duration of warfarin treatment before bleeding	
3 weeks-3 months	11 (10.1%)
3 months-1 year	32 (29.4%)
>1 year	66 (60.5%)
Treatment for bleeding	
FFP	104 (95.4%)
Blood transfusion	39 (35.8%)
Vitamin K	22 (20.2%)
Surgery	1 (0.9%)
Follow-up without treatment	1 (0.9%)
Duration of hospitalization (days)	2 (1-45)

[1] Data are expressed as n (%) and median (IQR)
[2] PPIs: Proton pump inhibitors, NSAIDs: Nonsteroidal anti-inflammatory drugs, FFP: Fresh frozen plasma

Indications for Warfarin Use and INR levels

Among the patients, 39 (35.8%) were using warfarin for atrial fibrillation (AF), 35 (32.1%) for atherosclerotic cardiovascular disease (ASCVD), 29 (26.6%) for stroke, 24 (22.0%) for prosthetic heart valves, 5 (4.6%) for pulmonary thromboembolism (PTE), and 4 (3.7%) for deep vein thrombosis (DVT). Some patients had more than one indication for warfarin treatment (Graph 1). Ninety-eight percent of the patients (n=107) experienced bleeding with supratherapeutic INR levels, whereas only 1.9% (n=2) experienced bleeding within the therapeutic INR range.

Bleeding Symptoms and Severity

Regarding presenting complaints, the most common symptom was ecchymosis (37.6%), followed by GI bleeding symptoms (28.4%) and hematoma (13.8%) (Table II).

Table II. Symptoms and frequency of bleeding

Clinical sign	n=109 (%)
Upper gastrointestinal bleeding	29 (26.6%)
Lower gastrointestinal bleeding	2 (1.8%)
Ecchymosis	41 (37.6%)
Hematoma	15 (13.8%)
Epistaxis	13 (11.9%)
Hematuria	12 (11.0%)
Gingival bleeding	11 (10.1%)
Vaginal bleeding	3 (2.8%)
Intracranial hemorrhage	1 (0.9%)
Hemarthrosis	1 (0.9%)

According to the Fihn criteria, 62 patients (56.9%) experienced minor bleeding, 28 (25.7%) experienced severe bleeding, and 19 (17.4%) experienced life-threatening or fatal bleeding. Overall, 6 patients (5.5%) died (Table III). Among the patients followed-up for bleeding, 22% (n=24) were concurrently using aspirin. Among patients with major bleeding, 61.7% (n=29) had upper GI bleeding. In these cases, the use of acetylsalicylic acid was observed in 44.8% (n=13) and NSAID use in 55.2% (n=16) of the patients, both of which were statistically significantly higher ($p<0.001$).

Table III. Severity of bleeding and mortality

Variable	n=109 (%)
Severity of bleeding	
[1] Minor bleeding	62 (56.9%)
[2] Severe bleeding	28 (25.7%)
[3] Life-threatening and fatal bleeding	19 (17.4%)
Mortality	6 (5.5%)

[1] Reported but not requiring additional testing, follow-up, or visits
[2] Requiring treatment, medical evaluation, or transfusion of at least two units of blood
[3] Causing myocardial infarction, surgical/angiographic intervention, or irreversible sequelae and bleeding that directly causes the patient's death

In the major bleeding group, a statistically significant difference in gender distribution was observed, with males exhibiting a higher prevalence of major bleeding ($p=0.046$). There was no statistically significant difference between the major bleeding group and the non-major bleeding group in terms of age, duration of warfarin use, presence of comorbid conditions, indications for drug use, or INR levels at presentation ($p>0.05$). The incidence of bleeding events in the therapeutic range (INR<4) was notably low (1.9%) at the study group due to the majority being managed as outpatients. The absence of statistically significant difference in INR levels between patients with and without major bleeding events may be attributed to the majority of patients

requiring hospitalization were in the supratherapeutic INR values (98.1%).

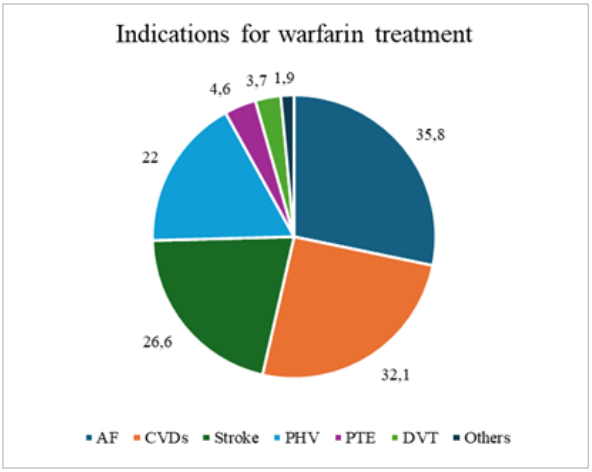
Factors Affecting Mortality

When evaluated separately, patients aged 65 years and older and those aged 80 years and older exhibited significantly higher mortality (p=0.029 and p=0.002, respectively). No significant difference in mortality was observed with regard to gender (p>0.05). Additionally, there were no statistically significant differences in mortality based on the duration of drug use, comorbid conditions, indications for drug use, presenting symptoms, or INR levels (p>0.05). The lack of statistical difference in mortality in patients with comorbidities may be related to the high rate of comorbid conditions in the patients included in the study. Factors affecting mortality are presented in Table IV.

Table IV. Factors affecting mortality

Variable	Mortality		*P value*
	No (n=103)	Yes (n=6)	
Age groups			**0.029**
<65 years	51 (49.5%)	0 (0%)	
≥65 years	52 (50.5%)	6 (100.0%)	
Gender			1.000
Female	53 (51.5%)	3 (50.0%)	
Male	50 (48.5%)	3 (50.0%)	
Duration of anticoagulation			0.110
3 weeks-3 months	11 (10.7%)	0 (0%)	
3 months-1 year	28 (27.2%)	4 (66.7%)	
>1 year	64 (62.1%)	2 (33.3%)	
Comorbid disease	72 (69.9%)	6 (100.0%)	0.180
Indications for warfarin			
AF	36 (34.9%)	3 (50.0%)	0.664
CVDs	33 (32.0%)	2 (33.3%)	1.000
Stroke	27 (26.2%)	2 (33.3%)	0.656
PHV	24 (23.3%)	0 (0%)	0.335
DVT	5 (4.9%)	0 (0%)	1.000
PTE	4 (3.9%)	0 (0%)	1.000
Symptoms			
Ecchymosis	40 (38.8%)	1 (16.7%)	0.406
Gastrointestinal bleeding	29 (28.2%)	2 (33.3%)	1.000
Hematoma	13 (12.6%)	2 (33.3%)	0.191
Hematuria	12 (11.7%)	0 (0%)	1.000
Gingival bleeding	11 (10.7%)	0 (0%)	1.000
INR			0.822
<4	2 (1.9%)	0 (0%)	
4-10	42 (40.8%)	2 (33.3%)	
>10	59 (57.3%)	4 (66.7%)	

AF: atrial fibrillation, CVDs: cardiovascular diseases, PHV: prosthetic heart valve, DVT: deep vein thrombosis, PTE: pulmonary thromboembolism



[1] *AF: atrial fibrillation, CVDs: cardiovascular diseases, PHV: prosthetic heart valve, PTE: pulmonary thromboembolism, DVT: deep vein thrombosis*
[2] *Some patients had multiple indications for warfarin treatment.*

Figure 1:
Distribution of indications for warfarin treatment

Discussion and Conclusion

Oral anticoagulant therapy is widely used in the treatment of deep venous thrombosis and pulmonary embolism, as well as in the prevention of systemic thromboembolism. The most common complication of long-term anticoagulant use is bleeding[11]. The number of patients presenting to emergency departments with life-threatening bleeding due to anticoagulant therapy is increasing every day[12]. Particularly in elderly patients, the risk of major bleeding is observed at a higher rate due to increased comorbidities and the concomitant prescription of multiple medications[6,13].

The most well-known risk factors for warfarin-related bleeding are treatment intensity and INR levels. Regardless of the indication, moderate-intensity warfarin therapy (INR 2.0-3.0) significantly reduces the risk of bleeding compared to high-intensity warfarin therapy (INR >2.5-3.0)[14,15]. In patients with prosthetic heart valves, an INR below 2 increases the incidence of thromboembolic events. While the frequency of major bleeding increases moderately when INR is in the range of 3-4, it rises markedly when INR exceeds 4[16]. One of the reasons for switching from warfarin to new-generation DOACs is that these drugs require 64% less monitoring and 32% have labile INR values[17]. In the present study, bleeding events were found to be significantly higher in patients with supratherapeutic INR values.

Clinical studies have reported that the incidence of serious bleeding episodes in patients on warfarin therapy ranges from 2% to 13%[18]. The frequency of major bleeding is higher in elderly patients (aged ≥65 years). As the incidence of comorbidities increases in

the elderly, the number of concomitantly used medications also rises. In a study evaluating 17,661 elderly patients, the rate of hospitalizations due to warfarin-related bleeding was calculated as 4.1 per 100 patient-years, and hospitalization rates were found to be significantly higher in those using aspirin and/or clopidogrel concurrently[19]. Advanced age was identified as an independent predictor of mortality (HR: 1.03) in patients using warfarin for mechanical heart valve[20]. Ron P. et al. introduced the HAS-BLED score (Hypertension, Abnormal renal/liver function, Stroke, Bleeding history or predisposition, Labile international normalized ratio, Elderly [>65 years], Drugs/alcohol concomitantly) to determine the risk of major bleeding in patients with AF receiving anticoagulant therapy. According to this scoring system, advanced age and labile INR are among the risk factors[21]. In our study, mortality was found to be significantly higher in the patient group aged 65 years and older.

NSAIDs are frequently prescribed medications that are associated with GI bleeding. In patients receiving warfarin therapy, the use of NSAIDs approximately doubles the probability of GI bleeding. This increased risk is especially pronounced in elderly patients and can occur even without changes in INR levels. Additionally, NSAIDs have been associated with an increased risk of bleeding through other mechanisms[22]. Polymorphisms in cytochrome enzymes (particularly CYP2C9) may increase the tendency to bleed by affecting the metabolism of both NSAIDs and warfarin[23,24]. Besides antiplatelet agents and NSAIDs, warfarin interacts with many other drugs—including various antimicrobial agents, SSRIs, mirtazapine, and loop diuretics—, which may increase the risk of bleeding. Proton pump inhibitors (PPIs), on the other hand, reduce hospitalizations due to GI bleeding[25]. In our study, concomitant medications most commonly used with warfarin were antihypertensive agents and PPIs.

Some studies have reported that bleeding events in patients on warfarin are more frequently observed in females, although other studies have not supported this finding[26,27]. In a study by Nekkanti et al., warfarin-related bleeding was detected more frequently in females (60.65%)[28]. However, another study evaluating 54,568 patients found that bleeding events were less frequent in females (HR: 0.52)[29]. A meta-analysis of 37 studies reported no statistically significant difference in warfarin-related major bleeding events between males and females[30]. In the this study, although the number of female patients was higher, there was no statistically significant difference in bleeding events between genders.

DOACs have become widely prescribed medications instead of warfarin therapy for AF and VTE. They have similar or better efficacy and safety profiles compared with warfarin therapy[31]. The incidence of major bleeding events in patients receiving DOACs is 2-3.5% annually. In a meta-analysis evaluating 4735 patients, the most common indication for DOACs was AF (82%), followed by VTE (14%). Intracranial hemorrhage (ICH) was the most frequently reported bleeding complication, accounting for 55% of cases, while other types of bleeding were comparatively less prevalent. Mortality was 20.2% in patients with ICH[32]. In a study evaluating 125,195 AF patients receiving warfarin treatment, the incidence of bleeding was 3.8% per year, and the most common bleeding events were in the first month of treatment[33]. In our study group, AF was the most frequent indication for warfarin therapy, consistent with findings reported in studies evaluating DOACs. The majority of bleeding events were observed after the first year of treatment.

The limitations of our study are its retrospective design and the fact that some patients may not have been included due to coding errors after diagnosis. Additionally, a relatively small number of cases were included, and the study was limited to a single medical center. As DOACs were not yet licensed during the study period, patients using these drugs could not be included in the study.

In conclusion, treatment intensity and INR levels, as well as age and drug interactions, influence the severity of warfarin-related bleeding events at varying degrees. Bleeding events remain the major complication of warfarin therapy and warrant careful management, especially in elderly patients due to the associated higher mortality rate.

Researcher Contribution Statement:
Idea and design: F.E., E.S.; Data collection and processing: F.E.; Analysis and interpretation of data: F.E.; Writing of significant parts of the article: F.E.; Review and editing: E.S.
Support and Acknowledgement Statement:
The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Ethics Committee of Ankara Numune Training and Research Hospital
Approval Date: 15 June 2011
Decision No: 2011-186

References

1. Lim GB. Milestone 2: Warfarin: from rat poison to clinical use. Nat Rev Cardiol. Published online December 14, 2017.
2. Hylek EM. Vitamin K antagonists and time in the therapeutic range: implications, challenges, and strategies for improvement. J Thromb Thrombolysis. 2013;35(3):333-335.
3. Wadsworth D, Sullivan E, Jacky T, et al. A review of indications and comorbidities in which warfarin may be the preferred oral anticoagulant. J Clin Pharm Ther. 2021;46(3):560-570.
4. Schulman S, Kearon C, Subcommittee on Control of Anticoagulation of the Scientific and Standardization

Committee of the International Society on Thrombosis and Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. J Thromb Haemost. 2005;3(4):692-694.

5. Merli GJ, Tzanis G. Warfarin: what are the clinical implications of an out-of-range-therapeutic international normalized ratio? J Thromb Thrombolysis. 2009;27(3):293-299.
6. Vitry AI, Roughead EE, Ramsay EN, et al. Major bleeding risk associated with warfarin and co-medications in the elderly population. Pharmacoepidemiol Drug Saf. 2011;20(10):1057-1063.
7. Comoretto RI, Rea F, Lucenteforte E, et al. Bleeding events attributable to concurrent use of warfarin and other medications in high-risk elderly: meta-analysis and Italian population-based investigation. Eur J Clin Pharmacol. 2018;74(8):1061-1070.
8. Halperin JL, Hankey GJ, Wojdyla DM, et al. Efficacy and safety of rivaroxaban compared with warfarin among elderly patients with nonvalvular atrial fibrillation in the Rivaroxaban Once Daily, Oral, Direct Factor Xa Inhibition Compared With Vitamin K Antagonism for Prevention of Stroke and Embolism Trial in Atrial Fibrillation (ROCKET AF). Circulation. 2014;130(2):138-146.
9. Ghate SR, Biskupiak J, Ye X, Kwong WJ, Brixner DI. All-cause and bleeding-related health care costs in warfarin-treated patients with atrial fibrillation. J Manag Care Pharm. 2011;17(9):672-684.
10. Fihn SD, McDonell M, Martin D, et al. Risk factors for complications of chronic anticoagulation. A multicenter study. Warfarin Optimized Outpatient Follow-up Study Group. Ann Intern Med. 1993;118(7):511-520.
11. Raj G, Kumar R, McKinney WP. Long-term oral anticoagulant therapy: update on indications, therapeutic ranges, and monitoring. Am J Med Sci. 1994;307(2):128-132.
12. Backus B, Beyer-Westendorf J, Body R, et al. Management of major bleeding for anticoagulated patients in the Emergency Department: a European experts consensus statement. Eur J Emerg Med. 2023;30(5):315-323.
13. Rydberg DM, Linder M, Malmström RE, Andersen M. Risk factors for severe bleeding events during warfarin treatment: the influence of sex, age, comorbidity and co-medication. Eur J Clin Pharmacol. 2020;76(6):867-876.
14. Raza S, Pinkerton P, Hirsh J, Callum J, Selby R. The historical origins of modern international normalized ratio targets. J Thromb Haemost. 2024;22(8):2184-2194.
15. Levine MN, Raskob G, Beyth RJ, Kearon C, Schulman S. Hemorrhagic complications of anticoagulant treatment: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest. 2004;126(3 Suppl):287S-310S.
16. Labaf A, Själander A, Stagmo M, Svensson PJ. INR variability and outcomes in patients with mechanical heart valve prosthesis. Thromb Res. 2015;136(6):1211-1215.
17. Bartholomew RR, Noble BN, Stanislaw JJ, et al. Frequency and clinical outcomes of pharmacist-driven switching from warfarin to direct oral anticoagulants in an underserved patient population: A retrospective cohort study. Am J Health Syst Pharm. 2023;80(Suppl 3):S103-S110.
18. Wiedermann CJ, Stockner I. Warfarin-induced bleeding complications - clinical presentation and therapeutic options. Thromb Res. 2008;122 Suppl 2:S13-8.
19. Vitry AI, Roughead EE, Ramsay EN, et al. Major bleeding risk associated with warfarin and co-medications in the elderly population. Pharmacoepidemiol Drug Saf. 2011;20(10):1057-1063.
20. Labaf A, Grzymala-Lubanski B, Stagmo M, et al. Thromboembolism, major bleeding and mortality in patients with mechanical heart valves- a population-based cohort study. Thromb Res. 2014;134(2):354-359.
21. Pisters R, Lane DA, Nieuwlaat R, et al. A novel user-friendly score (HAS-BLED) to assess 1-year risk of major bleeding in patients with atrial fibrillation: the Euro Heart Survey. Chest. 2010;138(5):1093-1100.
22. Villa Zapata L, Hansten PD, Panic J, et al. Risk of Bleeding with Exposure to Warfarin and Nonsteroidal Anti-Inflammatory Drugs: A Systematic Review and Meta-Analysis. Thromb Haemost. 2020;120(7):1066-1074.
23. Malhi H, Atac B, Daly AK, Gupta S. Warfarin and celecoxib interaction in the setting of cytochrome P450 (CYP2C9) polymorphism with bleeding complication. Postgrad Med J. 2004;80(940):107-109.
24. Zhou L, Ding Y, Gao Y, et al. Genetic influence on bleeding and over-anticoagulation risk in patients undergoing warfarin treatment after heart valve replacements. Expert Opin Drug Metab Toxicol. 2020;16(1):1-9.
25. Wang M, Zeraatkar D, Obeda M, et al. Drug-drug interactions with warfarin: A systematic review and meta-analysis. Br J Clin Pharmacol. 2021;87(11):4051-4100.
26. Barcellona D, Schirru P, Mameli A, et al. Over-anticoagulation by vitamin K antagonists and gender differences. Int J Cardiol. 2022;362:147-151.
27. Simon TA, Pan X, Kawabata H, Huang HY, Azoulay L. The Association Between Bleeding and the Incidence of Warfarin Discontinuation in Patients with Atrial Fibrillation. Cardiovasc Ther. 2016;34(2):94-99.
28. Nekkanti H, Mateti UV, Vilakkathala R, et al. Predictors of warfarin-induced bleeding in a South Indian cardiology unit. Perspect Clin Res. 2012;3(1):22-25.
29. Penttilä T, Lehto M, Niiranen J, et al. Differences in the risk of stroke, bleeding events, and mortality between female and male patients with atrial fibrillation during warfarin therapy. Eur Heart J Cardiovasc Pharmacother. 2019;5(1):29-36.
30. Pancholy SB, Sharma PS, Pancholy DS, et al. Meta-analysis of gender differences in residual stroke risk and major bleeding in patients with nonvalvular atrial fibrillation treated with oral anticoagulants. Am J Cardiol. 2014;113(3):485-490.
31. Ballestri S, Romagnoli E, Arioli D, et al. Risk and Management of Bleeding Complications with Direct Oral Anticoagulants in Patients with Atrial Fibrillation and Venous Thromboembolism: a Narrative Review. *Adv Ther*. 2023;40(1):41-66.
32. Gómez-Outes A, Alcubilla P, et al. Meta-Analysis of Reversal Agents for Severe Bleeding Associated With Direct Oral Anticoagulants. *J Am Coll Cardiol*. 2021;77(24):2987-3001.
33. Gomes T, Mamdani MM, Holbrook AM, et al. Rates of hemorrhage during warfarin therapy for atrial fibrillation. *CMAJ*. 2013;185(2):E121-E127.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
(51 (2) 193-200, 2025
DOI: https://doi.org/10.32708/uutfd.1653471

ORIGINAL RESEARCH

Clinical Presentation, Diagnosis, and Genetic Characteristics of Patients with Hypophosphatemic Rickets*

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ABSTRACT

Phosphate plays an essential role in bone mineralization. Hypophosphatemic rickets (HR) is a rare phosphate-wasting disorder that leads to rickets, which may be FGF23-dependent or independent. In this study, we aimed to evaluate the clinical features of HR patients with genotypic and phenotypic features. This study included 32 children. The suspected gene was primarily studied in cases meeting the clinical diagnosis. Variants were evaluated according to ACMG criteria. All HR patients' median ages at the onset of complaints and diagnosis were 1.5 and 5 years, respectively ($p<0.001$). The most common complaint is bowlegs. At the time of diagnosis, calcium was 9.70 mg/dl; phosphorus was 2.68±0.64 mg/dl; ALP was 530±245 u/l, PTH was 58.70 ng/l, tubular phosphate reabsorption was 74±17%, and TmP/GFR was 2.58±1.15. Five of 32 patients were diagnosed with McCune-Albright syndrome, and two patients were followed as HR secondary to chemotherapy. Variants of the *PHEX* gene were identified in 15 cases (X-linked HR). In X-linked HR patients, no statistically significant difference was found between the age of presentation and diagnosis. Diagnostic delay was observed in infantile hypercalcemia type 2 and hereditary HR with hypercalciuria patients who were followed with nephrocalcinosis from an early age. In conclusion, HR is rare, and patients with bowlegs, short stature, kidney stones, hypophosphatemia, and renal phosphate wasting should be classified as FGF-23 dependent or FGF-23 independent of initiating treatment as soon as possible. Delayed diagnosis is common in cases of nephrocalcinosis, so measurement of calcium and phosphate levels should be standard for these patients.

Keywords: Hypophosphatemic rickets. McCune Albright Syndrome. *PHEX. SLC34A3. SLC34A1.*

Hipofosfatemik Rikets Hastalarının Klinik Başvuru, Tanı ve Genetik Özellikleri

ÖZET

Fosfat kemik mineralizasyonunda önemli bir rol oynar. Hipofosfatemik rikets (HR), FGF23'e bağlı veya bağımsız olabilen riketse yol açan nadir bir fosfat kaybı bozukluğudur. Bu çalışmada, hipofosfatemik rikets hastalarının klinik özelliklerini genotip ve fenotipik özellikleriyle değerlendirmeyi amaçladık. Bu çalışmaya otuz iki hasta dâhil edildi. Klinik tanıyı karşılayan olgularda öncelikle şüpheli gen çalışıldı. Varyantlar ACMG kriterlerine göre değerlendirildi. Tüm HR hastalarının şikâyetlerin başlangıcındaki ve tanıdaki medyan yaşları sırasıyla 1,5 ve 5 yıl idi ($p<0,001$). En sık görülen şikâyet bacak eğriliğiydi. Tanı anında kalsiyum 9,70 mg/dl; fosfor 2,68±0,64 mg/dl; ALP 530±245 u/l, PTH 58,70 ng/l, tübüler fosfat reabsorbsiyonu %74±17 ve TmP/GFR 2,58±1,15 olarak bulundu. Otuz iki hastanın beşi McCune Albright sendromu tanısı aldı ve iki hasta kemoterapiye sekonder HR olarak takip edildi. On beş vakada *PHEX* gen varyantları saptandı (X'e bağlı HR). X'e bağlı HR hastalarında başvuru yaşı ile tanı yaşı arasında istatistiksel olarak anlamlı bir fark bulunmadı. Erken yaştan itibaren nefrokalsinozis ile takip edilen infantil hiperkalsemi tip 2 ve hiperkalsiürik herediter hipofosfatemik raşitizm hastalarında tanı gecikmesi gözlendi. Sonuç olarak hipofosfatemik raşitizm nadir görülür ve bacak eğriliği, boy kısalığı, böbrek taşı, hipofosfatemi ve renal fosfat kaybı olan hastalar mümkün olan en kısa sürede FGF-23 bağımlı veya FGF-23 bağımsız olarak sınıflandırılmalı ve tedavi planlanmalıdır. Nefrokalsinozis olgularında tanı gecikmesi sık görüldüğünden bu hastalarda kalsiyum ve fosfat düzeylerinin ölçümü standart olmalıdır.

Anahtar Kelimeler: Hipofosfatemik raşitizm. McCune Albright Sendromu. *PHEX. SLC34A3. SLC34A1.*

Date Received: 14.March.2025
Date Accepted: 10.June.2025

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* Presented as an oral presentation at the "4.Ege endokrin hastalıklar ve genetik sempozyumu" (2021, Online).

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Phosphate is involved in cell membrane integrity, nucleic acid skeleton, ATP production, cell signaling, buffering of acid-base balance, and bone mineralization. 80-85% of the phosphate filtered from the renal glomeruli is reabsorbed by the proximal tubule, and the remainder is excreted in urine. Serum phosphate levels (P) are influenced by dietary intake and regulated through feedback mechanisms involving parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and 1,25-dihydroxy vitamin D (1,25(OH)2D). FGF23 is a phosphatonin synthesized by a gene located on chromosome 12 in response to hyperphosphatemia and increased vitamin D. Klotho protein acts as a cofactor for the FGF23 to bind to its receptor on the cell membrane[1-2].

Hypophosphatemic rickets (HR) is a group of rare phosphate-wasting disorders with rickets or osteomalacia caused by genetic mutations, drug-induced or a part of syndromes that are FGF23-dependent or FGF23-independent[3-4].

The most common inherited FGF23-dependent HR is X-linked HR (XLH), in which inactivating mutations in the phosphate regulatory endopeptidase homologous X-linked *(PHEX)* gene lead to local and systemic effects[5-6]. Its prevalence is estimated to be 1/20,000-25,000[2].

Autosomal dominant hypophosphatemic rickets (ADHR) due to *FGF23* and *SGK3* mutations, autosomal recessive hypophosphatemic rickets 1 (ARHR1) due to *DMP1* mutations, autosomal recessive hypophosphatemic rickets 2 (ARHR2) due to *ENPP1* mutations, hypophosphatemic rickets with hyperparathyroidism (*KLOTHO*), osteoglophonic dysplasia (OGD) due to *FGFR1* mutations, Raines syndrome (*FAM20C*), tumor-induced osteomalacia, drug-induced HR, intravenous iron infusion, gain-of-function mutations in *GNAS, HRAS, or NRAS and* Neurofibromatosis type 1 are also cause of FGF23-dependent HR[2]. Whilst hereditary hypophosphatemic rickets with hypercalciuria (HHRH) (*SLC34A3*), infantile hypercalcemia type 2 (*SLC34A1*), Fanconi renotubular syndrome type 2 (*SLC34A1*), nephrolithiasis/osteoporosis hypophosphatemia type 1 (*SLC34A1*), nephrolithiasis/osteoporosis, hypophosphatemia type 2 (*SLC9A3R1*), and Fanconi syndrome (Dent disease, cystinosis, or drug-induced) are forms of FGF23-independent HR.

This study aimed to evaluate the clinical features of patients with HR and their genotypic and phenotypic characteristics.

Material and Method

Patient selection and laboratory

The study sample consists of 32 children aged under 18 years old from 27 families who were diagnosed with HR based on clinical and laboratory assessments. The diagnosis of hypophosphatemia was based on at least two P measurements. Patients were excluded from the study if their hypophosphatemia was attributable to calciopenic rickets, or nutritional phosphate deficiency.

The patients' age at which their complaints started, age at diagnosis, gender, diagnostic body weight (BW) standard deviation scores (SDS), height SDS, body mass index (BMI) SDS, calcium (Ca), P, PTH, and alkaline phosphatase (ALP) levels; tubular reabsorption of phosphate (TRP), and renal tubular maximum reabsorption rate/GFR (TmP/GFR) at diagnosis; genetic analysis, consanguinity, presence of a family member with similar complaints, additional findings (cafe au lait, fibrous dysplasia, bone fracture, bone deformities etc.) were evaluated. Height, weight, and BMI SDS were assessed according to Turkish child standards using 'child metrics'[7].

Genetic analysis

Genetic analysis was performed based on the clinical presentation. In patients without hypercalcemia, hypercalciuria, and with normal/high FGF-23 levels, Sanger sequencing of the *PHEX* gene was performed first. Multiplex ligation-dependent probe amplification (MLPA) of the *PHEX* gene was performed in clinically suspicious cases where a pathogenic/likely pathogenic variant was not detected. After that, if no clinically related pathogenic/likely pathogenic variant was detected, patients were included in the targeted next-generation sequencing (NGS) gene panel. In FGF-23 independent HR patients, a targeted NGS panel (nephrocalcinosis and hypophosphatemia) was performed. Whole-exome sequencing (WES) was performed if necessary. The pathogenicity of variants was evaluated according to the American College of Medical Genetics and Genomics (ACMG) criteria[8].

In cases where McCune-Albright syndrome (MAS) was considered, the diagnosis was made without genetic analysis, with café au lait, fibrous dysplasia, and additional endocrinological findings.

Statistics

Statistical analyses were performed using IBM SPSS 29.0.2.0 (IBM Corp. Released 2023. IBM SPSS Statistics for Windows, Version 29.0.2.0 Armonk, NY: IBM Corp.) statistical package program. Pearson's Chi-square tests were used in the analysis of categorical data. Wilcoxon tests were used to compare tests; $p<0.05$ was considered statistically significant.

Ethics

The study was ethically approved by the local ethics committee (Approval number 2021-17/28) and conducted following the Declaration of Helsinki. The patient's parents provided signed informed written consent.

Results

All HR patients

Data of the 32 children's data were evaluated. The median age at onset of complaints was 1.5 years (0.3-11 years), while the median age at diagnosis was 5 years (0.3-14.5 years). A significant difference was found *(p<0.001).* The female-to-male ratio was 1.46:1. The most common presenting complaints were bone-related: bowlegs (34.4%), in-toeing (9.4%), bone fractures (6.3%), and hip pain (3.2%). Besides, positive family HR history (15.6%), kidney stones (9.4%), suspicion of precocious puberty (9.4%), hypercalcemia (3.2%), and growth retardation (9.1%) were noted as presenting factors. The median calcium was 9.70 (8.80-15.30) mg/dl; the mean phosphorus was 2.68±0.64 mg/dl; the mean ALP was 530±245 U/L, and the median PTH was 58.70 ng/l (6.60-273.00 ng/l) at diagnosis. The mean tubular reabsorption of phosphate (TRP) was 74±17%, and the mean TmP/GFR was calculated as 2.58±1.15. Of the 32 patients, five were diagnosed with MAS, and two patients were followed up as HR secondary to chemotherapy (drug-induced HR). Genetic analyses were performed on 24 of the remaining 25 cases. Variants of the *PHEX* gene were identified in fifteen cases, variants of the *OCRL* gene in two cases, variants of the *SLC34A3* gene in two cases, variants of the *SLC34A1* gene in two cases, and a variant of the *SLC2A2* gene in one case (Figure 1). The last two patients are the dizygotic twin brothers, who both had hypophosphatemic rickets and normal/elevated intact FGF23 (iFGF23) levels. Their physical examination met the XLH phenotype. The sequencing of the *PHEX* gene, the NGS panel (*PHEX, CLCN5, FGF23, SLC34A1*, and *SLC34A3),* MLPA of the *PHEX* gene, and whole-exome analysis (WES) were studied. However, no variant was detected. 24 patients had been following as FGF23-dependent, and eight patients were FGF23-independent HR.

FGF23-dependent HR

PHEX patients (XLH): Fifteen patients have several *PHEX* gene variants (Table I). No statistical difference was found in the age of onset of complaints and diagnosis. Phosphate and calcitriol treatments (conventional) were started on all patients first.

Others: Dizygotic twin brothers with no *PHEX* variant were diagnosed with FGF23-dependent HR due to normal-elevated intact FGF23 levels (133 and 223 RU/ml- reference value <230). Their calcium was 10.10±0.14 mg/dl; phosphorus was 2.24±0.22 mg/dl; ALP was 415±2 U/L; PTH was 21±0 ng/l. Tubular reabsorption of phosphate (TRP) 63.0±11.3% and TmP/GFR were calculated as 2.78±0.58. Their admission complaint was bowlegs; O-Bain. Although they met the XLH phenotype, Sanger sequencing of the *PHEX* gene, the hypophosphatemia panel (*PHEX, CLCN5, FGF23, SLC34A1,* and *SLC34A3*), MLPA of the *PHEX* gene, and WES were studied.

Treatment: Phosphate and calcitriol treatments (conventional) were started for all XLH patients and dizygotic twin brothers. During follow-up, Burosumab treatment was recommended to them due to the side effects of conventional treatment, such as nephrocalcinosis, inadequacy in increasing phosphorus levels, difficulty in compliance with 6*1 phosphate and 2*1 calcitriol daily, and proven positive results in patients using it. Due to a lack of insurance approval and difficulty obtaining it, burosumab was started in only 8 of 17 patients. On Burosumab treatment, patient compliance and phosphorus levels were more stable, and no significant side effects were observed.

Chemotherapy-induced HR: Long bone fracture and HR were detected in an 11-year-old female patient who received chemotherapy with the diagnosis of Ewing sarcoma. Vertebral compression fracture and HR were detected in a 5-year-old female patient who received chemotherapy for neuroblastoma. Phosphate and calcium treatments were started in patients who were thought to have HR secondary to chemotherapy, and bisphosphonate treatment was started in the first patient because of the DEXA Z score of -3.4.

McCune Albright Syndrome (MAS): The median age at diagnosis of the five patients followed up with MAS was 8 years (6-10 years). Three patients were presented with suspicion of precocious puberty, one with a bone fracture, and one with hip pain. Diagnostic mean Ca 9.42±0.41 mg/dl, P 3.1.4±0.92, ALP 799 U/L (180-1203), and PTH 50.1 ng/l (27.2-84) were found. The TRP and TmP/GFR were calculated as 85.2±10% and 4.26±3.29, respectively. In all cases, café au lait spots and polyostotic fibrous dysplasia were detected at the time of diagnosis, while three cases presenting with precocious puberty had ovarian cysts (peripheral precocious puberty). Two cases were started on bisphosphonate due to recurrent bone fractures and DEXA Z scores of -2.7 and -5.2, and four cases were started on calcitriol and phosphate.

FGF23-independent HR

Fanconi-Reno tubular syndrome: In the case of those who applied with bowlegs at the age of six, there were proximal renal tubular acidosis (RTA) findings other than HR and a second-degree cousin marriage, and there was no similar complaint in the family. Genetic studies could not be performed in this case, who was followed up with a preliminary diagnosis of the "Fanconi-Reno tubular syndrome."

Oculocerebrorenal (Lowe) syndrome: In a 5-month-old male patient, syndromic facial appearance, history of clavicle fracture, bilateral cataract, nephrocalcinosis, and HR were detected. In the

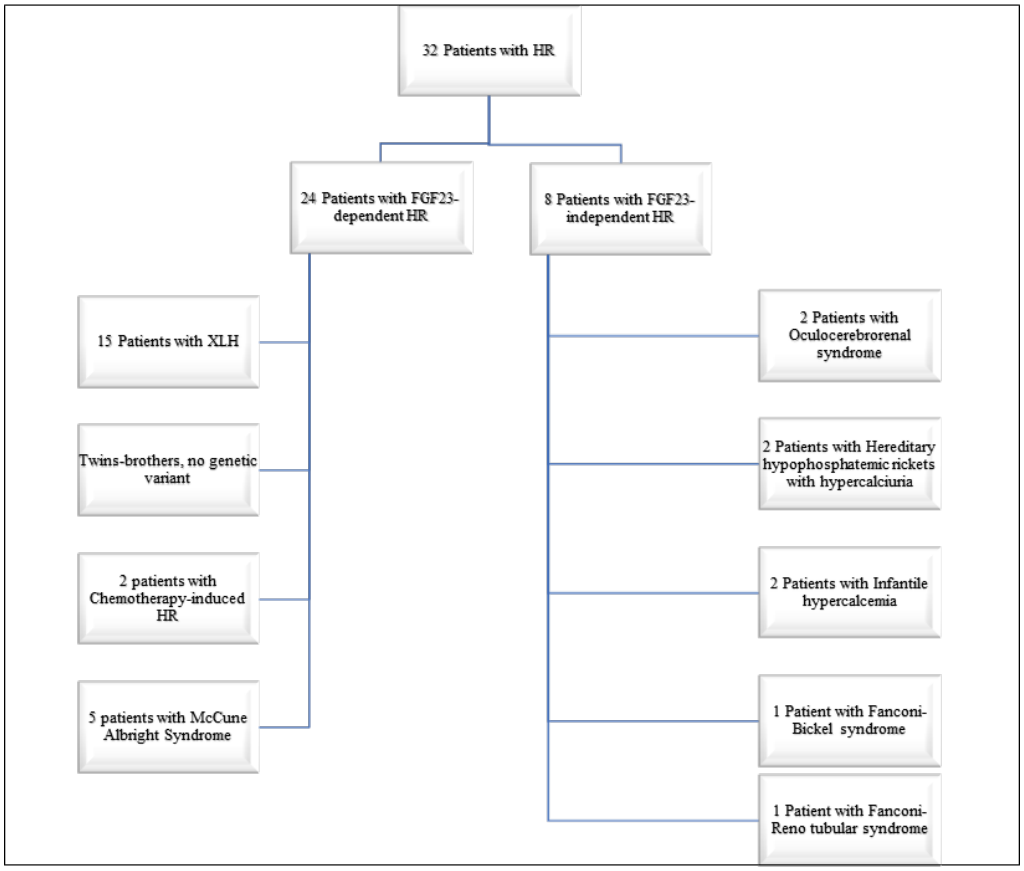

Figure 1:
The distribution of patients according to FGF23-dependent and FGF23-independent groups is shown in Figure 1.

physical examination, weight and height were -3.36 and -4.18 SDS, respectively. Calcium 9.8 mg/dl (8.8-10.8), phosphorus 3.2 mg/dl (3.7-5.6), ALP 767 U/L (156-369), PTH 8.2 ng/L (16-63), TRP: 84%, TmP/GFR: 3.79, and urine Ca/Cre 1.5. A genetic study was performed, and the *OCRL* gene c.1467-1G>C variant was found.

A 14.5-year-old male was admitted with short stature and *OCRL* gene variant results. He was diagnosed with proteinuria at 7 years old. In the physical examination, weight and height were -1.42 and -3.24 SDS, respectively, and no skeletal dysplasia. Hemizygous C.560+1G>A variant detected in the *OCRL* gene. Calcium 8.9 mg/dl (8.8-10.8), phosphorus 2.5 mg/dl (3.7-5.6), ALP 436 U/L (156-369), PTH 25.7 ng/L (16-63), TRP: 88%, TmP/GFR: 4.26, and urine Ca/Cre 0.42.

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH): A 9-year-old girl presented to the outpatient clinic with growth retardation. She had been diagnosed with kidney stones at 6 months of age, and she was receiving hydrochlorothiazide treatment. On physical examination, her weight was -2.97 SDS, and her height was -3.23 SDS. Laboratory tests revealed calcium 10.9 mg/dl (8.8-10.8), phosphorus 2.6 mg/dl (3.7-5.6), ALP 287 U/L (156-369), PTH 9.9 ng/L (16-63), 25-OH vitamin D 22.9 µg/L (>15), 1.25-OH vitamin D 26.44 pg/mL (16-65), Somatomedin-C 70 µg/L (-1.8 SDS), free t4 1.35 ng/dL, TSH 2.931 mU/L, bone age 7 years 2 months, TRP: 51%, TmP/GFR: 1.35 and urine Ca/Cre 0.01 (under treatment). With these findings, genetic analysis was performed for hereditary hypophosphatemic rickets and hypercalciuria. Heterozygous pathogenic variant C.100_1003delGTGC is detected in the *SLC34A3* gene. Oral phosphate (20 mg/kg/day) was started, and dietary calcium was restricted.

An 11.8-year-old female was referred to us with the diagnosis of nephrocalcinosis with the *SLC34A3* gene variant. She had been followed up with kidney stones since the age of two and had a family history of kidney stones. She was receiving hydrochlorothiazide treatment. In her physical examination, her weight was -1.08 SDS, and her height was SDS -0.39, which was within the normal range, with no skeletal deformity. Laboratory tests revealed calcium 10.5 mg/dl (8.8-10.8), phosphorus 3.7 mg/dl (3.7-5.6), ALP 266 U/L (156-369), PTH 8.6 ng/L (16-63), TRP: 93%, TmP/GFR: 4.47 and urine Ca/Cre 0.02 (under treatment). Oral phosphate (25 mg/kg/day) was started.

Table I. Clinical, laboratory, genetic, and family characteristics of patients with *PHEX* gene mutations (XLH).

Patient no	Family no	Gender	Onset of complaints (years)	Age of diagnosis (years)	Weight at diagnosis (SDS)	Height at diagnosis (SDS)	Body mass index at diagnosis (SDS)	Complaints at admission	lower bone deformity	Additional deformities	Calcium (mg/dl)	Phosphorus (mg/dl)	Alkaline phosphatase (ALP) (U/L)	Parathyroid hormone (PTH) (ng/l)	Tubular reabsorption of phosphate (TRP) (%)	TmP/GFR	DNA description	Protein description	Variant	Pathogenicity: According to ACMG criteria (7)	Clinvar	Franklin	HGMD	Zygosity	Family members with XLH	Consequence
3	2	Male	1.0	2.5	n/a	n/a	n/a	Bowlegs	X-bain	Frontal bossing and pectus excavatum	8.9	2.2	349	273	71	3.2	g.22016715_22056805del		Novel (10)	Pathogenic	0	0	0	Heterozygous	No	No
4	3	Male	1.5	1.5	0.48	-1.72	2.28	In-toeing	O-bain	Frontal bossing	9.8	2.3	707	102.3	66	1.53	c.281_281delTTCCCGAA	p.Ile94Argfs*14	Novel (11)	Likely pathogenic	0	LP	0	Hemizygous	Mother	No
5	4	Female	3.0	6.0	-2.26	-5.56	1.58	In-toeing	O-bain	No	9.8	2.2	374	35.6	72	1.65	c.1645+1G>A	no transcript	Reported	Pathogenic	P	P	DM Rickets, hypophosphataemic	Heterozygous	No	No
6	5	Male	1.0	10.0	-2.29	-4.08	-0.07	Bowlegs	X-bain	No	9.0	1.7	345	69.1	68	1.15	c.2242_2243delCT	p.L748fs*48	Reported	Likely pathogenic	0	P	0	N/a	No	No
7	6	Female	2.0	2.0	-1.68	-2.91	-0.03	Bowlegs	O-bain	No	9.3	1.9	485	109.9	40	1.20	c.1806G>A	p.Trp602*	Reported	Pathogenic	P	P	DM Rickets, hypophosphataemic	Heterozygous	Mother, uncle, grandmother, cousins	No
8	6	Female	0.5	0.5	-0.31	-0.84	0.26	XLH of family members	No	No	10.5	3.1	685	109	85	2.64	c.1806G>A	p.Trp602*	Reported	Pathogenic	P	P	DM Rickets, hypophosphataemic	Heterozygous	Father, aunt, grandmother, sister	No
9	6	Female	0.7	0.7	-0.32	-1.18	0.48	XLH of family members	No	No	10.4	2.5	418	107.8	53	1.49	c.1806G>A	p.Trp602*	Reported	Pathogenic	P	P	DM Rickets, hypophosphataemic	Heterozygous	Father, aunt, grandmother, sister	No
10	7	Male	0.3	0.3	-1.62	-1.68	-0.88	XLH of family members	No	No	9.9	3.1	730	137	91	3.18	c.1899+5G>T	IVS14+1G>A	Reported	Pathogenic	0	VUS	0	Hemizygous	Mother, aunt, grandfather	No
11	7	Female	0.4	0.4	-0.41	1.36	-1.80	XLH of family members	No	No	9.7	3.3	686	104.2	88	2.91	c.1899+5G>T	IVS14+1G>A	Reported	Pathogenic	0	VUS	0	Heterozygous	Mother, aunt, grandfather	No
12	8	Female	1.5	8.0	1.02	-1.19	1.51	Bowlegs	O-bain	No	9.4	2.7	535	72.1	82	2.2	c.1735G>A	p.Gly579Arg	Reported	Pathogenic	0	P	DM Rickets, hypophosphataemic	Heterozygous	Mother	No
13	9	Female	2.0	2.0	n/a	n/a	n/a	Bowlegs	O-bain	No	9.2	2.8	497	118	80	2.08	c.448A>T	p.Lys150Ter	Reported	Pathogenic	P	P	0	Heterozygous	No	No
14	8	Female	2.1	2.1	1.64	-1.29	3.13	XLH of family members	O-bain	No	10.1	2.80	642	54.9	75	2.12	c.1735G>A	p.gly579Arg	Reported	Pathogenic	0	P	DM Rickets, hypophosphataemic	Heterozygous	Sister	No
15	10	Female	1.0	8.1	-0.56	-2.82	1.17	Bowlegs	O-bain	No	9.5	3.0	928	164	79	2.39	c.2247G>T	p.W749C	Novel	Pathogenic	0	LP	0	Heterozygous	No	No
16	11	Male	1.8	3.2	0.28	-1.99	2.20	Bowlegs	O-bain	No	9.1	2.1	570	69.7	84	2.11	Exons 8 and 9 deletions	n/a	n/a	Pathogenic			DM Rickets, hypophosphataemic	Hemizygous	Mother, grandmother	No
17	12	Female	1.5	2.0	-2.73	-2.60	-1.59	Bowlegs	O-bain	No	9.3	2.37	n/a	n/a	84	2.1	c.1461_1465del	p.H487Qfs*2	Novel	Likely pathogenic	0	LP	0	Heterozygous	No	No

Table II. Genetic and diagnostic characteristics of patients with FGF23-independent HR

Patient no	Family no	Disease	Age at presentation (years)	Age at diagnosis (years)	Complaint	Gene	DNA description	Pathogenicity: According to ACMG criteria (7)	Zygosity
18	13	Fanconi-Reno tubular syndrome	6	6	O Bain	n/a	n/a	n/a	n/a
19	14	Oculocerebrorenal (Lowe) syndrome	0.4	0.6	Nephrolithiasis and fracture	*OCRL1*	c.1467-1G>C	pathogenic	Homozygous
20	15	Oculocerebrorenal (Lowe) syndrome	7	14	Family history	*OCRL1*	c.560+1G>a		Hemizygous
21	16	Hereditary hypophosphatemic rickets with hypercalciuria	0.5	9	Growth retardation	*SLC34A3*	c.100_1003delGTGC	pathogenic	Heterozygous
22	17	Hereditary hypophosphatemic rickets with hypercalciuria	9	11.8	Nephrolithiasis	*SLC34A3*	c.846G>A	pathogenic	Homozygous
23	18	Infantile hypercalcemia type 2	0.4	5	Nephrolithiasis and genetic result	*SLC34A1*	c.272_292del c.644+1G>A	pathogenic	Compound Heterozygous Maternal (Heterozygous) *SLC34A1* c.272_292del Paternal (Heterozygous) *SLC34A1* c.644+1G>A
24	19	Infantile hypercalcemia type 2	0.3	14	Hypercalcemia	*SLC34A1*	c.713A>C c.1449G>A	pathogenic	Compound Heterozygous Maternal (Heterozygous) *SLC34A1* c.713A>C Paternal (Heterozygous) *SLC34A1* c.1449G>A
25	20	Fanconi-Bickel syndrome	0.83	1.2		*SLC2A2*	c.835_836del	pathogenic	Homozygous

Infantile hypercalcemia type 2: A 5-year-old boy who had been diagnosed with nephrocalcinosis at 5 months. He was admitted with genetic analysis c.272_292del and c.644+1G>A variants found in the *SLC34A1* gene. His weight is 0.41 SDS, and his height SDS is 0.57, within the normal range, and he has no skeletal deformity. Laboratory tests revealed calcium 11.5 mg/dl (8.8-10.8), phosphorus 4.2 mg/dl (3.7-5.6), ALP 234 U/L (156-369), PTH <3 ng/L (16-63), TRP: 90%, TmP/GFR: 4.22, and urine Ca/Cre 0.3.

A 4-month-old male was admitted with vomiting. In the physical examination, weight and height were -1.98 and -0.26 SDS, respectively. No skeletal deformity. Calcium 15.3 mg/dl (8.8-10.8), phosphorus 1.7 mg/dl (3.7-5.6), ALP 248 U/L (156-369), PTH 39.8 ng/L (16-63), TRP: 86%, TmP/GFR: 3.39, and urine Ca/Cre 0.8. Oral phosphate (40 mg/kg/day) was started immediately. In the follow-up, nephrolithiasis was detected. After a long follow-up period when he was fourteen, *SLC34A1* gene variants (heterozygous c.713A>C and c.1449G>A) were found (In segregation analyses, the father had a heterozygous c.713A>C variant, and the mother had a c.1449G>A variant).

Fanconi-Bickel syndrome: She initially was admitted to an external center at the age of 1 with growth retardation, and since Calcium 9.5 mg/dl (8.8-10.8), phosphorus 2.5 mg/dl (3.7-5.6), ALP 558 U/L (156-369), PTH 85.7 ng/L (16-63), and TPR were 45%. Her cousin was diagnosed with Fanconi-Bickel syndrome; she was diagnosed at the age of 14 months, and calcitriol and phosphate treatments were started immediately (c.835_836del variant homozygous in the *SLC2A2* gene). In her physical examination, weight -2.65 SDS, height SDS -3.55, and O-Bain were detected. FGF23-independent patients were described in Table II.

Discussion and Conclusion

We presented single-center experiences via diagnostic characteristics and genetic analyses of HR in thirty-two children. Most patients were in the FGF23-dependent group; the pathogenic/likely pathogenic *PHEX* gene variants (47%) were the most common, similar to the literature[3-4,9]. While the median age at diagnosis was 5 years in all HR patients, it was 2.05 years in XLH patients, which is very similar to previous Turkish HR surveys[3]. However, a Norwegian study found a median age of 2.1 and 0.9[4]. The very high rate of family history in this study (78%) may explain the earlier diagnosis. The fact that no non-XLH case had a skeletal deformity at presentation in our study suggests that XLH cases may be diagnosed earlier due to their more severe skeletal deformities.

Four novel *PHEX* mutations were found in our study-g.22016715_22056805del,

c.281_281delTTCCCGAA, c.2247G>T, c.1461_1465 del- two of which we reported previously[10,11]. There were also many novel *PHEX* variants that had been published before from Türkiye: c.1217G>A, c.2078G>T, IVS13+1G>T, IVS13–2A>G, c.684_685dup, c.1900-6T>A, c.1162G>T, c.1309G>T, c.1392delA, c.1313T>A, c.1173+5G>A, c.1899+5G>T, c.154G>T, c.401_402insGCCAAA, c.1600C>T, c.2242_2243delCT, c.1382_1391del, c.1111_1112insTT, c.983_987dupCTACC, c.1586+2T>G, c.1206delA, c.436+1G>T, c.1217G>T, and g.22,215,887±22,395,767del[11-16].

No significant difference was found between the age of symptom onset and diagnosis in HR cases with *PHEX* mutation. In contrast, a significant difference was found when all cases were considered. Diagnosis may be delayed, especially in HR cases accompanied by hypercalciuria that presents with nephrocalcinosis (esp. *SLC34A1* and *SLC34A3* cases)[4,13]. In another study, Broadman-Pretty speculated on "a diagnostic odyssey" in XLH patients and emphasized the prevention of diagnostic delay and the importance of early initiation of proper treatment[17]. Although symptoms appear early in most cases, diagnosis may be delayed. One study similarly states that HHRH patients were misdiagnosed with FGF-dependent HR before targeted next-generation sequencing, were given the wrong treatment, and received revised treatment immediately[13]. While PTH was normal in patients, ALP was found to be high. In this patient group, especially when PTH is normal, ALP elevation is important; it responds to phosphorus loss[12,18], and despite this, secondary hyperparathyroidism can be seen during treatment. In our study, while skeletal deformities were very evident in XLH cases, it was noted that skeletal findings were not apparent in the remaining group, and short stature was mainly seen. Notably, the fact that non-XLH patients present with non-specific findings such as short stature, nephrocalcinosis, and growth retardation indicate that calcium metabolism tests, including phosphorus, should be evaluated at least once in all these patients. In cases with precocious puberty, hyperthyroidism, and bone fractures, skin findings should be carefully examined, and, if necessary, bone radiographs should be taken to exclude MAS. Calcitriol and phosphate (conventional treatment) are started once the diagnosis is made (only phosphate in hypocalciuric cases)[5,19]. Although it has been known as an initial treatment for a long time, many studies showed that there were complications: nephrolithiasis, secondary hyperparathyroidism, and persistence of renal phosphate wasting and hypophosphatemia were seen[4-5,20]. It has been reported that the new treatment modality, burosumab[5,20,21], increases serum phosphorus by increasing TPR, improves growth, and helps recover rickets[5,9,20,22]. An international multicenter phase 3 study demonstrated that burosumab was superior to conventional therapy in improving rickets scores, growth, limb deformities, and mobility in children ages 1 to 12. This was supported by real-world studies reporting promising improvements in linear growth, rickets scores, and laboratory values in pediatric patients treated with burosumab. The FDA expanded approval to children ages 6 months and older in 2022[20,23]. This treatment is now recommended as a first-line treatment in symptomatic patients[22]. In our cases, due to the risk of nephrocalcinosis, skeletal deformities, and persistent low phosphate levels, Burosumab treatment has been started in most XLH cases. No complications or side effects were seen with this treatment.—FGF-23 independent causes leading to hypophosphatemia are often followed by other clinics, with different findings: nephrocalcinosis, hypercalcemia, kidney failure, developmental delay, and psychomotor delay. Firstly, FGF-23 dependent and independent distinctions should be made in hypophosphatemic cases to use accurate treatment (for example, HHRH patients must not use calcitriol treatment, and Burosumab is effective in XLH patients)[17,19-21]. Gene panels of possible causes should be designed, or NGS should be performed to prevent diagnosis delay and incorrect treatment[12-13]. Eltan et al. have recently reported that MLPA may provide an additional explanatory value of 10% regarding molecular etiology in HR cases[12]. Despite all analyses, this method should be considered when the diagnosis cannot be established.

Hypophosphatemic rickets is a rare disease. When hypophosphatemia and increased renal phosphate wasting are detected in patients presenting with bowlegs, short stature, and kidney stones, it should be determined whether they are in the FGF-23 dependent or FGF-23 independent group, and appropriate treatment should be initiated. Since the delay in diagnosis is particularly evident in the group presenting with nephrocalcinosis, it should be routine to measure calcium and phosphate levels in patients diagnosed with nephrocalcinosis.

Strengths and limitations

Although HR is a rare disease, the number and variety of patients are superior to many studies.

The limitation of this study is that it is a descriptive study conducted through retrospective file scanning.

Researcher Contribution Statement:

Idea and design: Y.D.Ö., E.E.; Data collection and processing: Y.D.Ö.; Analysis and interpretation of data: Y.D.Ö., Ş.G.T., Ö.T., Ş.Ö.S., E.E.; Writing of significant parts of the article: Y.D.Ö., Ş.G.T., Ö.T., Ş.Ö.S., E.E.

Support and Acknowledgement Statement:

Special thanks to Prof. Serap Demircioğlu for the genetic analysis of our twin HPR patient.

Conflict of Interest Statement:

The authors of the article have no conflict-of-interest declarations.

Ethics Committee Approval Information:

Approving Committee: Uludağ Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik kurul

Approval Date: 15.11.2021

Decision No: 2021-KAEK-26

Revision No: 2025/559-6/23

References

1- Kritmetapak K, Kumar R. Phosphatonins: From Discovery to Therapeutics. Endocr Pract. 2023;29(1):69-79.

2- Imel EA. Congenital Conditions of Hypophosphatemia in Children. Calcif Tissue Int. 2021;108(1):74-90.

3- Şıklar Z, Turan S, Bereket A, et al. Nationwide Turkish Cohort Study of Hypophosphatemic Rickets. J Clin Res Pediatr Endocrinol. 2020;12(2):150-159.

4- Rafaelsen S, Johansson S, Ræder H, Bjerknes R. Hereditary hypophosphatemia in Norway: a retrospective population-based study of genotypes, phenotypes, and treatment complications. Eur J Endocrinol. 2016;174(2):125-136.

5- Lambert AS, Zhukouskaya V, Rothenbuhler A, Linglart A. X-linked hypophosphatemia: Management and treatment prospects. Joint Bone Spine. 2019;86(6):731-738.

6- Rothenbuhler A, Schnabel D, Högler W, Linglart A. Diagnosis, treatment-monitoring and follow-up of children and adolescents with X-linked hypophosphatemia (XLH). Metabolism. 2020;103S:153892.

7- Demir K, Özen S, Konakçı E, Aydın M, Darendeliler F. A comprehensive online calculator for pediatric endocrinologists: ÇEDD Çözüm/TPEDS Metrics. J Clin Res Pediatr Endocrinol 2017;9(2):182-184.

8- Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine. 2015;17(5):405-23.

9- Capelli S, Donghi V, Maruca K, et al. Clinical and molecular heterogeneity in a large series of patients with hypophosphatemic rickets. Bone. 2015;79:143-149.

10- Demirbaş Ö, Eren E, Öngen YD, et al. A Novel PHEX Mutation in A Case Followed Up with A Diagnosis of X-linked Hypophosphatemic Rickets. J Curr Pediatr. 2023 Apr;21(1):98-101.

11- Guven A, Al-Rijjal RA, BinEssa HA, et al. Mutational analysis of PHEX, FGF23 and CLCN5 in patients with hypophosphataemic rickets. Clin Endocrinol (Oxf). 2017;87(1):103-112.

12- Eltan M, Alavanda C, Abali ZY, et al. Clinical and molecular genetic characteristics of patients with hereditary hypophosphatemia. J Clin Endocrinol Metab. Published online December 19, 2024.

13- Turan I, Erdem S, Kotan LD, et al. Experience with the targeted next-generation sequencing in the diagnosis of hereditary hypophosphatemic rickets. J Pediatr Endocrinol Metab. 2021;34(5):639-648. Published 2021 Apr 13. doi:10.1515/jpem-2020-0624.

14- Durmaz, Erdem et al. "Novel and de novo PHEX mutations in patients with hypophosphatemic rickets." Bone vol. 52,1 (2013): 286-91.

15- Alikasifoglu A, Unsal Y, Gonc EN, Ozon ZA, Kandemir N, Alikasifoglu M. Long-term effect of conventional phosphate and calcitriol treatment on metabolic recovery and catch-up growth in children with PHEX mutation. J Pediatr Endocrinol Metab. 2021;34(12):1573-1584. Published 2021 Sep 16.

16- Acar S, BinEssa HA, Demir K, et al. Clinical and genetic characteristics of 15 families with hereditary hypophosphatemia: Novel Mutations in PHEX and SLC34A3. PLoS One. 2018;13(3):e0193388. Published 2018 Mar 5.

17- Boardman-Pretty F, Clift AK, Mahon H, Sawoky N, Mughal MZ. The Diagnostic Odyssey in Children and Adolescents With X-linked Hypophosphatemia: Population-Based, Case-Control Study. J Clin Endocrinol Metab. 2024;109(8):2012-2018.

18- Wu H, Ying H, Zhao W, et al. Characterization of Novel PHEX Variants in X-linked Hypophosphatemic Rickets and Genotype-PHEX Activity Correlation. J Clin Endocrinol Metab. 2024;109(9):2242-2255.

19- Bergwitz C, Miyamoto KI. Hereditary hypophosphatemic rickets with hypercalciuria: pathophysiology, clinical presentation, diagnosis, and therapy. Pflugers Arch. 2019;471(1):149-163.

20- Imel EA, Glorieux FH, Whyte MP, et al. Burosumab versus conventional therapy in children with X-linked hypophosphataemia: a randomised, active-controlled, open-label, phase 3 trial [published correction appears in Lancet. 2019 Jul 13;394(10193):120.

21- Kamenický P, Briot K, Munns CF, Linglart A. X-linked hypophosphataemia. Lancet. 2024;404(10455):887-901. doi:10.1016/S0140-6736(24)01305-9.

22- Haffner, D., Emma, F., Seefried, L. et al. Clinical practice recommendations for the diagnosis and management of X-linked hypophosphataemia. Nat Rev Nephrol (2025).

23- US Food and Drug Administration. Crysvita (burosumab-twza) Marketing Approval.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 201-208, 2025
DOI: https://doi.org/10.32708/uutfd.1666702

ORIGINAL RESEARCH

The Impact of Regional Anesthesia Techniques on Pain Control and Opioid Consumption in Sleeve Gastrectomy

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ABSTRACT

This study aims to evaluate the effectiveness of erector spinae block (ESP) and transversus abdominis plane (TAP) blocks in reducing postoperative opioid requirements and enhancing pain control in laparoscopic sleeve gastrectomy (LSG) patients. This retrospective study included 90 patients undergoing LSG. The patients were equally allocated into three groups—ESP block, TAP block, and control (no regional anesthesia applied)—with 30 patients in each group. During the allocation process, patients were matched for age and gender. Pain levels were assessed using the Visual Analog Scale (VAS) at 0, 2, 4, 8, 12, and 24 hours postoperatively. Opioid consumption, side effects, patient, and surgeon satisfaction were recorded. Statistical analyses were conducted to compare pain scores, opioid use, and satisfaction levels among the groups. The ESP block group reported the lowest VAS scores, indicating superior pain control. Opioid consumption was significantly reduced in both ESP and TAP block groups compared to the control group, with the ESP group showing the greatest reduction. There was a significant relationship between the amount of opioid used and side effects. Patient satisfaction was highest in the ESP block group, followed by the TAP block group, and lowest in the control group. The ESP and TAP blocks are effective in reducing postoperative opioid consumption and providing better pain control in LSG patients. The ESP block, in particular, offers superior analgesia and higher patient satisfaction compared to the TAP block and no block.

Keywords: Erector spinae plane block. Laparoscopic sleeve gastrectomy. Opioid consumption. Postoperative pain. Transversus abdominis plane block.

Regionel Anestezi Tekniklerinin Sleeve Gastrektomide Ağri Kontrolü ve Opioid Tüketimi Üzerindeki Etkisi

ÖZET

Bu çalışma, laparoskopik mide tüpü gastrektomi (LSG) hastalarında erector spinae bloğu (ESP) ve transversus abdominis plane (TAP) bloklarının postoperatif opioid gereksinimlerini azaltma ve ağrı kontrolünü iyileştirme etkinliğini değerlendirmeyi amaçlamaktadır. Bu retrospektif çalışmaya, LSG uygulanan 90 hasta dahil edilmiştir. Hastalar, her bir grup için 30 hasta olacak şekilde üç gruba eşit olarak dağıtılmıştır—ESP bloğu, TAP bloğu ve kontrol (regionel anestezi uygulanmayan)—ve hastalar yaş ve cinsiyet açısından eşleştirilmiştir. Ağrı düzeyleri, postoperatif 0, 2, 4, 8, 12 ve 24 saatlerde Görsel Analog Skala (VAS) kullanılarak değerlendirilmiştir. Opioid tüketimi, yan etkiler, hasta ve cerrah memnuniyeti kaydedilmiştir. Gruplar arasında ağrı skorları, opioid kullanımı ve memnuniyet düzeylerini karşılaştırmak için istatistiksel analizler yapılmıştır. ESP grubu, kontrol grubuna kıyasla anlamlı şekilde daha düşük VAS skorları göstermiştir. ESP bloğu grubunda en düşük VAS skorları bildirilmiş, bu da üstün ağrı kontrolünü göstermektedir. Opioid tüketimi, ESP ve TAP bloğu gruplarında kontrol grubuna göre anlamlı şekilde azalırken, ESP grubunda en büyük azalma gözlemlenmiştir. Kullanılan opioid miktarı ile yan etkiler arasında anlamlı bir ilişki bulunmuştur. Hasta memnuniyeti, en yüksek ESP bloğu grubunda, ardından TAP bloğu grubunda ve en düşük kontrol grubunda kaydedilmiştir. ESP ve TAP blokları, LSG hastalarında postoperatif opioid tüketimini azaltmada ve daha iyi ağrı kontrolü sağlamada etkilidir. Özellikle ESP bloğu, TAP bloğuna ve herhangi bir blok uygulanmayan gruba kıyasla üstün analjezi ve daha yüksek hasta memnuniyeti sunmaktadır.

Anahtar Kelimeler: Erectör spina plane bloğu. Laparoskopik sleeve gastrektomi. Opioid tüketimi. Postoperatif ağrı. Transversus abdominis plane bloğu.

Date Received: 28.March.2025
Date Accepted: 10.June.2025

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Obesity is a global health issue associated with significant morbidity and mortality due to its related comorbidities.[1] Sleeve gastrectomy (SG) is the most commonly performed bariatric surgery for managing obesity.[2]

Following tissue damage, local inflammatory mediators, such as bradykinin, substance P, prostaglandins, histamine, and serotonin, are released at the surgical site, inducing pain. Impulses from these inflammatory mediators upon tissue destruction activate nociceptors, i.e., free nerve endings that sense

pain. Fast, sharp pain is transmitted by myelinated A-delta fibers, whereas slow, chronic pain is carried by unmyelinated C fibers via the spinal cord anterolateral pathway.[3]

The perioperative period is characterized by immune system suppression due to inflammatory pain and surgical stress, which inhibits cytotoxic cells.[4] Multimodal analgesia strategies during this period can benefit immune modulation and minimize immune suppression, thereby reducing postoperative morbidities.[5] Regional anesthesia techniques as part of multimodal analgesia also modulate the immune response by reducing surgical stress and opioid use, which are known to impair cellular and humoral immunity. This helps protect patients from opioid-related side effects and prevents immunosuppression caused by pain and surgical stress. Effective management of immunosuppressive factors is crucial for shorter hospital stays, a more comfortable postoperative process, and reduced hospital costs.[4]

Postoperative pain management can be complicated by serious side effects, such as respiratory depression, cardiopulmonary arrest, urinary retention, ileus, and addictive potential, of high-dose opioids used for analgesia in patients who undergo bariatric surgery.[6,7] Opioids also suppress the immune system by weakening the cytotoxic functions of natural killer cells.[8] Therefore, it is important to reduce opioid use and explore alternative pain management methods that can replace or support opioid therapy.[9]

The Visual Analog Scale (VAS) is a reliable and valid scale for assessing pain severity in postoperative patients.[10]

The Transversus Abdominis Plane (TAP) block, first described by Rafi in 2001, is an intervention that creates a peripheral nerve block in the anterolateral abdominal wall.[11] TAP is the space between the internal oblique and transversus abdominis muscles. An anesthetic is injected into this site for the procedure. Initially, the procedure was performed blindly, but its reliability and success rate have improved with ultrasonography (USG) guidance.[12] The Erector Spinae Plane (ESP) block, introduced as a technique for managing thoracic neuropathic pain and pain associated with thoracic trauma or surgery[13] is based on the columnar arrangement of the erector spinae muscle and retinaculum. This concept suggests that ESP block applications at lower thoracic levels might also provide abdominal analgesia.[14]

This study aims to compare the efficacy of the Erector Spinae Plane (ESP) block and the Transversus Abdominis Plane (TAP) block in managing postoperative pain following sleeve gastrectomy (SG), one of the most commonly performed bariatric surgeries for obesity. Specifically, the study evaluates the effectiveness of both regional anesthesia techniques in reducing postoperative analgesic requirements, opioid consumption, pain intensity, and opioid-related side effects. Additionally, as part of a multimodal analgesia approach, the potential of these blocks to attenuate surgical stress and mitigate immunosuppression will also be indirectly assessed. The ultimate goal is to contribute to the identification of a more effective, safer, and immune-supportive pain management strategy for patients undergoing SG.

Material and Method

Study Design and Patient Population

The study was reviewed and approved by the Harran Universisty Clinical Research Ethics Committee (28.12.2023-25).

The study was designed as a retrospective study. The patients included in the study were selected from those who underwent laparoscopic sleeve gastrectomy (LSG) for morbid obesity in our clinic between January 1, 2023, and June 30, 2023. The patients were equally allocated into three groups—ESP block, TAP block, and control (no regional anesthesia applied)—with 30 patients in each group. During the allocation process, patients were matched for age and gender.

All patients underwent LSG by a single general surgeon. Due to the potential need for analgesics in the postoperative recovery unit, the patients were accompanied by the anesthesiologist, who performed the block procedure. Postoperative pain assessment was performed using VAS score at 0, 2, 4, 8, 12, and 24 hours after the patient was returned to their room.

A Likert-type survey was used to assess patient and physician satisfaction with postoperative analgesia. This survey is a routine assessment tool applied to patients who have undergone obesity surgery during their outpatient follow-up visits in our clinic. During the first outpatient clinic control after discharge, the patient was asked, “Considering the pain I experienced after surgery, I would have surgery again if I went back to the preoperative period”; and the physician was asked, “When I evaluate the patient in terms of pain control, the patient was easy to manage.” They were asked to respond to both questions with one of the following responses, which were scored thereafter;

Strongly disagree (1 point)

Disagree (2 points)

Neither agree nor disagree (3 points)

Agree (4 points)

Strongly agree (5 points)

Patients were evaluated by age, sex, body mass index (BMI), American Society of Anesthesiologists physical status classifications (ASA) score,

comorbidity, amount of opioid use, VAS score, side effects (urinary retention, cardiopulmonary arrest, respiratory depression and ileus were considered as side effects), patient, and physician satisfaction. Data other than the survey responses were obtained from patient records.

Inclusion and Exclusion Criteria

Pursuant to the inclusion criteria, patients aged 18–60 years, patients with ASA scores of I, II, and III, and a BMI of >35 kg/m^2 were included in the study.

For the exclusion criteria, patients with missing data in their records, patients who were allergic to local anesthetics, refused a regional block, underwent concomitant surgeries, experienced intraoperative or postoperative complications within the first 24 hours, had a platelet count of <100,000, had known coagulopathy, or had a history of opioid addiction. Patients who underwent concomitant surgeries (e.g., cholecystectomy) were excluded from the study due to the potential for increased surgical morbidity and higher analgesic requirements. Additionally, patients with coagulopathy or low platelet counts were excluded due to the elevated risk of bleeding, and individuals with opioid dependence were excluded as they may require higher opioid doses for effective analgesia.

General Anesthesia, ESP, and TAP Block Applications

At induction of anesthesia, all patients received 2 mg/kg propofol, 0.5 mg/kg atracurium, and 2 mcg/kg fentanyl based on their corrected ideal weight. No additional analgesics or anti-inflammatories were administered. 1000 mg of 1st generation cephalosporin was administered intravenously for preoperative antibiotic prophylaxis. The same anesthesiologist performed regional anesthesia to ensure consistency in the study results. All regional anesthesia procedures were performed on the operating table upon induction of anesthesia. The control group did not receive regional anesthesia. Intravenous (IV) opioids were used for postoperative analgesia in all groups based on the patient's needs and VAS scores. If required, postoperative patients received intravenous morphine sulfate infusion at a dose of 0.1 mg/kg of body weight.

ESP Block Application

ESP block was performed immediately after induction of anesthesia to investigate its effect on postoperative analgesia. It was performed on the operating table before induction of general anesthesia. Overall, 10% povidone–iodine was used for skin preparation. The probe was covered with a sterile drape. All ESP blocks were performed by the same anesthesiologist, who was experienced in ultrasound-guided regional anesthesia techniques. A wide-bandwidth (1–8 MHz) convex probe (Esaote®, MyLab5, Italy) and a 22G, 50 or 100 mm, isolated facet-type needle (Braun Sonoplex, Melsungen, Germany) were used to perform the multifrequency block. Blocks were performed bilaterally using an intraplane approach at the T8 level with the patient in the prone position. The convex probe was placed 2–3 cm lateral to the spine using a sagittal approach. The needle was inserted deeply into the erector spinae muscle after the erector spinae muscle and transverse processes were identified (Figure 1). The correct position of the needle tip was verified by 0.5–1 ml of local anesthetic (LA). 20 ml of 0.25% bupivacaine was administered as local anesthetic to perform the ESP block. local anesthetic spread was confirmed in both cranial and caudal directions.

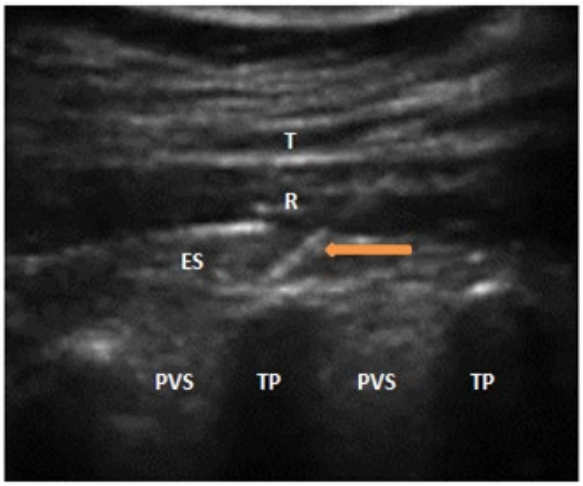

Figure 1:
Identifying the layers and determining the needle position in ESP Block application (T: Trapezius muscle, R: Rhomboid muscle, ES: Erector Spinae muscle, TP: Transverse process, PVS: Paravertebral space, area marked with an arrow: Needle insertion and drug administration)

TAP Block Procedure

Following the induction of general anesthesia, the surgical site was cleaned with an antiseptic solution on the operating table, and a sterile cover was placed over the linear ultrasound probe (Esaote®, MyLab5, Italy). To capture the optimal image, the probe was moved vertically or tilted up and down along the abdominal wall toward the costal margin and iliac crest. Once it obtained a clear view, the probe was secured in position, spanning from the superficial to deep layers and displaying the skin, subcutaneous fat tissue, external oblique muscle, internal oblique muscle, transversus abdominis muscle fascia, and peritoneum. A 100-mm, 22-G needle (Braun Sonoplex, Melsungen, Germany) was advanced anterior to posterior using an in-plane technique under ultrasound guidance. Careful aspiration was performed to confirm

proper placement of the needle tip between the fascial planes of the internal oblique and transversus abdominis muscles. Subsequently, a test dose of 1 mL of 0.25% bupivacaine was administered to confirm proper needle localization (Figure 2). Following this, 20 mL of 0.25% bupivacaine solution was injected simultaneously on both the right and left sides under ultrasound guidance, after which the surgical procedure was initiated.

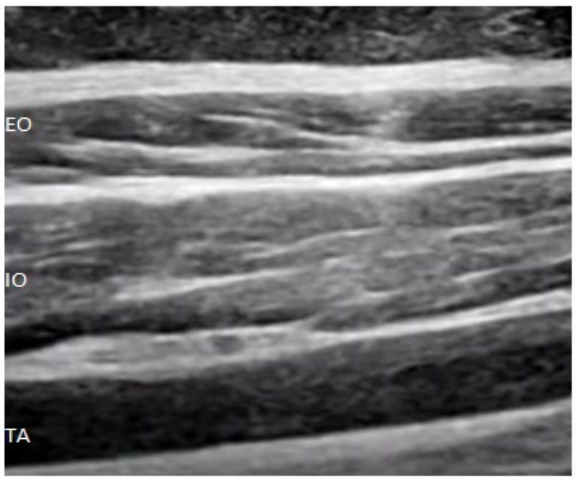

Figure 2:
Identifying layers in the TAP Block application (EO: External oblique muscle, IO: Internal oblique muscle, TA: Transversus abdominis muscle)

All patients underwent LSG, performed by a single general surgeon. Following surgery, the anesthesiologist who performed the block procedure accompanied the patients in the post-anesthesia care unit to address any potential analgesic needs. Once the patient was transferred to the regular ward, pain assessments were conducted based on the VAS score at 0, 2, 4, 8, 12, and 24 hours after surgery.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows, version 25.0 (IBM SPSS Inc., Chicago, IL, USA). The Shapiro–Wilk test was employed to assess the normality of the data distribution. As the data did not follow a normal distribution, continuous variables are presented as medians and minimum-maximum, while categorical variables are reported as counts (n) and percentages (%). Between-group comparisons involving more than two independent groups were conducted using the Kruskal–Wallis H test. The Friedman test was applied to evaluate changes in intragroup VAS scores over time (at 2, 4, 8, 12, and 24 hours), followed by post-hoc pairwise comparisons with Bonferroni correction. The Chi-square test was used for the analysis of categorical variables. All analyses were performed using a 95% confidence interval, and a two-tailed p-value less than 0.05 was considered statistically significant.

Results

The study including 25 (27.8%) male and 65 (72.2%) female patients. The mean age of the patients was 33.2 ± 9.5 years, where the mean BMI was 46.3 ± 5.3 kg/m². Overall 46 (51.1%) patients were classified as ASA 2 and 44 (48.9%) as ASA 3. There were no significant intergroup differences by age, sex, BMI, ASA score, and comorbidity variables ($p > 0.05$ for each) (Table I).

Table I. Comparison of Clinical and Demographic Characteristics based on Postoperative Analgesia Methods

	Overall (n = 90)	Group			p
		Control (n = 30)	ESP (n = 30)	TAP (n = 30)	
Age †	33.2 ±9.5	34.5 ±9.9	29.9 ±8.6	35.2 ±9.5	0.054*
Sex ‡					
Male	25 (27.8)	7 (23.3)	10 (33.3)	8 (26.7)	0.679**
Female	65 (72.2)	23 (76.7)	20 (66.7)	22 (73.3)	
BMI †	46.3 ±5.3	46.3 ±6.1	46.3 ±5.0	46.2 ±4.7	0.992*
ASA Score ‡					
2	46 (51.1)	13 (43.3)	14 (46.7)	19 (63.3)	0.252**
3	44 (48.9)	17 (56.7)	16 (53.3)	11 (36.7)	
Comorbid Conditions, *present* ‡	12 (13.3)	4 (13.3)	4 (13.3)	4 (13.3)	0.999**

ESP: Erector Spinae Plane, TAP: Transversus Abdominis Plane, BMI: Body Mass Index, ASA: American Society of Anesthesiologists

*. One-way ANOVA. **. Pearson's Chi-Squared/Fisher–Freeman–Halton test, ‡: n (%), †: Mean ± Standard deviation

There were significant differences between the groups by mean and maximum VAS scores, opioid use, patient, and physician satisfaction (Table II).

The number of patients with comorbid diseases was 12 (13.3%). The median and maximum VAS value was 3.7 and 6, respectively. Opioid use was necessary in 70 patients (77.8%), with a median opioid dose of 200 mg. Side effects associated with opioid use were observed in a total of 35 patients. There was no significant intergroup difference by the incidence of side effects associated with opioid use ($p = 0.311$). In terms of the patient satisfaction scores, 1 (1.1%) participant scored 1 point, 6 (6.7%) scored 2 points, 43 (47.8%) scored 3 points, 32 (35.6%) scored 4 points, and 8 (8.9%) scored 5 points. In terms of physician satisfaction, 6 (6.7%) participants scored 2 points, 33 (36.7%) scored 3 points, 32 (35.6%) scored 4 points and 19 (21.1%) scored 5 points.

Table II. Comparison of VAS Scores, Opioid Use and Satisfaction based on Postoperative Analgesia Methods

	Overall (n = 90)	Group			p
		Control (n = 30)	ESP (n = 30)	TAP (n = 30)	
VAS Median §	3.7 [0.3 – 7.5]	4.3 [1.3 – 6.5]	3.2 [0.3 – 5.2]	3.9 [2.2 – 7.5]	0.037*
VAS Max. §	6.0 [2.0 – 10.0]	7.0 [2.0 – 10.0]	5.0 [2.0 – 9.0]	6.0 [4.0 – 10.0]	0.016*
Opioid Consumption, *yes* ‡	70 (77.8)	30 (100.0)	16 (53.3)	24 (80.0)	<0.001**
Amount of Opioids (*mg*) §	200.0 [100.0 – 300.0]	200.0 [100.0 – 300.0]	100.0 [100.0 – 300.0]	200.0 [100.0 – 300.0]	<0.001*
Postoperative Side Effects, *present* ‡	35 (38.9)	15 (50.0)	10 (33.3)	10 (33.3)	0.311**
Patient Satisfaction ‡					
1	1 (1.1)	0 (0.0)	0 (0.0)	1 (3.3)	
2	6 (6.7)	3 (10.0)	2 (6.7)	1 (3.3)	
3	43 (47.8)	20 (66.7)	5 (16.7)	18 (60.0)	<0.001**
4	32 (35.6)	7 (23.3)	18 (60.0)	7 (23.3)	
5	8 (8.9)	0 (0.0)	5 (16.7)	3 (10.0)	
Physician Satisfaction ‡					
2	6 (6.7)	5 (16.7)	1 (3.3)	0 (0.0)	
3	33 (36.7)	16 (53.3)	5 (16.7)	12 (40.0)	<0.001**
4	32 (35.6)	8 (26.7)	10 (33.3)	14 (46.7)	
5	19 (21.1)	1 (3.3)	14 (46.7)	4 (13.3)	

ESP: Erector Spinae Plane, TAP: Transversus Abdominis Plane, VAS: Visual Analogue Scale,
*. Kruskal–Wallis test. **, Pearson's Chi-Squared/Fisher–Freeman–Halton test, ‡: n (%), §: Median [Min–Max]

For group comparisons, there were significantly lower scores in the ESP group compared to the control group by mean value of VAS and maximum VAS value ($p = 0.037$, $p = 0.016$, respectively). The mean VAS value and maximum VAS value were lower in the TAP group compared to the control group. However, this difference was not significant ($p < 0.05$, $p < 0.05$, respectively). The comparison of intra-group and inter-group VAS scores over time is presented in Table III (In light of a significant intergroup difference at baseline [0 hours], delta values were derived by subtracting the 0-hour measurements from subsequent time points; these delta values were then subjected to further statistical analyses).

A comparison of median VAS values over time by groups is shown in Figure 3.

There was also a significant intergroup difference by opioid use. ESP and TAP block treatments significantly reduced postoperative opioid consumption compared to the control group ($p < 0.001$, $p < 0.001$, respectively). Opioid use in the ESP group was significantly lower compared to the TAP group ($p = 0.001$). Postoperative opioid analgesic treatment was necessary in all patients in the control group, 53.3% in the ESP group, and 80% in the TAP group.

Table III. Comparison of VAS Scores Over Time Across Groups

	Group			p*
	Control (n = 30)	ESP (n = 30)	TAP (n = 30)	
VAS				
2 hours	1.0 [0.0 – 5.0]	1.0 [-3.0 – 4.0]	1.0 [-2.0 – 3.0]	0.047 α
4 hours	1.0 [-1.0 – 6.0]	1.0 [-2.0 – 4.0]	2.0 [-3.0 – 4.0]	0.019 α
8 hours	2.50.0 [0.0 – 6.0]	1.0 [-4.0 – 6.0]	2.0 [-2.0 – 6.0]	0.039 α
12 hours	4.0 [0.0 – 8.0]	2.0 [-2.0 – 6.0]	2.5 [-2.0 – 4.0]	0.001 α, §
24 hours	4.0 [0.0 – 9.0]	2.0 [-2.0 – 6.0]	3.0 [-1.0 – 5.0]	0.005 α
p**	**<0.001** a,b,c,d	**<0.001** c,d,e	**<0.001** b,c,d	

VAS: Visual Analogue Scale, ESP: Erector Spinae Plane, TAP: Transversus Abdominis Plane,
α: Control vs ESP, **§:** Control vs TAP, **a:** 2. Hours vs 8. Hours, **b:** 2. Hours vs 12. Hours, **c:** 2. Hours vs 24. Hours, **d:** 4. Hours vs 24. Hours, **e:** 8. Hours vs 24. Hours
*Kruskal–Wallis H test., **Friedman test, Median [Min–Max] (Post-Hoc with Bonferroni correction)

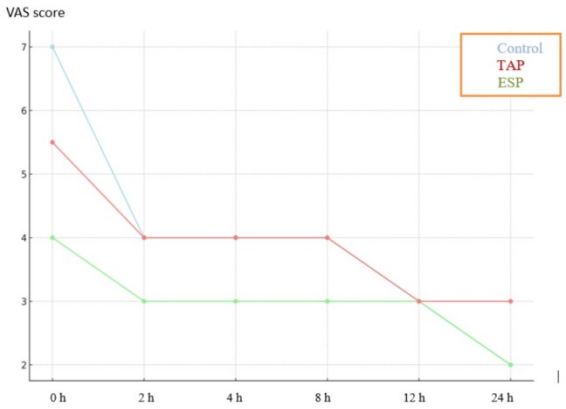

Figure 3:
Temporal Changes in Median VAS Scores Across Groups

Postoperative side effects associated with opioid use were observed in a total of 35 patients: 15 in the control group and 10 each in the ESP and TAP groups. This difference was not statistically significant ($p = 0.311$). However, when comparing the opioid doses used in patients with side effects to those without, the opioid use was significantly higher in the group with side effects ($p = 0.026$).

Patient satisfaction was significantly higher in the ESP group compared to both the control and TAP groups ($p < 0.001$). Specifically, patient satisfaction in the ESP group increased by 58% ($p = 0.009$). No significant difference in patient satisfaction was observed between the TAP and control groups ($p > 0.05$). Advanced statistical analyses further confirmed that the ESP group exhibited significantly higher

levels of patient satisfaction (p = 0.009). Regarding physician satisfaction, the ESP group demonstrated significantly higher scores than the TAP and control groups (p < 0.001). No significant difference in satisfaction was observed between the TAP and control groups. Advanced statistical analyses identified maximum VAS scores, opioid dose, and patient satisfaction as significant factors influencing physician satisfaction, with each variable showing statistical significance (p < 0.05). Opioid use (r = −0.472) and maximum VAS values (r = −0.451) had a significant adverse effect on physician satisfaction, while patient satisfaction had a significant positive relationship (r = 364). Correlations between the opioid dose, physician and patient satisfaction, and clinical variables are summarized in Table IV (The correlation coefficient, denoted as r, ranges from -1 to +1. A value of r close to -1 indicates a negative correlation between the variables, while a value close to +1 suggests a positive correlation).

Table IV. Correlations Between Clinical Variables and Opioid Consumption, Patient Satisfaction, and Physician Satisfaction

	Opioid (Milligram)		Patient Satisfaction		Physician Satisfaction	
	r	P	r	p	r	p
VAS Median	0.364	0.002	-0.250	0.018	-0.359	<0.001
VAS Max.	0.327	0.006	-0.266	0.011	-0.451	<0.001
Age	0.179	0.139	-0.236	0.025	-0.195	0.066
BMI	-0.004	0.973	0.108	0.311	0.015	0.886
ASA	0.033	0.788	0.007	0.951	0.174	0.100
Patient Satisfaction	-0.213	0.077				
Physician Satisfaction	-0.472	<0.001	0.364	<0.001	-	-
Sex	0.075	0.539	0.113	0.290	0.124	0.246
Comorbid Conditions	0.028	0.815	0.054	0.613	0.066	0.538
Postoperative Side Effects	-0.267	0.026	0.140	0.187	0.178	0.093

VAS: Visual Analogue Scale, BMI: Body Mass Index, ASA: American Society of Anesthesiologists, Spearman's rho.

Discussion and Conclusion

Postoperative surgical stress and pain induce the release of corticotropin-releasing hormone (CRH) from the hypothalamus, which in turn causes the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary lobe and ultimately cortisol from the adrenal cortex. These two factors also induce the release of vasopressin from the posterior pituitary. Another effect is on the sympathetic nervous system. By stimulating the sympathetic nervous system, it causes activation in the renin–angiotensin–aldosterone axis, leading to an increase in epinephrine and glucagon release, a decrease in insulin levels, and a decrease in renal blood flow.[15] All these neuroendocrine-metabolic responses and alterations in the hypothalamic–pituitary–adrenal axis result in immunosuppression. Regional anesthesia provides significant advantages for intraoperative management of the hypothalamic–pituitary–adrenal axis, including reduced stress response, better glycemic control, improved recovery, and potentially lower risks of chronic pain and recurrence of cancer.[3] These factors help regional anesthesia serve an appropriate choice in various surgical contexts.

The efficacy of ESP and TAP blocks in postoperative pain management was demonstrated in various previous studies and they were recommended for multimodal analgesia management.[16,17] The results of the present study on the reduction of postoperative opioid use using ESP and TAP blocks in patients, who underwent LSG, was largely consistent with the previous studies.

There are recent studies in the relevant literature on the positive efficacy of the postoperative multimodal approach on analgesia management.[18,19] Yashraj Jain et al. suggested that the use of multimodal analgesia strategies in postoperative pain management was important to reduce opioid consumption and side effects.[9] Upon a literature review by Yuliana et al. on postoperative pain management, it was reported that appropriate management strategies could optimize pain relief and reduce side effects.[20] This strategy is consistent with the results of the present study that ESP and TAP blocks were effective in reducing opioid consumption.

Elshazly et al. compared the feasibility and efficacy of ESP and TAP nerve blocks in patients undergoing bariatric surgery and reported statistically significant lower VAS scores in the ESP group at 30 min, 18 h, and 24 h after extubation.[1] Conversely, Saber et al.[21] and Wassef et al.[22] reported that TAP nerve blocks were not effective in reducing pain scores in LSG and bariatric surgery patients, respectively. In the present study, maximum and mean VAS values were significantly lower in the ESP group compared to the control group, while there was no statistically significant difference between the TAP and control groups. ESP nerve blocks may be more effective in pain management as they provide both visceral and somatic analgesia. The effectiveness of the TAP block in reducing VAS scores may have been limited, likely due to its selective blockade of somatic pain originating from the abdominal wall.

Elshazly et al. also reported that the opioid requirements were significantly higher in patients who received TAP blocks compared to those who received ESP blocks.[1] Similarly, Altıparmak et al. compared TAP and ESP blocks in laparoscopic cholecystectomy and found that ESP blocks were more effective in reducing opioid use.[23] In this study, ESP and TAP

block groups had significantly reduced postoperative opioid consumption compared to the control group, with the ESP group requiring less opioid than the TAP group. Postoperative opioid analgesic treatment was necessary for all patients in the control group, 53.3% in the ESP group, and 80% in the TAP group.

Eadie et al. reported that regional anesthesia applications provided significant benefits to avoid the side effects of opioid use.[24] These results were consistent with a study by Bolesta et al.[25] However, in this study, there were no significant intergroup differences by side effects. Notwithstanding the above, the opioid dose was significantly higher in favor of the group with side effects.

Sinha and Mittal reported that patient satisfaction scores associated with analgesia were significantly higher in patients who underwent bariatric surgery and received TAP block compared to the control group.[12,26] The present study investigated both patient and physician satisfaction and found that ESP block application significantly increased the level of satisfaction compared to TAP and control groups.

In conclusion, our study demonstrated that both ESP and TAP blocks are effective in reducing pain and opioid consumption following LSG. Notably, the ESP block was more effective in decreasing opioid requirements and postoperative VAS scores. This may be attributed to the blockade of both the dorsal and ventral branches of the spinal nerves by the ESP block, which provides analgesia to both the abdominal wall and internal organs, thus targeting both somatic and visceral pain. In comparison, TAP block application reduced postoperative opioid consumption and nearly significantly decreased VAS scores in our study relative to the control group. We hypothesize that with larger sample sizes in future studies, the reduction in VAS scores with TAP block may reach statistical significance. The lower effectiveness of the TAP block compared to the ESP block may be explained by the fact that the TAP block provides analgesia only to the abdominal wall.

This study has several limitations. First, the relatively small sample size may restrict the generalizability of the findings. Moreover, no power analysis was conducted to determine the adequacy of the sample size, which limits the confidence in the statistical robustness of the results. Future studies with larger cohorts and appropriate power calculations are needed to enhance the accuracy and reliability of the findings. Second, the retrospective design of the study inherently limits the ability to establish causal relationships and increases the risk of selection bias. Third, the study did not include randomization, which may have introduced confounding variables that could affect the outcomes. Additionally, our analysis did not separate patients into subgroups based on conditions such as diabetes, which may cause neuropathy and influence pain perception and analgesic response. Grouping such comorbidities together without stratification may obscure the effects of the nerve blocks in specific populations. Furthermore, the study focused exclusively on patients undergoing laparoscopic sleeve gastrectomy (LSG), which limits the applicability of the results to other surgical procedures. Future research should investigate the efficacy of ESP and TAP blocks in a wider variety of surgeries to improve the external validity of the findings. Lastly, long-term outcomes were not assessed in this study. Long-term follow-up studies are essential to evaluate the sustained effectiveness of these nerve blocks and to monitor for potential delayed adverse effects.

Furthermore, subjective measurements were used in our study. Individual differences and patient perceptions played an important role in the evaluation of subjective data such as pain and satisfaction. This is a factor that should be considered when interpreting the results. The time difference between the administration of the ESP block prior to anesthesia induction and the TAP block following induction may be considered another limitation of the study.

The study found that VAS scores were significantly lower in the ESP group, and both ESP and TAP blocks significantly reduced postoperative opioid consumption. This reduction in pain and opioid use is important for preventing immunosuppression and minimizing-related side effects. Further comprehensive, large-scale studies are needed to confirm these findings and enhance clinical applications.

Researcher Contribution Statement:
Idea and design: H.E., M.S.B, H.Y.; Data collection and processing: H.E., M.S.B., A.A.; Analysis and interpretation of data: M.S.B., H.Y., A.A.; Writing of significant parts of the article: H.E., M.S.B., H.Y., A.A.
Support and Acknowledgement Statement:
Non-applicable
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Harran Üniversitesi Klinik Araştırmalar Etik Kurulu
Approval Date: 04.01.2024
Decision No: 25

References

1. Elshazly M, El-Halafawy YM, Mohamed DZ, Wahab KAE, Mohamed TMK. Feasibility and efficacy of erector spinae plane block versus transversus abdominis plane block in laparoscopic bariatric surgery: a randomized comparative trial. Korean J Anesthesiol. 2022; 75: 502–509.
2. Qanaq D, O'Keeffe M, Cremona S, Bernardo WM, McIntyre RD, Papada E et al. The Role of Dietary Intake in the Weight Loss Outcomes of Roux-en-Y Gastric Bypass and Sleeve Gastrectomy: A Systematic Review and Meta-analysis. Obes Surg. 2024;34:3021–3037.

3. Ivascu R, Torsin L.I, Hostiuc L, Nitipir C, Corneci D, Dutu M. The Surgical Stress Response and Anesthesia: A Narrative Review. J Clin Med. 2024;13:3017.
4. Lisowsk, B, Jakubiak J, Siewruk K, Sady M, Kosson D. Which idea is better with regard to immune response? Opioid anesthesia or opioid free anesthesia. J Inflamm Res. 2020;13:859–869.
5. Lee M, Ayad M, Diz Ferre JL, Oliver LA, Swerchowsky N, Ayad S. Ultrasound-Guided External Oblique Intercostal Block as Part of Multimodal Analgesia for Unplanned Open Cholecystectomy. Cureus. 2024;16:e61606.
6. Joshi GP. Rational Multimodal Analgesia for Perioperative Pain Management. Curr Pain Headache Rep. 2023;27:227–237.
7. Chaouch MAMA, Daghmouri MA, Boutron MC, Ferraz JM, Usai S, Soubrane O et al. Ketamine as a component of multimodal analgesia for pain management in bariatric surgery: A systematic review and meta-analysis of randomized controlled trials. Ann Med Surg (Lond). 2022;78:103783.
8. Wolf A, Unterberg M, Witowski A, Adamzik M, Wolf A. Efficacy, safety, and side effects of oliceridine in acute postoperative pain, a protocol for a systematic review and meta-analysis. PLoS One. 2024;19:e0299320.
9. Jain Y, Lanjewar R, Lamture Y, Bawiskar D. Evaluation of Different Approaches for Pain Management in Postoperative General Surgery Patients: A Comprehensive Review. Cureus. 2023;15:e48573.
10. Kindler CH, Harms C, Amsler F, Ihde-Scholl T, Scheidegger D. The visual analog scale allows effective measurement of preoperative anxiety and detection of patients' anesthetic concerns. Anesth Analg. 2000;90:706–712.
11. Rafi AN. Abdominal field block: a new approach via the lumbar triangle. Anaesthesia. 2001;56:1024–1026.
12. Sinha A, Jayaraman L, Punhani D. Efficacy of ultrasound-guided transversus abdominis plane block after laparoscopic bariatric surgery: a double blind, randomized, controlled study. Obes Surg. 2013;23:548–553.
13. Forero M, Adhikary SD, Lopez H, Tsui C, Chin K.J. The Erector Spinae Plane Block: A Novel Analgesic Technique in Thoracic Neuropathic Pain. Reg Anesth Pain Med. 2016;41:621–627.
14. Chin KJ, Malhas L, Perlas A. The Erector Spinae Plane Block Provides Visceral Abdominal Analgesia in Bariatric Surgery: A Report of 3 Cases. Reg Anesth Pain Med. 2017;42:372–376.
15. Vicković S, Zdravković R, Maričić-Prijić S, Nikolić D, Pap D, Čolak E et al. Salivary cortisol as a biomarker of stress in surgical patients. J Med Biochem. 2023;42:469–475.
16. Sia CJ, Wee S, Au-Yong AP, Lie SA, Tan WJ, Foo FJ et al. Analgesia efficacy of erector spinae plane block in laparoscopic abdominal surgeries: a systemic review and meta-analysis. Int J Surg. 2024;110:4393–4401.
17. Richards DC, Dunn BA, Chellappa VR, John CR, Davis WB. Postoperative pain control and opioid use with transversus abdominis plane block and scheduled multimodal pain management in patients undergoing cesarean section. Int J Gynaecol Obstet. 2024;167:668–674.
18. Sia CJ, Wee S, Au-Yong AP, Lie SA, Tan WJ, Foo FJ et al. Analgesia efficacy of erector spinae plane block in laparoscopic abdominal surgeries: a systemic review and meta-analysis. Int J Surg. 2024;110:4393–4401.
19. Földi M, Soós A, Hegyi P, Kiss S, Szakács Z, Solymár M et al. Transversus Abdominis Plane Block Appears to Be Effective and Safe as a Part of Multimodal Analgesia in Bariatric Surgery: a Meta-analysis and Systematic Review of Randomized Controlled Trials. Obes Surg. 2021;31:531–543.
20. Yuliana Silitonga YA, Wahyuni A. Manajemen Nyeri Pasca Operasi: Tinjauan Pustaka. J Nutrient. 2022;2:27-37.
21. Saber AA, Lee YC, Chandrasekaran A, Olivia N, Asarian A, Al-Ayoubi S et al. Efficacy of transversus abdominis plane (TAP) block in pain management after laparoscopic sleeve gastrectomy (LSG): A double-blind randomized controlled trial. Am J Surg. 2019;217:126–132.
22. Wassef M, Lee DY, Levine JL, Ross RE, Guend H, Vandepitte C et al. Feasibility and analgesic efficacy of the transversus abdominis plane block after single-port laparoscopy in patients having bariatric surgery. J Pain Res. 2013;6:837–841.
23. Altıparmak B, Korkmaz Toker M, Uysal AI, Kuşçu Y, Gümüş Demirbilek S. Ultrasound-guided erector spinae plane block versus oblique subcostal transversus abdominis plane block for postoperative analgesia of adult patients undergoing laparoscopic cholecystectomy: Randomized, controlled trial. J Clin Anesth. 2019;57:31–36.
24. Eadie R, McKenzie CA, Hadfield D, Kalk NJ, Bolesta S, Dempster M et al. UK ALERT-ICU study investigators. Opioid, sedative, preadmission medication and iatrogenic withdrawal risk in UK adult critically ill patients: a point prevalence study. Int J Clin Pharm. 2023;45:1167–1175.
25. Bolesta S, Burry L, Perreault MM, Gélinas C, Smith KE, Eadie R et al. AduLt iatrogEnic withdRawal sTudy in the ICU (ALERT-ICU) Study Investigators. International Analgesia and Sedation Weaning and Withdrawal Practices in Critically Ill Adults: The Adult Iatrogenic Withdrawal Study in the ICU. Crit Care Med. 2023;51:1502–1514.
26. Mittal T, Dey A, Siddhartha R, Nali A, Sharma B, Malik V. Efficacy of ultrasound-guided transversus abdominis plane (TAP) block for postoperative analgesia in laparoscopic gastric sleeve resection: a randomized single blinded case control study. Surg Endosc. 2018;32:4985–4989.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 209-215, 2025
DOI: https://doi.org/10.32708/uutfd.1641884

ORIGINAL RESEARCH

In Vitro Study of the Effect of 1.0% Sodium Hyaluronate on Bacterial Strains and Antibiotics*

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ABSTRACT

During anterior segment eye surgeries, ophthalmic viscosurgical devices (OVDs), primarily containing hyaluronic acid, protect the corneal endothelium and help maintain the anterior chamber with reduced trauma. We aimed to investigate the interaction of sodium hyaluronate 1.0% with Gram-positive and Gram-negative bacteria, and its interactions with prophylactic intracameral antibiotics moxifloxacin and cefuroxime. Four quality control bacterial strains were used in vitro to conduct five experiments using either cefuroxime or moxifloxacin. Our experiment included five stages: 1. Testing the ability of OVD to retain bacteria before antibiotic exposure. 2. Examining antibiotic-bacteria interactions in the presence of OVD. 3. Simulating aqueous humor circulation. 4. Evaluating the effect of OVD residuals on antibiotic retention. 5. Analyzing the interaction of bacteria and antibiotics within the OVD. Results showed a significant decrease in bacterial counts ($p<0.001$) between the initial stage and subsequent groups, with moxifloxacin consistently demonstrating lower bacterial counts and greater effectiveness compared to cefuroxime ($p<0.001$). Further in vivo studies are recommended to validate these results.

Key words: Sodium hyaluronate 1.0%. Cefuroxime. Moxifloxacin. Ophthalmic viscosurgical devices.

%1.0 Sodyum Hyaluronatın Bakteriler ve Antibiyotikler Üzerindeki Etkisinin İn Vitro Çalışması

ÖZET

Ön segment göz ameliyatları sırasında, öncelikli olarak hyaluronik asit içeren oftalmik viskocerrahi cihazlar (OVD'ler), kornea endotelini korur ve ön bölmenin daha az travma ile korunmasına yardımcı olur. Çalışmamızda %1.0 Sodyum hyaluronat içeren OVD'nin Gram pozitif ve Gram negatif bakterilerle etkileşimini ve profilaktik olarak kullanılan intrakamaral moksifloksasin ve sefuroksim ile etkileşimlerini araştırmayı amaçladık. Sefuroksim veya moksifloksasin kullanılarak beş deney yürütmek için dört kalite kontrol bakteri suşu in vitro kullanıldı. Deneyimiz beş aşamadan oluşmaktadır; 1. Antibiyotik maruziyetinden önce OVD'nin bakterileri tutma yeteneğini test etme, 2. OVD varlığında antibiyotik-bakteri etkileşimlerini inceleme, 3. Humor aköz dolaşımını simüle etme, 4. OVD kalıntılarının antibiyotik tutması üzerindeki etkisini değerlendirme, 5. OVD içindeki bakteri ve antibiyotik etkileşimini analiz etme. İlk deney grubundaki bakteri sayısı ve sonraki aşamalar arasındaki bakteri sayılarında önemli bir azalma olduğu ($p<0,001$) saptandı. Moksifloksasin, sefuroksim ile karşılaştırıldığında daha fazla etkinlik gösterdi ($p<0,001$). Çalışmamız invitro bir çalışma olup, sonuçları doğrulamak için daha fazla in vivo çalışma önerilmektedir.

Anahtar Kelimeler: %1 Sodyum hyaluronat. Sefuroksim. Moksifloksasin. Oftalmik viskocerrahi cihaz.

Date Received: 25.February.2025
Date Accepted: 2.July.2025

* Presented as an oral presentation at the "40th Congress of the European Society of Cataract & Refractive Surgeons" (16-20 September 2022, Milan, Italy).

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Hyaluronic acid (HA) is a linear polysaccharide composed of alternating β-D (1→3) glucuronic acid and β-D (1→4) N-acetyl-β-D-glucosamine units. HA, a biodegradable and biocompatible carbohydrate polymer, is present throughout the eye[1,2]. Sodium hyaluronate accelerates wound healing by promoting intercellular interaction, cell-matrix adhesion, cell motility, and extracellular organization[3]. In the 1980s, the US Food &Drug Administration (FDA) approved the first ophthalmic medical device containing HA. HA is primarily produced through the fermentation of *Streptococcus zooepidemicus* rather than being extracted from rooster combs. This method ensures minimal contaminants in the final product, such as proteins, pathogens, or endotoxins. CD44 is a primary cell surface receptor for HA and is responsible for internalizing HA-coated nanoparticles to the cornea and conjunctiva. HA is highly susceptible to degradation through enzymatic, chemical, and physical pathways[1,2]. Ophthalmic viscosurgical devices (OVD)primarily contain HA andprotect the corneal endothelium and maintain the anterior chamber during anterior segment eye surgeries, with less trauma[1,2].

Infection is an uncommon but severe complication that may occur after cataract surgery. One of the most critical complications is post-operative endophthalmitis, which can result in irreversible blindness or even loss of the eye. The reported incidence rates of post-operative endophthalmitis vary significantly across medical centers worldwide, ranging from 0.28 to 1.6 per 1000 cataract surgeries[4]. Many cases of sporadic endophthalmitis are caused by pathogens from a patient's eyelid and periocular flora. *Staphylococcus epidermidis* is the most commonly identified organism in such cases. Patients who have a compromised immune system may be more susceptible to developing infections from endogenous pathogens. Intraocular surgery, which includes cataract surgery, is a possible means of transmitting external pathogens. A cluster of multiple cases of post-operative endophthalmitis in the same surgical center is a significant cause for concern. It can often be traced back to transmitting pathogens from external sources such as surgical instruments, contaminated fluid solutions, and the surgical environment[4]. Therefore, administering antibiotics during the perioperative period is a reasonable strategy for reducing the occurrence of post-operative endophthalmitis. In daily practice, various antibiotics have been used to prevent endophthalmitis and different antibiotic administration routes have been proposed accordingly. However, the effectiveness of antibiotic use has not been established until recently[5].

In the current study we created a non-surgical simulation environment to showcase how a commercial product containing sodium hyaluronate 1.0% (an OVD) interacts with bacteria and antibiotics because of its polysaccharide structure. Our objective was to demonstrate how this device interacts with gram-positive and gram-negative bacteria and interacts with moxifloxacin and cefuroxime, commonly used intracameral antibiotics as prophylactics.

Material and Method

We conducted an in vitro laboratory study consisting of five experimental steps (Figure 1). *Staphylococcus aureus* (ATCC 25923), two strains of *S. epidermidis* (ATCC 12228 and ATCC 35984), and *Pseudomonas aeruginosa* (ATCC 27853) were used in this study. Two antibiotics, cefuroxime (Cefeye, Deva, Istanbul, Turkey) and moxifloxacin (Moxai %0.5, Abdi İbrahim, Istanbul, Turkey), were also used.

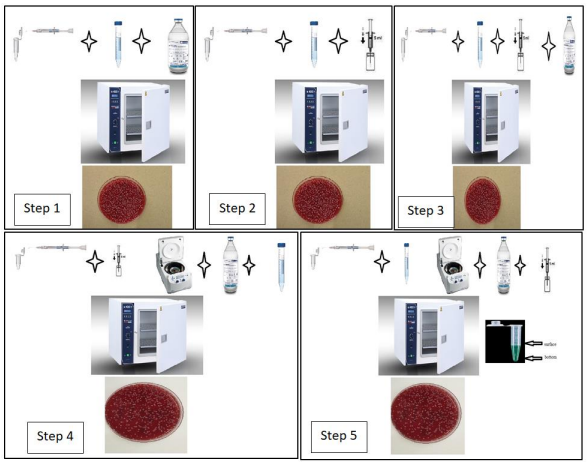

Figure 1.
The design of the experimental process

Sterile Eppendorf tubes (1.5 milliliters) represented the eye's anterior chamber. After every experimental step, the number of bacteria in the Eppendorf tubes was determined. To do this, 5% sheep blood agar was streaked and incubated at 35°C for 16-18 hours. To ensure even distribution across the entire plate in the colony counting process, we took 10 µl of the solutions created in each experimental step, diluted it with 990 µl of physiological saline, and spread the mixture onto a 5% sheep blood agar plate, making sure to wet the entire surface evenly. Each experimental step was repeated five times, and the average bacterial colony numbers per milliliter were calculated. The sodium hyaluronate 1.0% was studied in five experimental steps, and all procedures were repeated for both antibiotics using four quality control strains. When evaluating bacterial counts calculated by the colony counting method, percentage reduction and logarithmic reduction formulas were used.

1.0% Sodium Hyaluronate

Before conducting the experiments, we performed antibiotic susceptibility testing for each bacterium using the disc diffusion method with the antibiotics we intended to use. The results were evaluated based on the Clinical and Laboratory Standards Institute (CLSI) criteria[6]. We used antibiotic concentrations of 4 µg/ml for moxifloxacin and 16 µg/ml for cefuroxime in staphylococci, and 8 µg/ml for moxifloxacin and 64 µg/ml for cefuroxime in *P. aeruginosa*. CLSI criteria determine these concentrations.

Experimental steps

The initial experiment aimed to assess the ability of OVD (Healon Pro - sodium hyaluronate 1.0%) to retain bacteria prior to antibiotic exposure. The experiment also aimed to determine the impact of antibiotics on other stages where they were used. To begin, 500 µl of bacterial solution with a density of 1/10000 McFarland was added to a drop of viscoelastic material in an Eppendorf tube. As antibiotics were not used in this stage, 500 µl of sterile irrigation fluid (BSS Ocrosol-Polifarma) (SIF) was added to maintain a constant bacterial concentration. After an hour of incubation at 37°C, the mixture was streaked onto 5% sheep blood agar using the colony count method (Figure 1- step 1).

In the second stage of the experiment, the objective was to showcase the interaction between antibiotics and bacteria in the presence of OVD. Initially, a drop of OVD was added to an Eppendorf tube, followed by 500 µl of antibiotic. The mixture was then incubated at 35°C for an hour. After that, bacterial solution at 1/10000 McFarland density was added to the mixture. The tube was then incubated again at 35°C for another hour. Finally, the colony count method was used to determine bacterial count (Figure 1- step 2).

During the third experimental stage, an additional step was taken in addition to the second stage. The mixture was washed with SIF to simulate aqueous humor circulation before spreading in the medium. To prevent the removal of OVD during the washing process, 500 µl of liquid was taken from the surface of the mixture in an Eppendorf tube and replaced with 500 µl of SIF. This mixture was gently shaken by hand and incubated for 15 minutes. The process was repeated eight times, and the colony count method was used (Figure 1- step 3).

During the fourth experimental step, we performed a centrifugation and washing step. The aim was to evaluate the antibiotic retention effect of the OVD left at the bottom of the tube. We incubated the antibiotics with the OVD in this step, as in the second step. Next, we centrifuged the Eppendorf at 1000 rpm for 10 seconds to ensure the precipitation of the OVD. After centrifugation, we discarded 300 µl of the supernatant remaining on the surface and added 300 µl of SIF instead. We repeated this cycle three times. Then, we added 500 µl of bacterial solution with a density of 1/10000 McFarland and incubated it for 1 hour at 35°C. Finally, the colony count method was used (Figure 1- step 4).

In the fifth experimental step, we applied the centrifugation process at different stages to evaluate the antibiotic and bacteria-retaining effect of OVD. We also examined the interaction of bacteria and antibiotics within the OVD. This step demonstrates the effectiveness of post-operative antibiotics in preventing surgical site infections caused by contamination during surgery in patients who did not receive preoperative prophylaxis. To perform this step, we added 500 µl of bacterial solution at 1/10000 McFarland density onto a drop of OVD added to the Eppendorf tube. We then incubated it for 1 hour at 35°C. After incubation with the bacterial solution, we repeated the centrifuge and washing cycle described in the fourth experimental step three more times. Then, we added 500 µl of antibiotic and incubated it for another 1 hour. After the second incubation, we centrifuged the OVD at 1000 rpm for 10 seconds to ensure precipitation of OVD. Next, we discarded 500 µl of the supernatant remaining on the surface and added 500 µl of SIF instead. We repeated this cycle four times. After the fourth centrifuge, we made surface and bottom inoculations separately on 5% sheep blood agar without washing. We left them for incubation for 16-18 hours at 35°C and evaluated them by the colony count method. With the first centrifuge-washing process in this step, the supernatant was removed, and the OVD and the bacteria precipitated and remained mainly at the bottom of the Eppendorf. During the incubation phase with antibiotics, the antibiotic was expected to affect the bacteria present with OVD. With the centrifuge washing process in the last stage, we removed the supernatant and the antibiotics. This step helps us understand how OVD interacts with bacteria and antibiotics and evaluate the antibiotic and bacteria-retaining effect of OVD (Figure 1- step 5).

The design of the experimental process is summarized below, with Figure 1 for visualization:

1. OVD+bacteria+SIF; incubation; cultivation

2. OVD+antibiotic; incubation; +bacteria; incubation; cultivation

3. OVD+antibiotic; incubation; +bacteria; incubation; irrigation; cultivation

4. OVD+antibiotic; incubation; centrifugation; +bacteria; incubation; cultivation

5. OVD+bacteria; incubation; centrifugation; + antibiotic; incubation; centrifugation; cultivation (deep and supernatant)

Statistical analysis

The statistical data analysis was conducted using IBM SPSS 28.0 (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.) software. The Shapiro-Wilk test was employed to determine whether the data exhibited a normal distribution. Descriptive statistics were presented as mean, standard deviation, or median (interquartile range) for quantitative data. For data that showed normal distribution, a one-way analysis of variance was used to compare more than two groups. At the same time, the Kruskal-Wallis test was employed for non-normal distribution. In the case of significance, one of the multiple comparison tests, the Bonferroni test, was used. The level of significance was set at p=0.05.

Results

Before conducting the experiments, we performed antibiotic susceptibility testing for each bacterium using the disk diffusion method. The antibiotics we used were evaluated according to CLSI criteria. We found that *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 were sensitive (S) to cefuroxime and moxifloxacin. However, *S. epidermidis* ATCC 35984 and *P. aeruginosa* ATCC 27853 were resistant (R) to cefuroxime. Because no CLSI data were available to evaluate *P. aeruginosa* with moxifloxacin, we evaluated it according to levofloxacin. We found that it was sensitive at high doses (I).

Tables I and II show the logarithmic and percentage reductions in the number of bacteria for each quality control bacterium tested with moxifloxacin and cefuroxime, respectively, compared to the initial step.

A significant difference ($p<0.001$) was observed between step 1 and the subsequent steps when comparing moxifloxacin and cefuroxime against all bacteria (Table III). In step 1, bacteria were incubated solely with OVD. It was noted that when OVD was exposed to antibiotics in any of the following steps, there was a significant reduction in the number of bacteria.

The moxifloxacin group showed a lower bacterial count at all stages compared to the cefuroxime group. However, there were statistically significant differences ($p<0.001$) in bacterial counts, specifically at step 2 and step 4 (see Table III).

During step 3 of the experiment, the circulation was utilized to simulate aqueous humor. Step 2 demonstrated static conditions, while step 3 involved dynamic conditions. The number of bacteria in the *P. aeruginosa* group also decreased during step 3; however, this decrease was not statistically significant when moxifloxacin was used. It was observed that the number of bacteria decreased significantly ($p < 0.001$) in all other groups.

Table I. The logarithmic and percentage reduction in the number of bacteria for moxifloxacin

MOXIFLOXACIN								
	S. aureus		*S. epidermidis* 35984		*S. epidermidis* 12228		*P. aeruginosa*	
	%	*log*	*%*	*log*	*%*	*log*	*%*	*log*
Second step	-72.93	-0.57	-76.98	-0.64	-59.25	-0.39	-98.01	-1.70
Third step	-98.77	-1.91	-95.79	-1.38	-92.00	-1.10	-99.97	-3.54
Fourth step	-57.96	-0.38	-46.95	-0.28	-54.29	-0.34	-99.30	-2.16
Fifth step supernatant	-99.73	-2.56	-99.23	-2.12	-97.87	-1.67	-99.99	-4.01
Fifth step deep	-99.44	-2.25	-98.60	-1.85	-95.28	-1.33	-99.78	-2.65

Table II. The logarithmic and percentage reduction in the number of bacteria for cefuroxime

CEFUROXIME								
	S. aureus		*S. epidermidis* 35984		*S. epidermidis* 12228		*P. aeruginosa*	
	%	*log*	*%*	*log*	*%*	*log*	*%*	*log*
Second step	-41.87	-0.24	-1.71	-0.01	-5.91	-0.03	-36.66	-0.20
Third step	-95.43	-1.34	-90.82	-1.04	-89.95	-1.00	-97.53	-1.61
Fourth step	-39.82	-0.22	-32.92	-0.17	-39.41	-0.22	-36.95	-0.20
Fifth step supernatant	-99.22	-2.11	-99.15	-2.07	-98.51	-1.83	-99.53	-2.33
Fifth step deep	-97.62	-1.62	-98.77	-1.91	-97.05	-1.53	-98.26	-1.76

1.0% Sodium Hyaluronate

Table III. The average number of bacteria colonies observed in the experimental steps

	S. aureus (ATCC 25923)		*S. epidermidis* (ATCC 35984)		*S. epidermidis* (ATCC 12228)		*P. aureginosa* (ATCC 27853)	
	Moxifloxacin	Cefuroxime	Moxifloxacin	Cefuroxime	Moxifloxacin	Cefuroxime	Moxifloxacin	Cefuroxime
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
First step	205040±12969.5	205040±12969.5	91240±5781.7	91240±5781.7	92760±5485.25	92760±5485.25	206200±19976.86	206200±19976.86
Second step	55500±3708.1	119200±5761.94	21000±1421.27	89680±5655.71	37800±1923.54	87280±3048.28	4100±1208.3	130600±17854.97
Third step	2520±676.02	9380±957.6	3840±981.33	8380±1961.38	7420±641.87	9320±370.14	60±89.44	5100±234.52
Fourth step	86200±1254.99	123400±5549.77	48400±5140.53	61200±1104.54	42400±1557.24	56200±3053.69	1440±512.84	130000±5000
Fifth step supernatant	560±461.52	1600±696.42	700±187.08	780±44.72	1980±83.67	1380±311.45	20±44.72	960±638.75
Fifth step deep	1140±260.77	4880±216.79	1280±334.66	1120±130.38	4380±258.84	2740±384.71	460±114.02	3580±676.02

In Step 4, we measured the number of bacteria remaining at the bottom of the Eppendorf after exposure to antibiotics using OVD. This was done by discarding the surface layer following centrifugation. This step aimed to simulate the effects of prophylactic antibiotics administered before surgery. In Step 1, only the bacteria were incubated with OVD. In Step 4, we observed a significant decrease in bacteria ($p<0.001$) across all tested microorganisms compared to Step 1. Additionally, moxifloxacin proved more effective than cefuroxime ($p<0.001$).

Step 5 simulated the adhesive effect of antibiotics and bacteria on OVD, and deep cultivation was conducted to test for potential infections due to residual OVD in the environment after surgery. This step aimed to evaluate the effectiveness of antibiotics following surgery involving contaminated OVD. The results showed a significant decrease ($p<0.001$) in the number of bacteria observed in the deep cultivation during Step 5 compared to Step 1 for both antibiotics across all tested bacteria.

Staphylococcus epidermidis (ATCC 35984) and *P. aureginosa* (ATCC 27853) were resistant to cefuroxime. Although moxifloxacin led to a significant decrease ($p<0.001$) in the number of bacteria in all steps compared to the first step, cefuroxime resistance prevented any significant decrease from being observed in the second step.

Discussion and Conclusion

During cataract surgery, OVDs are used to coat surgical instruments. The primary purpose of these devices is to minimize the risk of damage to intraocular tissue. They also help to create space, maintain stability in the anterior chamber, and protect the endothelium against fluid turbulence, free radicals, air bubbles, and lens fragmentation. OVDs are well-established and commonly used during cataract surgery[7]. One of the serious but uncommon complications that can occur during cataract surgery is a surgical site infection. This type of infection can lead to bacterial endophthalmitis, which can cause significant damage to the eye. Bacterial endophthalmitis progresses quickly and can affect the retina, leading to vision loss[8]. The purpose of this study was to gain a better understanding of how OVD interacts with bacteria and antibiotics. Specifically, we investigated how 1% sodium hyaluronate interacts with the bacteria that most commonly cause endophthalmitis and the antibiotics most frequently used to prevent it. To achieve this, we conducted five separate experimental steps.

One of the most concerning issues after cataract surgery is the increased risk of developing post-operative endophthalmitis. This condition often begins during surgery when a corneal incision is made in the anterior chamber of the eye to remove the cataract lens. This incision allows ocular surface fluid containing bacteria to enter the eye[9]. Bacteria mainly come from the conjunctiva and are inoculated into the eye during ocular surgery, but can also come from the skin[10]. Different types of eye surgeries are associated with various bacterial strains, but most are Gram-positive. Two studies, the Endophthalmitis Vitrectomy Study and the French Institutional Endophthalmitis Study, discovered that Gram-positive bacteria caused 94% of acute cases following cataract surgery. Among these bacteria, 70% were coagulase-negative staphylococci (CoNS), normal bacteria in the body's commensal flora[11,12]. CoNS is the most frequently identified pathogen, followed by *S. aureus* and *Streptococcus spp*. About 20% of infections are caused by Gram-negative microorganisms[13]. Therefore, we chose CoNS, *S. aureus* and *P. aureginosa*.

Post-operative endophthalmitis refers to severe inflammation in both the anterior and posterior segments of the eye following intraocular surgery[14]. According to a 2014 survey by the American Society of Cataract and Refractive Surgery (ASCRS),

approximately 90% of respondents reported using antibiotics regularly during the peri-operative period. However, there is no universally agreed-upon regimen for administering these antibiotics. According to the ASCRS survey, the most commonly used medication was a fourth-generation fluoroquinolone, such as moxifloxacin or gatifloxacin[15]. Moxifloxacin has a broad spectrum of activity and is effective against Gram-positive and Gram-negative bacteria, including *P. aeruginosa*[14]. In 2005, the European Society of Cataract and Refractive Surgeons (ESCRS) conducted a significant randomized clinical trial to evaluate the routine use of topical and intracameral antibiotics during cataract surgery. The study revealed that prophylactic intracameral injections of cefuroxime significantly reduced the incidence of post-operative endophthalmitis (POE) by almost five times, decreasing the rate from 0.33% to 0.07%. Enterococci are resistant to cefuroxime, and cefuroxime's narrow spectrum of activity is not ideal for treating infections caused by Staphylococcus species. In Sweden, routine use of intracameral cefuroxime has led to an increase in cases of Enterococci endophthalmitis, and its use has also been associated with an increase in fungal endophthalmitis cases[14]. The occurrence of endophthalmitis was found to be significantly lower in patients who were administered intracameral cefuroxime (5/6836; 0.07%) as compared to those who did not receive the same (23/6862; 0.33%) in a single randomized clinical trial by the ESCRS in 2007. However, the incidence of endophthalmitis was not significantly different between patients who received levofloxacin eye drops (12/6852; 0.18%) and those who did not receive them (16/6846; 0.23%)[16]. In our laboratory study, we found that moxifloxacin was effective against all tested strains, while cefuroxime showed resistance in *S. epidermidis* ATCC 35984 and *P. aeruginosa*. Throughout the experiments, the bacterial counts for moxifloxacin were consistently lower than those for cefuroxime across all steps and bacterial types. Notably, the differences observed were statistically significant ($p<0.001$) in steps 2 and 4 (see Table III). Based on these findings, moxifloxacin is considered to be more effective than cefuroxime in addressing both Gram-positive and Gram-negative bacterial contamination.

The aqueous humor is a fluid that comprises organic and inorganic ions, carbohydrates, amino acids, glutathione, carbon dioxide, oxygen, and water. Its primary function is to supply nutrients and oxygen to the avascular tissues of the eye, such as the cornea and the lens. Additionally, it helps remove waste products, blood, macrophages, and other debris from the anterior lens and posterior cornea. It also helps to maintain the shape of the eyeball shape and intraocular pressure[17]. Astafurov et al.[18] found that patients with glaucoma had a higher oral bacterial load compared to those without glaucoma. Moreover, the immune system successfully eliminates a low amount of bacteria. The number of bacteria in the aqueous humor is reduced by the circulation of aqueous humor and the immune system. In step 3, even though we could not simulate the immune system, we saw that the number of bacteria was reduced by stimulating the circulation of the aqueous humor.

A meta-analysis indicated that the rates of post-operative endophthalmitis using intracameral cefuroxime, moxifloxacin, and vancomycin were 0.0332%, 0.0153%, and 0.0106%, respectively. Moxifloxacin provides a wider range of bacterial coverage than cefuroxime and vancomycin[19]. During the fourth step, the number of bacteria decreased significantly ($p<0.001$) in all microorganisms compared to the first. Furthermore, moxifloxacin proved more effective than cefuroxime ($p<0.001$). These findings highlight the significance of prophylactic antibiotics in case of endogenous or exogenous contamination during surgery. In step 5 of the study, the effectiveness of antibiotics was checked after surgery involving contaminated OVD. The results showed a significant decrease ($p<0.001$) in the number of bacteria during step 5 deep cultivation as compared to step 1 for both antibiotics in all bacteria. This indicates that OVD, which can remain as a residue for days after the surgery, is less likely to cause an infection.

Staphylococcus epidermidis (ATCC 35984) and *P. aureginosa* (ATCC 27853) were resistant to cefuroxime. Moxifloxacin significantly reduced the number of bacteria ($p<0.001$) at all steps when compared to the first. Despite cefuroxime resistance, there was no significant reduction in the second step. However, a decrease in the number of bacteria was observed in other steps due to the implementation of washing procedures in the cefuroxime group. It is worth noting that ATCC 35984 is a biofilm-forming strain. If extracellular polymeric substances do not protect the bacteria, they can be removed by the circulation of body fluids or through irrigation during surgery. Unfortunately, cefuroxime is not effective in eliminating this particular strain of bacteria.

It is important to recognize some limitations of this study. First, it is an in vitro study, meaning an accurate anterior chamber simulation may not have been achieved at all stages.

In conclusion, we found that moxifloxacin is more effective than cefuroxime against Gram-positive or Gram-negative bacterial contamination. Additionally, we found that it is unlikely that contaminated OVD may cause an infection when antibiotics are present. These findings emphasize the importance of using prophylactic or post-operative antibiotics in endogenous or exogenous contamination cases. Further, in vivo studies should support our results.

1.0% Sodium Hyaluronate

Researcher Contribution Statement:
Idea and design: N.U.T., M.B., E.S.S., T.T., K.E., G.Ö. and C.O.; Data collection and processing: N.U.T., T.T. and K.E.; Analysis and interpretation of data: G.Ö.; Writing of significant parts of the article: N.U.T., M.B., E.S.S., T.T., K.E., G.Ö. and C.O.
Support and Acknowledgement Statement:
none
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
No human or animal was used in this study. This is experimental study that does not require ethics committee approval.

References

1. Huerta Ángeles G, Nešporová K. Hyaluronan and its derivatives for ophthalmology: Recent advances and future perspectives. Carbohydr Polym. 2021;259:117697. doi:10.1016/j.carbpol.2021.117697.
2. Zhang X, Wei D, Xu Y, Zhu Q. Hyaluronic acid in ocular drug delivery. Carbohydr Polym. 2021;264:118006. doi:10.1016/j.carbpol.2021.118006.
3. Borkenstein AF, Borkenstein EM, Malyugin B. Ophthalmic Viscosurgical Devices (OVDs) in Challenging Cases: a Review. Ophthalmol Ther. 2021;10(4):831-843. doi:10.1007/s40123-021-00403-9.
4. Park J, Popovic MM, Balas M, El-Defrawy SR, Alaei R, Kertes PJ. Clinical features of endophthalmitis clusters after cataract surgery and practical recommendations to mitigate risk: systematic review. J Cataract Refract Surg. 2022;48(1):100-112. doi:10.1097/j.jcrs.0000000000000756.
5. Kato A, Horita N, Namkoong H, et al. Prophylactic antibiotics for postcataract surgery endophthalmitis: a systematic review and network meta-analysis of 6.8 million eyes. Sci Rep. 2022;12(1):17416. doi:10.1038/s41598-022-21423-w.
6. Clinical and Laboratory Standards Institute (CLSI) Guidelines—Performance Standards for Antimicrobial Susceptibility Testing (2023) CLSI Document M100 ED33.
7. Hsiao CW, Cheng H, Ghafouri R, Ferko NC, Ayres BD. Corneal Outcomes Following Cataract Surgery Using Ophthalmic Viscosurgical Devices Composed of Chondroitin Sulfate-Hyaluronic Acid: A Systematic Review and Meta-Analysis. Clin Ophthalmol. 2023;17:2083-2096. doi:10.2147/OPTH.S419863.
8. Matsuo T, Iguchi M, Morisato N, Murasako T, Hagiya H. Are Prophylactic Systemic Antibiotics Required in Patients with Cataract Surgery at Local Anesthesia?. Int J Environ Res Public Health. 2022;19(23):15796. doi:10.3390/ijerph192315796.
9. Althiabi S, Aljbreen AJ, Alshutily A, Althwiny FA. Post-operative Endophthalmitis After Cataract Surgery: An Update. Cureus. 2022;14(2):e22003. doi:10.7759/cureus.22003.
10. Chiquet C, Bron AM, Lundström M, Maurin M. Acute post-operative endophthalmitis: Microbiology from the laboratory to the bedside. Surv Ophthalmol. 2022;67(6):1698-1710. doi:10.1016/j.survophthal.2022.07.001.
11. Chiquet C, Cornut PL, Benito Y, et al. Eubacterial PCR for bacterial detection and identification in 100 acute postcataract surgery endophthalmitis. Invest Ophthalmol Vis Sci. 2008;49(5):1971-1978. doi:10.1167/iovs.07-1377.
12. Endophthalmitis Vitrectomy Study Group. Results of the Endophthalmitis Vitrectomy Study. A randomized trial of immediate vitrectomy and of intravenous antibiotics for the treatment of post-operative bacterial endophthalmitis. Arch Ophthalmol.1995;113(12):1479-96.
13. Simina DS, Larisa I, Otilia C, Ana Cristina G, Liliana MV, Aurelian MG. The ocular surface bacterial contamination and its management in the prophylaxis of post cataract surgery endophthalmitis. Rom J Ophthalmol. 2021; 65(1):2-9. doi: 10.22336/rjo.2021.2.
14. George NK, Stewart MW. The Routine Use of Intracameral Antibiotics to Prevent Endophthalmitis After Cataract Surgery: How Good is the Evidence? Ophthalmol Ther. 2018;7(2):233-245. doi: 10.1007/s40123-018-0138-6.
15. Chang DF, Braga-Mele R, Henderson BA, Mamalis N, Vasavada A. ASCRS Cataract Clinical Committee Antibiotic prophylaxis of post-operative endophthalmitis after cataract surgery: Results of the 2014 ASCRS member survey. J Cataract Refract Surg. 2015;41(6):1300-5. doi: 10.1016/j.jcrs.2015.01.014.
16. Endophthalmitis Study Group, European Society of Cataract & Refractive Surgeons. Prophylaxis of post-operative endophthalmitis following cataract surgery: results of the ESCRS multicenter study and identification of risk factors. J Cataract Refract Surg. 2007;33(6):978-88. doi: 10.1016/j.jcrs.2007.02.032.
17. Sunderland DK, Sapra A. Physiology, Aqueous Humor Circulation. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. (2023 Mar 12) 2024 Jan–. PMID: 31985990.
18. Astafurov K, Elhawy E, Ren L, Dong CQ, Igboin C, Hyman L, Griffen A, Mittag T, Danias J. Oral microbiome link to neurodegeneration in glaucoma. PLoS One. 2014;9(9):e104416. doi: 10.1371/journal.pone.0104416.
19. Bowen RC, Zhou AX, Bondalapati S, Lawyer TW, Snow KB, Evans PR, Bardsley T, McFarland M, Kliethermes M, Shi D, Mamalis CA, Greene T, Rudnisky CJ, Ambati BK. Comparative analysis of the safety and efficacy of intracameral cefuroxime, moxifloxacin and vancomycin at the end of cataract surgery: a meta-analysis. Br J Ophthalmol. 2018;102(9):1268-1276. doi: 10.1136/bjophthalmol-2017-311051.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 217-221, 2025
https://doi.org/10.32708/uutfd.1650479

ORIGINAL RESEARCH

Dupuytren's Contracture: A 10-Year Retrospective Study

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ABSTRACT

Dupuytren's contracture is a progressive, benign fibroproliferative disease characterized by abnormal collagen accumulation in the palmar region of the hand and flexor surfaces of the fingers. This study retrospectively reviewed 257 patients who underwent surgery at our clinic between January 2014 and December 2024 using electronic hospital records. The analysis focused on age, sex, number of operated hands, affected fingers, annual surgical distribution, skin closure techniques, and comorbidities. Among the 257 patients, 24 underwent bilateral surgery, and five required reoperation due to recurrence, resulting in a total of 286 operated hands. The study cohort consisted of 196 male and 61 female patients. Bilateral surgery was performed in 21 male and three female patients, while five male patients required a second operation due to recurrence. The mean age of the patients was 60 years (range: 34-83). In 73.4% of cases, both the hand and fingers were affected, while 25.8% had isolated palmar involvement. The fourth finger was the most frequently affected (78.5%), followed by the fifth finger (57.6%). Z-plasty was used for skin closure in 222 out of 286 operated hands. This study aims to explore the risk factors associated with Dupuytren's contracture and contribute to the understanding of its prevalence in our region, enhancing regional data.

Keywords: Dupuytren's contracture. Palmar fibromatosis. Open partial fasciectomy. Finger involvement.

Dupuytren Kontraktürü: 10 Yıllık Retrospektif Çalışma

ÖZET

Dupuytren kontraktürü, ilerleyici benign fibroproliferatif bir hastalık olup elin palmar bölgesi ile parmakların fleksör yüzlerinde anormal kolajen birikimi ile karakterizedir. Bu çalışmada Ocak 2014 – Aralık 2024 yılları arasında kliniğimizde opere edilmiş 257 hasta, hastanemiz elektronik arşivi kullanılarak retrospektif olarak incelendi. Yaş, cinsiyet, opere edilen el sayısı, hangi el ve parmakların tutulduğu, yıllara göre opere edilen hasta sayısı, cilt kapatma teknikleri ve eşlik eden morbiditeler açısından tarama yapıldı. 257 hastanın 24'ü bilateral ellerinden opere olurken 5 hasta da nüks nedeniyle ikinci kez opere edildi. Toplam 286 ele Dupuytren cerrahisi uygulanmış oldu. Opere edilen hastaların 196'sı erkek, 61'i kadındı. Erkek hastaların 21'ine bilateral cerrahi uygulanırken 5'ine nüks nedeniyle ikinci kez cerrahi uygulandı. Kadın hastaların 3'üne bilateral cerrahi uygulandı, kadın hastalarda hiç nüks görülmedi. Hastaların ortalama yaşı 60 olarak bulundu (34-83). Opere edilen ellerin %73,4'ünde el+parmak tutulumu birlikte görülürken, %25,8'inde yalnızca el (palmar bölge) tutulumu mevcuttu. El+parmak tutulumu olan hastalarda ise %78,5 oranında tek başına veya diğer parmaklara eşlik edecek şekilde 4.parmağın tutulmuş olduğu görüldü. Bunu %57,6 ile 5.parmak tutulumu takip etti. Opere edilen 286 elin 222'sine ise cilt kapamada z-plasti uygulandı. Bu çalışmada son 11 yılda kliniğimizde Dupuytren kontraktürü nedeniyle opere edilen hastalar risk faktörleri ile birlikte araştırılmış, hastalığın bölgemizdeki görülme prevalansına katkı yapmak amaçlanmıştır.

Anahtar Kelimeler: Dupuytren kontraktürü. Palmar fibromatozis. Açık parsiyel fasyektomi. Parmak tutulumu.

Date Received: 10.March.2025
Date Accepted: 2.July.2025

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Dupuytren's contracture is a progressive, benign fibroproliferative disorder characterized by abnormal collagen accumulation in the palmar region of the hand and the flexor surfaces of the fingers.[1-3] It is a connective tissue disorder affecting between 0.6% and 31.6% of the global population.[4] The condition was first described in 1831 by the French surgeon Baron Guillaume Dupuytren.[1]

Collagen exists in 28 different types and two forms. It is a structural protein found in various tissues, including skin, blood vessels, muscles, bones, cartilage, and tendons. Under normal conditions, type I collagen is predominant in the tissues of the hand; however, in Dupuytren's contracture, there is an increase in the ratio of immature type III collagen to

mature type I collagen.[5,6] Fibroblast proliferation is regulated by the Wnt signaling pathway,[7] which is excessively activated in Dupuytren's contracture.[8] Consequently, histological findings include hyperactivated myofibroblasts, increased tissue proliferation, and enhanced extracellular matrix synthesis. Although the precise trigger of this signaling pathway remains unclear, clinical, biomechanical, epidemiological, and morphological changes have been reported in the literature.[9] Because the palmar aponeurosis is extremely adherent to the overlying skin, it is also difficult to distinguish whether Dupuytren's disease first begins in the dermis of the skin or in the palmar aponeurosis.[4]

Although Dupuytren's contracture is considered a genetic disorder, several environmental risk factors, such as alcohol and tobacco use, diabetes mellitus (DM), anticonvulsant drug use, liver disease, HIV infection, and chronic trauma, have been implicated. While Dupuytren's disease can affect individuals of all ethnicities, it is most prevalent among individuals of Northern European descent. Its incidence increases with age, predominantly affecting men aged 50–70 years, with a higher prevalence in males compared to females.[2,3]

This study aims to retrospectively analyze patients who underwent surgery for Dupuytren's contracture at our clinic, thereby delineating the clinical profile of the disease in our patient population and providing insights into its prevalence in our region.

Material and Method

A retrospective analysis was conducted on 257 patients who underwent surgery for Dupuytren's contracture at our clinic between January 2014 and December 2024 using the hospital's electronic archive. The study was approved by Bursa Uludag University Faculty of Medicine Health Research Ethics Committee (approval number: 2025/4-28). The patients were evaluated in terms of age, sex, number of operated hands, affected hands and fingers, annual distribution of surgical cases, skin closure techniques, and associated comorbidities.

Among the 257 patients, 24 underwent bilateral hand surgery, while 5 patients required a second operation due to recurrence. Consequently, a total of 286 hands underwent Dupuytren's surgery. All patients underwent open partial fasciectomy, which involved the excision of abnormal fascia responsible for the contracture. Various skin closure techniques were employed to prevent recurrence and to correct existing skin contracture (Figure 1). All procedures were performed under a tourniquet with the aid of loop magnification.

At the end of the surgery, after appropriate wound dressing, patients with both hand and finger involvement were fitted with a volar splint to maintain the interphalangeal and metacarpophalangeal joints in extension. In contrast, patients with isolated palmar involvement did not receive a splint; instead, a padded dressing was applied to the palm.

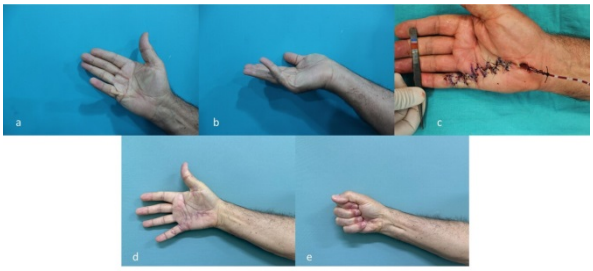

Figure 1. a-b:
Hand with 5th finger and palmar involvement ***c:*** *Perioperative view. Skin closed with multiple Z-plasties in the same area* ***d-e:*** *Postoperative 2nd month view. Skin and joint contractures are resolved, movements are normal.*

Results

Of the operated patients, 196 were male and 61 were female. Bilateral surgery was performed in 21 male patients, while 5 male patients required a second operation due to recurrence. As a result, a total of 222 hands underwent Dupuytren's surgery in male patients. Among female patients, three underwent bilateral surgery, and no recurrence was observed. Consequently, a total of 64 hands were operated on in female patients. A total of 164 right hands and 122 left hands were operated on, with all five recurrences occurring in the right hand.

The mean age of the patients was 60 years (range: 34–83). The annual distribution of operated hands is presented in Figure 2. The highest number of surgeries was performed in 2019–2020 (52 hands), while the lowest number was recorded in 2020–2021 (9 hands). This decline in surgical cases is attributed to the decrease in hospital admissions during the COVID-19 pandemic, which affected both Türkiye and the world.

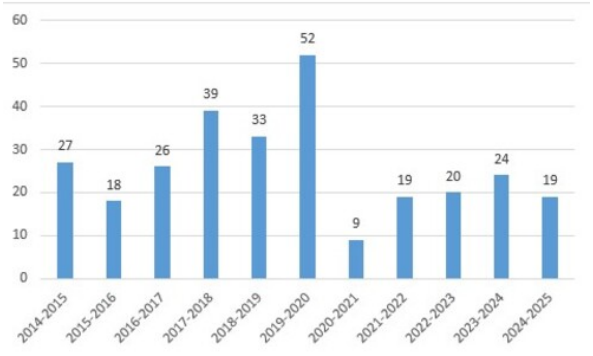

Figure 2:
The Annual Distribution of Operated Hands

The distribution of operated hands according to the affected regions is shown in Table I. In 73.4% of cases, both the palmar and fingers were involved, whereas 26.2% of cases exhibited isolated palmar involvement. Among patients with both hand and finger involvement, 86 had a single affected finger, 67 had two affected fingers, 49 had three affected fingers, and 8 had four affected fingers. The most commonly affected digit in single-finger involvement was the fourth finger. In two-finger involvement, the fourth and fifth fingers were most frequently affected, while in three-finger involvement, the third, fourth, and fifth fingers were predominantly involved. Overall, the fourth finger was the most frequently affected in cases of palmar and finger involvement (78.5%).

Table I. The distribution of operated hands according to the affected regions.

AFFECTED AREA	NUMBER OF OPERATED HANDS *(n=286)*
Palmar + Finger	**210**
One Finger	*86*
D1	1
D2	1
D3	9
D4	47
D5	28
Two Fingers	*67*
D1-2	1
D1-4	1
D3-4	19
D3-5	3
D4-5	43
Three Fingers	*49*
D1-2-3	1
D1-3-4	2
D1-4-5	1
D2-3-4	7
D2-3-5	1
D3-4-5	37
Four Fingers	*8*
D1-3-4-5	4
D2-3-4-5	4
Palmar	**75**
Palmar + Plantar	**1**

D: Digit

During surgery, open partial fasciectomy was performed to remove all contracted and abnormal fascial structures. Various skin closure techniques were employed to prevent recurrence and to correct existing skin contracture (Table II). Among the operated hands, 77.6% underwent skin closure using various Z-plasty techniques, while 12.5% were closed using Brunner's (zig-zag) incision. Skin grafting was required in five patients.

Given that Dupuytren's contracture may be associated with various environmental factors, patients were also assessed for comorbidities. Hypertension (HT) was present in 98 patients, and diabetes mellitus (DM) was present in 75 patients. Additionally, 12 patients had both HT and DM. Asthma and chronic obstructive pulmonary disease (COPD) were each observed in seven patients, while hyperlipidemia was identified in eight.

Table II. Distribution of operated hands according to skin closure techniques.

Skin Closure Technique	Number of Operated Hands *(n=286)*
Primary Repair	23
Brunner's Incision	36
Z-plasty *(n=222)*	
Multiple Z-plasty (MZP)	167
MZP + Full Thickness Skin Graft (FTSG)	1
MZP + Split Thickness Skin Graft (STSG)	1
One z-plasty	28
Two z-plasty	16
Three z-plasty	9
Fillet flap	2
FTSG	3

Discussion and Conclusion

Genetic predisposition is one of the most significant risk factors for Dupuytren's Contracture. Individuals with a first-degree relative affected by Dupuytren's Disease are at a substantially higher risk.[10] Being male is another crucial risk factor, with studies indicating that the disease is 2–7 times more common in men than in women and follows a more aggressive course in males.[5,11,12] In our study, 76% of patients were male and 24% were female. Bilateral involvement was more frequent in males, and all five cases of recurrence were observed in male patients. Advanced age is another important risk factor.[11,12] In accordance with the literature, the mean age in our study was 60 years for male patients and 61 years for female patients.

Environmental risk factors such as alcohol and smoking, diabetes mellitus (DM), anticonvulsant medication use, liver disease, HIV infection, and chronic trauma have also been reported.[2,3] In our study, DM was present in 33.8% of patients, and hypertension (HT) in 42.8%. While the prevalence of DM was consistent with the literature, the prevalence of HT was higher than reported in previous studies. A 2008 survey study investigated the association between Dupuytren's Contracture and occupational exposure, finding that manual labor and the use of vibrating tools were significant risk factors.[13]

A review by Khaliq and Orji examined conditions that can be confused with Dupuytren's Contracture based on clinical presentation, identifying trigger finger, flexor tendon contracture, and cubital tunnel syndrome as differential diagnoses.[14] It was also noted that

Dupuytren's Contracture can be associated with plantar fibromatosis, necessitating clinical differentiation for appropriate treatment.[15] During our retrospective review, one patient was identified with isolated plantar fibromatosis and no palmar involvement. As no hand surgery was performed, this patient was excluded from the study cohort. Another patient presented with both palmar and plantar involvement; however, only the palmar component was surgically treated and thus included in the analysis.

Previous studies have reported that the fourth finger is the most commonly affected in Dupuytren's Contracture.[16,17] In our study, the fourth finger was affected in 78.5% of cases, either alone or in combination with other fingers. The fifth finger was the second most frequently affected, with an involvement rate of 57.6%, consistent with the literature.

Z-plasty was performed in 77.6% of the operated hands in the study. A study by Elmelegy and Nader[4] emphasized the necessity of Z-plasty to prevent scar recurrence.

In our series, recurrence requiring revision surgery was observed in five male patients (1.9%) during the follow-up period. Although this rate is lower than what has been reported in the literature, it is consistent with studies highlighting the benefit of early-stage intervention and standardized surgical technique. Hindocha et al.[18] noted that recurrence rates are significantly higher in patients with early-onset disease, bilateral involvement, and positive family history, suggesting a genetic predisposition to more aggressive disease behavior. Dominguez-Malagón et al.[19] further emphasized the role of persistent myofibroblast activity at the cellular level, which may contribute to recurrence even after complete fasciectomy.

While complications were not systematically evaluated in our study, the risk profile of limited fasciectomy has been well described. Denkler's 20-year review reported complication rates ranging from 3.6% to 39.1%, with frequent issues including digital nerve injury (up to 7.7%), digital artery injury (up to 2.0%), wound healing problems (up to 86%), hematoma, infection, and complex regional pain syndrome.[20] These findings reinforce the need for meticulous dissection, careful patient selection, and long-term follow-up to optimize surgical outcomes in Dupuytren's disease.

While our study focused exclusively on open fasciectomy, a range of alternative treatments has gained attention in recent years for selected cases of Dupuytren's disease. Minimally invasive techniques such as collagenase Clostridium histolyticum (CCH) injection and needle aponeurotomy have been proposed for early-stage or less aggressive disease, offering reduced recovery times but higher recurrence rates—reported up to 35–58% in the literatüre. In contrast, partial fasciectomy remains the gold standard for patients with advanced or functionally limiting contractures due to its lower long-term recurrence (10–15% after five years) and ability to directly address spiral cords and displaced neurovascular structures. However, total fasciectomy, while rarely performed, is reserved for diffuse or recurrent disease and carries a higher complication profile.[21] The choice of treatment must be individualized, considering disease severity, patient age, recurrence risk, and surgeon experience. Future directions may involve combination protocols or biologic therapies targeting the myofibroblastic process underlying the disease.

Our study has certain limitations. Family history and occupational exposure, which are important risk factors, were not evaluated. Additionally, in patients with both palmar and finger involvement, the degree of loss in joint range of motion was not assessed. Future studies could incorporate these factors for a more comprehensive analysis.

Dupuytren's Contracture is a progressive fibroproliferative disease influenced by various factors, including age, sex, genetic predisposition, occupational exposure, and comorbid systemic diseases. Our study retrospectively analyzed patients who presented to our clinic between 2014 and 2024 to assess the prevalence and risk factors of Dupuytren's Contracture in our region. We believe that our findings contribute to the existing literature by providing insight into the regional prevalence and associated risk factors of Dupuytren's Contracture.

Researcher Contribution Statement:
Idea and design: M.K.; S.Ç.; Data collection and processing: M.K., K.Y., O.A.G.; Analysis and interpretation of data: M.K., Süleyman Çeçen, Güzin Yeşim Özgenel; Writing of significant parts of the article: M.K., S.A., M.U.O.
Support and Acknowledgement Statement:
The authors declare that the work has not received any funding.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Bursa Uludag University Faculty of Medicine Health Research Ethics Committee
Approval Date: 19.02.2025
Decision No: 2025/4-28

References

1. Khaliq F, Orji C. Dupuytren's Contracture: A Review of the Literature. *Cureus*. 2024;16(12):e74945. doi:10.7759/cureus.74945
2. Şakı MC, Ersen B, Tunalı O, Aksu İ, Kahveci R. Dupuytren Kontraktürü: 87 Olgunun Retrospektif İncelenmesi. *Uludağ Üniversitesi Tıp Fakültesi Derg*. 2014;40(1):19-21.
3. Lurati AR. Dupuytren's Contracture. *Work Heal Saf*. 2017;65(3):96-99. doi:10.1177/2165079916680215

4. Elmelegy NG, Nader D. The importance of multiple Z- plasty-assisted physical therapy in the treatment of Dupuytren's contracture. *J Orthop Surg Res*. 2025;20(1). doi:10.1186/s13018-024-05387-3
5. Hindocha S, John S, Stanley JK, Watson SJ, Bayat A. The Heritability of Dupuytren's Disease: Familial Aggregation and Its Clinical Significance. *J Hand Surg Am*. 2006;31(2):204-210. doi:10.1016/J.JHSA.2005.09.018
6. Thorne CH. Grabb and Smith's Plastic Surgery. In: Freshwater M.felix. Dupuytren's Disease, 6th edition. Philadelphia: Lippincott Williams & Wilkins, a Wolters Kluwer business; 2007.864-868.
7. Dolmans GH, Werker PM, Hennies HC, et al. Wnt Signaling and Dupuytren's Disease. *N Engl J Med*. 2011;365(4):307-317. doi:10.1056/nejmoa1101029
8. ten Dam EJPM, van Beuge MM, Bank RA, Werker PMN. Further evidence of the involvement of the Wnt signaling pathway in Dupuytren's disease. *J Cell Commun Signal*. 2016;10(1):33-40. doi:10.1007/s12079-015-0312-8
9. Özkaya Ö, Karasoy Yeşilada A, Karşıdağ S, et al. Dupuytren Kontraktürü: Etiyoloji, Tanı ve Cerrahi Tedavisi, On Yıllık Retrospektif Analiz. *Turkiye Klinikleri J Med Sci*. 2010;30(2):553-8.
10. Burge P. Genetics of Dupuytren's disease. *Hand Clin*. 1999;15(1):63-71.
11. Anthony SG, Lozano-Calderon SA, Simmons BP, Jupiter JB. Gender Ratio of Dupuytren's Disease in the Modern U.S. Population. *HAND*. 2008;3(2):87-90. doi:10.1007/s11552-007-9076-9
12. Mikkelsen OA. Dupuytren's Disease—Initial Symptoms, Age of Onset and Spontaneous Course. *Journal of Hand Surgery (European Volume)*. 1977;os-9(1):11-15. doi:10.1016/S0072-968X(77)80023-5
13. Lucas G, Brichet A, Roquelaure Y, Leclerc A, Descatha A. Dupuytren's disease: personal factors and occupational exposure. *Am J Ind Med*. 2008;51(1):9-15. doi:10.1002/ajim.20542
14. Vargas A, Chiapas-Gasca K, Hernández-Díaz C, et al. Clinical anatomy of the hand. *Reumatol Clin*. 2012;8 Suppl 2:25-32. doi:10.1016/j.reuma.2012.10.004
15. Walthall J, Anand P, Rehman UH. Dupuytren Contracture. [Updated 2023 Feb 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK526074/
16. Dominguez-Malagon HR, Alfeiran-Ruiz A, Chavarria-Xicotencatl P, Duran-Hernandez MS. Clinical and cellular effects of colchicine in fibromatosis. *Cancer*. 1992;69(10):2478-2483. doi:10.1002/1097-0142(19920515)69:10<2478::aid-cncr2820691016>3.0.co;2-v
17. Luck JV. Dupuytren's contracture; a new concept of the pathogenesis correlated with surgical management. *J Bone Joint Surg Am*. 1959;41-A(4):635-664.
18. Hindocha S, John S, Stanley JK, Watson SJ, Bayat A. The heritability of Dupuytren's disease: familial aggregation and its clinical significance. *J Hand Surg Am*. 2006;31(2):204-210. doi:10.1016/j.jhsa.2005.09.018
19. Dominguez-Malagon HR, Alfeiran-Ruiz A, Chavarria-Xicotencatl P, Duran-Hernandez MS. Clinical and cellular effects of colchicine in fibromatosis. Cancer. 1992;69(10):2478-2483. doi:10.1002/1097-0142(19920515)69:10<2478::aid-cncr2820691016>3.0.co;2-v
20. Denkler K. Surgical complications associated with fasciectomy for dupuytren's disease: a 20-year review of the English literature. *Eplasty*. 2010;10:e15. Published 2010 Jan 27.
21. Walthall J, Anand P, Rehman UH. Dupuytren Contracture. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; February 26, 2023.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 223-231, 2025
DOI: https://doi.org/10.32708/uutfd.1691390

ORIGINAL RESEARCH

Anti-Fibrotic and Regenerative Potential of Mesenchymal Stem Cell-Derived Exosomes in Cisplatin-Induced Kidney Injury

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ABSTRACT

Cisplatin is a widely used chemotherapeutic agent with potent antitumor activity; however, its nephrotoxicity limits clinical use, affecting 30–40% of treated patients. This study aimed to investigate the effects of mesenchymal stem cell-derived exosomes on cisplatin-induced nephrotoxicity and fibrosis in rat kidney tissue. Rats were divided into Control, Cis, Exo, and Cis+Exo groups. Nephrotoxicity was induced by a single dose Cis. Exosomes were isolated using a commercial kit and characterized by nanoparticle tracking analysis. Histopathological evaluations were performed Hematoxylin&Eosin and Periodic Acid-Schiff. Fibrosis markers were assessed by immunohistochemistry. Statistical analyses were conducted using one-way ANOVA and Kruskal-Wallis tests with Bonferroni and Dunn's post-hoc tests, considering $p<0.05$ as statistically significant. In the Cis group, significant tubular degeneration, necrosis, and fibrosis were observed compared to the Control group. TGF-β1, α-SMA, and TLR-4 expressions were markedly increased in the Cis group ($p<0.001$). Exo treatment significantly reduced the expression levels of these fibrosis markers compared to the Cis group (TGF-β1 and TLR-4, $p<0.001$; α-SMA, $p<0.05$). Histopathological analysis revealed that Exo administration mitigated nephrotoxic damage and supported tissue regeneration, with tissue architecture resembling that of the Control group. This study demonstrates that MSC-derived exosomes alleviate not only acute cisplatin-induced injury but also the associated fibrotic response. A single dose of exosome treatment significantly modulated the fibrotic response and reduced oxidative stress-induced damage. These findings indicate that MSC-derived exosomes, known for their regenerative and tissue-repairing properties, also possess significant potential as antifibrotic therapeutic agents, highlighting the need for further research toward clinical applications.

Keywords: Exosomes. Fibrosis. Mesenchymal stem cell. Nephrotoxicity. Cisplatin.

Sisplatinle İndüklenen Böbrek Hasarında Mezankimal Kök Hücre Kökenli Eksozomların Anti-Fibrotik ve Rejeneratif Potansiyeli

ÖZET

Sisplatin, güçlü antitümöral aktiviteye sahip, yaygın olarak kullanılan bir kemoterapi ajanıdır; ancak nefrotoksisite oluşturması, klinik kullanımını sınırlamakta ve tedavi edilen hastaların %30–40'ında görülmektedir. Bu çalışma, mezankimal kök hücre kaynaklı eksozomların, sisplatin ile indüklenen nefrotoksisite ve fibrozis üzerindeki etkilerini sıçan böbrek dokusunda araştırmayı amaçlamıştır. Sıçanlar kontrol, Cis, Exo ve Cis+Exo olmak üzere dört gruba ayrıldı. Nefrotoksisite, tek doz sisplatin uygulanarak indüklendi. Eksozomlar ticari bir kit kullanılarak izole edildi ve nanoparçacık izleme analizi ile karakterize edildi. Histopatolojik değerlendirmeler Hematoksilen&Eozin, Periyodik Asit-Schiff ile yapıldı. Fibrozis belirteçleri immünohistokimyasal boyama ile değerlendirildi. İstatistiksel analizler, çoklu grup karşılaştırmaları için tek yönlü ANOVA ve Kruskal-Wallis testleri kullanılarak, Bonferroni ve Dunn post-hoc testleri ile gerçekleştirildi. $p<0,05$ değeri istatistiksel olarak anlamlı kabul edildi.Cis grubunda, kontrol grubuna kıyasla anlamlı düzeyde tübüler dejenerasyon, nekroz ve fibrozis gözlendi. Histopatolojik analizler, Exo uygulamasının nefrotoksik hasarı azalttığını ve doku rejenerasyonunu desteklediğini, doku mimarisinin Kontrol grubuna benzer hale geldiğini ortaya koydu. TGF-β1, α-SMA ve TLR-4 ekspresyonları Cis grubunda belirgin şekilde artmıştı ($p<0,001$). Exo tedavisi, bu fibrozis belirteçlerinin ekspresyon seviyelerini Cis grubuna göre anlamlı düzeyde azalttı (TGF-β1 ve TLR-4 için $p<0,001$; α-SMA için $p<0,05$). Bu çalışma, MSC kaynaklı eksozomların yalnızca akut sisplatin hasarını değil, aynı zamanda ilişkili fibrotik süreci de hafiflettiğini göstermektedir. Tek doz Exo tedavisi, fibrotik yanıtı anlamlı şekilde modüle etmiş ve oksidatif stres kaynaklı doku hasarını azaltmıştır. Bu bulgular, rejeneratif ve doku onarıcı etkileri iyi bilinen MSC kaynaklı eksozomların, aynı zamanda belirgin bir antifibrotik bir tedavi ajanı olma potansiyeline de sahip olduğunu göstermekte ve klinik uygulamalara yönelik ileri araştırmaların gerekliliğini ortaya koymaktadır**.**

Anahtar Kelimeler: Eksozom. Fibrozis. Mezenkimal kök hücre. Nefrotosisite. Sisplatin.

Date Received: 4.May.2025
Date Accepted: 2.July.2025

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Extracellular vesicles (EVs) are lipid bilayered nanovesicles of varying sizes, contents, and mechanisms, secreted by nearly all cell types. Based on current knowledge regarding their biogenesis, these vesicles are classified into two main categories: exosomes (Exo) and microvesicles[1]. Exo are the smallest extracellular carriers, with diameters ranging between 30 and 120 nm, enclosed by a phospholipid bilayer[2]. Owing to their versatile roles in intercellular communication, Exo are critically involved in numerous biological processes, ranging from physiological regulation and disease progression to modulation of immune responses and pathogenesis[3]. Their high biocompatibility, low toxicity, and ability to traverse biological membranes have positioned Exo as a promising tool, particularly in the field of cancer therapy[4]. The biological functions of Exo are determined by the bioactive molecules they carry, including lipids, metabolites, proteins, and nucleic acids, which exert effects on target cells[5]. In this context, Exo are explored in cancer treatment within two main strategies: as carriers for therapeutic agents and as modulators of the tumor microenvironment to inhibit tumor growth and metastasis[3,5].

The kidney is a crucial excretory organ responsible for maintaining homeostasis and receives approximately 25% of the cardiac output. Consequently, it is one of the primary target organs in drug-induced toxicity[6]. The most significant dose-limiting side effect of cisplatin (Cis) is nephrotoxicity[7]. Cis-induced nephrotoxicity is dose-dependent, which in turn limits the drug's therapeutic efficacy[8]. Since Cis is not absorbed through the gastrointestinal tract, it can only be administered intravenously or intraperitoneally[9]. Approximately 90% of Cis binds to plasma proteins, and platinum residues have been reported in renal tissue even four months after administration[10]. The mechanisms underlying Cis nephrotoxicity include oxidative stress, apoptosis, necrosis, inflammation, fibrogenesis, and mitochondrial damage, all of which are interconnected[11]. Following kidney injury, damaged cells secrete various soluble factors that trigger fibroblast activation and inflammatory responses[12]. Renal fibrosis is characterized by persistent fibroblast activation leading to excessive extracellular matrix (ECM) accumulation, which results in scar formation, structural damage to the renal parenchyma, and ultimately, renal dysfunction[4,13]. In this process, transforming growth factor-beta (TGF-β) plays a pivotal role in driving fibrosis through both Smad-dependent and alternative signaling pathways, promoting the differentiation of fibroblasts into myofibroblasts. As a hallmark of myofibroblast activation, alpha-smooth muscle actin (α-SMA) is widely used as a marker for the assessment of fibrogenic activity in various kidney injury models[14]. Additionally, Toll-like receptor 4 (TLR4) has been shown to mediate inflammation-driven fibrosis by activating innate immune signaling pathways, linking tissue injury to fibrotic remodeling[15]. Therefore, in this study, TGF-β1, α-SMA, and TLR4 were selected as key molecular markers to evaluate the antifibrotic effects of exosomes in a cisplatin-induced nephrotoxicity model.

The therapeutic, immunomodulatory, and regenerative effects of mesenchymal stem cells (MSCs) are believed to be mediated predominantly through paracrine mechanisms involving cytokines, growth factors, hormones, and Exo[16]. These molecules promote angiogenesis, stimulate extracellular matrix production, and regulate immune responses by reducing apoptosis and fibrosis[17]. Exo derived from MSCs contributesto maintaining tissue homeostasis and supporting cellular functions by initiating regenerative processes. Compared to MSC transplantation, exosome-based therapies are considered a superior clinical alternative due to their low immunogenicity, high safety profile, absence of tumorigenic potential, and ethical advantages[5,18,19].

Because of their anti-inflammatory, anti-apoptotic, and anti-fibrotic properties, Exo may contribute to the preservation of renal function and the suppression of fibrotic processes. In this context, the therapeutic potential of Exo in cisplatin-induced kidney injury has attracted increasing attention. This study aims to investigate the reparative effects of mesenchymal stem cell-derived Exo on cisplatin-induced renal fibrosis and to highlight the potential of exosome-based therapeutic strategies as a safe and effective alternative to current treatment modalities.

Material and Method

Experimental Groups and Treatment Protocols

The experiments were conducted in accordance with The ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments). Ethics Committee Approval:

Erciyes University Animal Experiments Local Ethics Committee (decision no: 24/030, date: 07.02.2024). The study does not require patient consent.

The study was initiated with 32 male Wistar albino rats, aged 8 to 10 weeks. The rats were kept in a 25°C room with a 12-hour light/dark cycle, free access to water, and a standard diet from the Experimental and Clinical Research Center at Erciyes University, Kayseri, Turkiye.

In this study, the rats were randomly divided into four groups (n = 8 per group):

Group 1 (Control, C): No treatment was administered.

Group 2 (Exosome, Exo): The group treated with Exo received a single intravenous injection (i.v.) of exosome ($8x10^7$/100 µl) on the sixth day of the experiment[20].

Group 3 (Cisplatin, Cis): The Cis group was administered a single intraperitoneal (i.p.) injection of Cis (7.5mg/kg) (Kocak Pharma, Istanbul, Türkiye) on the first day of the experiment to induce nephrotoxicity[21].

Group 4 (Cisplatin + Exosome, Cis+Exo): Rats were administered a single i.p. injection of Cis (7.5mg/kg) on the first day to induce nephrotoxicity. On the sixth day, a single i.v. dose of Exo ($8x10^7$/100 µl) was administered to the rats.

All procedures were carried out at the same time of day to ensure consistency in experimental conditions. On the 8th day, tissue samples were collected under general anesthesia (xylazine;10 mg/kg, and ketamine;60 mg/kg, i.p.), and the animals were sacrificed.

Surgical Procedure

On the 8th day of the experiment, all groups were anesthetized intraperitoneally with ketamine hydrochloride (60 mg/kg) and xylazine hydrochloride (10 mg/kg, 2% solution). Under sterile conditions, a midline incision was made to perform a laparotomy. After opening the subcutaneous connective tissue and abdominal muscles, the left kidney was carefully dissected. The kidney was then placed in formaldehyde for subsequent histopathological and immunohistochemical evaluations.

Exosome Isolation

MSCs derived from the bone marrow of Wistar albino rats were obtained from the Genome and Stem Cell Center (GENKÖK) at Erciyes University, Türkiye[22]. Exosomes (Exo) were subsequently isolated from the secretomes of these cells Previously isolated and frozen MSCs were thawed in a 37°C water bath and seeded into 75 cm² cell culture flasks (TP Inc, Rochester, NY, USA). When the MSCs reached over 90% confluency, a serum-free medium (MEM-α, Cat. No: BI01-042-1A; Biological Industries, Beit HaEmek, Israel) was added, and the secretomes were collected after 24 hours.

A commercial kit (ExoQuick-TC Exosome Precipitation Solution Kit, Palo Alto, California, United States) was used to perform standard exosome isolation[23]. For exosome isolation, collected secretomes were centrifuged at 3000 × g for 15 minutes, and the supernatants were transferred into sterile tubes. To each 10 ml of supernatant, 2 ml of ExoQuick-TC solution was added, and the mixture was incubated overnight. Following incubation, the ExoQuick-TC/supernatant mixture was centrifuged at 1500 × g for 30 minutes. The supernatants were removed, and the Exo formed a pellet at the bottom of the tube. For exosomal protein analysis, the resulting pellet was resuspended in 100–500 µl of ExoQuick-TC solution. The quantity of Exo was determined using a microvesicle measurement kit (ExoQuick-TC Exosome Precipitation Solution Kit). The average particle size of the secretomes was measured using the Nanoparticle Tracking Analysis system (NTA, Malvern Instrument Nanosight NS300, Malvern, UK). The live particle imaging settings were adjusted according to the manufacturer's software manual (NanoSight NS300 User Manual, MAN0541-01-TR-00, 2017), and the measurements were completed (n = 3). Exosome quantification was performed using the ExoCet Exosome Quantitation Kit (EXOCET96A-1; System Biosciences, Palo Alto, CA, USA) on a Glomax® Multi Detection System microplate reader (Promega, Madison, WI, USA). This kit is an antibody-free, colorimetric enzymatic assay based on the activity of acetylcholinesterase (AChE), an enzyme highly enriched in the exosomal membrane. The enzymatic reaction produces a colorimetric signal that is measured at 405 nm. All absorbance readings were conducted at this wavelength using the Glomax® system. A standard curve was generated from known exosome standards provided with the kit, and exosome concentrations in the samples were calculated accordingly[24]. Exo diluted to a concentration of $8X10^7$ particles/100 µl were injected into the Exo and Cis + Exo groups in the study.

Histopathological Analysis

In order to histologically evaluate kidney defects in each experimental group, tissue samples collected at the end of the experiment were fixed in 10% formaldehyde solution. After 72 hours of fixation, the tissues were washed under running tap water, dehydrated through a graded series of alcohol, cleared in xylene, and embedded in paraffin. Sections of 5 µm thickness were obtained from the paraffin blocks and placed onto slides (Leica, Autocut, 14051956472, Germany). The prepared slides were deparaffinized

using xylene, rehydrated through a graded series of alcohols (100%, 96%, 80%, 70%, 50%), and washed in water following a standard histological staining protocol. For general histological assessment, the sections were stained with Hematoxylin and Eosin (H&E) (Bio-Optica 05-06004/L Harris' Hematoxylin & Bio-Optica 05-10002/L Eosin Y 1%) and Periodic Acid-Schiff (PAS) (Best Lab, Türkiye). After staining, the sections were dehydrated through ascending alcohol series, cleared with xylene, and mounted with coverslips using Entellan. The sections were then evaluated under a light microscope. Degenerative changes in the tubular and intertubular areas were assessed semi-quantitatively. In each kidney section, 10 different fields were evaluated for each damage parameter, and average percentage values within each group were calculated. Histopathological changes were scored as follows: changes observed in less than 25% of tubular epithelial cells were scored as 1 (mild), 25–50% as 2 (moderate), 50–75% as 3 (severe), and 75–100% as 4 (very severe) (absence=0, mild=1, moderate=2, severe=3, very severe=4)[25].

Immunohistochemical Analysis

The immunohistochemical staining kit (Lab VisionTM UltraVisionTM Large Volume Detection System: anti-polyvalent, HRP, TA-125-HL) was used in conjunction with the streptavidin-biotin-peroxidase method. Using this method, the expression levels of fibrotic markers TGF-β1, α-SMA, and TLR4 in kidney tissues were demonstrated immunohistochemically. For immunohistochemical staining, 5 µm sections were obtained from paraffin-embedded blocks prepared by routine processing following 10% formalin fixation. The sections were incubated overnight at 56°C, then deparaffinized in xylene for 15 minutes, dehydrated through descending alcohol series (%99, %96, and %70), and rehydrated by immersion in distilled water for 10 minutes. For antigen retrieval, the sections were boiled in 5% citrate buffer in a microwave oven at 600W for 5 minutes and subsequently washed with PBS. To block endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide (H_2O_2) for 20 minutes. After washing with PBS, blocking serum was applied at room temperature for 10 minutes to prevent non-specific binding.

The sections were incubated overnight at 4°C with primary antibodies against TGF-β (Proteintech, Cat No. 21898-1-AP, 1:250), α-SMA (Proteintech, Cat No. 80008-1-RR, 1:2500), and TLR-4 (Proteintech, Cat No. 19811-1-AP, 1:400). Following washing, the sections were incubated with a biotinylated secondary antibody for 20 minutes, washed again, and then treated with streptavidin-peroxidase (Thermo Scientific, SHRP248-B) for 20 minutes before a final wash. Diaminobenzidine (DAB) solution was applied, and the tissues were monitored under a light microscope until a visible signal developed, after which all groups were washed simultaneously with tap water.

The sections were counterstained with Mayer's hematoxylin, rinsed with distilled water, passed through ascending alcohol series (5 minutes in each concentration), cleared in xylene for 15 minutes, and finally mounted with Entellan without air bubbles. The immunohistochemically stained sections were examined under a light microscope, and microscopic images were captured from 20 different fields. Immunoreactivity intensities of the targeted markers in the captured images were quantified using the ImageJ software program (NIH, Washington, USA), and the results were evaluated[26].

Statistical Analyses

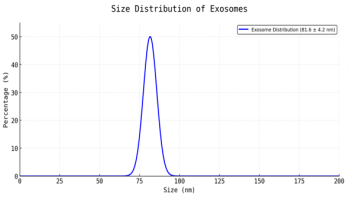
The results obtained from the analyses were evaluated using the GraphPad Prism 9.0 statistical software. The Shapiro-Wilk test was performed to assess the normality of data distribution. For comparisons involving multiple groups, one-way analysis of variance (ANOVA) and the Kruskal-Wallis test were used. Post hoc analyses were conducted using the Bonferroni test following ANOVA and the Dunn test following the Kruskal-Wallis test, both of which identified significant differences between variables. A p-value of less than 0.05 was considered statistically significant for all analyses.

Results

Quantification and Characterization of Isolated Exosomes

Nanoparticle tracking analysis (NTA) revealed that the isolated secretomes had an average particle diameter of 81.6 ± 4.2 nm, consistent with the expected size range of exosomes. A representative NTA size distribution image is shown in Figure 1. These findings confirmed the successful isolation of exosome-sized vesicles from the secretomes.

Exosome quantification was performed based on a standard curve generated using exosome solutions with known concentrations. The absorbance of the samples was measured at 405 nm using the ExoCet Exosome Quantitation Kit, and the obtained value was approximately 0.1136 OD. This value was analyzed using the linear regression equation provided by the kit ($y = 0.0012x + 0.0176$; $R^2 = 0.9976$). According to this equation, the exosome concentration in the sample was calculated to be approximately 8×10^7 particles/100 µl.



Measurement	Mean Size ± SD (nm)
1st Measurement	79.4 ± 3.9
2nd Measurement	84.1 ± 4.8
3rd Measurement	81.3 ± 3.9
Average	**81.6 ± 4.2**

Figure 1.
Size distribution of isolated exosomes measured by nanoparticle tracking analysis (NTA). Representative histogram showing the size distribution of exosomes (mean ± SD,). The majority of particles were within the 50–150 nm range, consistent with the expected size of exosomes.

Histological Results

Examination of kidney tissues from the control group rats revealed that the glomeruli, cortical, and medullary tubules maintained normal histological structures. Similarly, the Exo-only group exhibited nearly normal histological architecture, with no significant pathological findings except for mild tubular dilation (1.0 [0.0–2.0]) in some specimens. The Cis group, which received cisplatin to induce nephrotoxicity, showed severe pathological changes. These included prominent tubular degeneration (4.0[3.0–4.0]), tubular necrosis (4.0[3.0–4.0]) and tubular dilation (4.0[3.0–4.0]), along with extensive vascular hyperemia (3.0[2.0–4.0]) and collagen/ glycogen accumulation (3.0[2.0–4.0]). These changes indicate widespread structural damage in both parenchymal and stromal regions.

In the group treated with Exo following Cis administration (Cis+Exo group), a substantial reduction in tissue damage was observed compared the Cis group, with notable restoration in stromal and parenchymal areas and only a few degenerated and necrotic cells remaining. Tubular degeneration (1.0[0.0–2.0]), necrosis (0.0[0.0–1.0]), and dilation (1.0[0.0–2.0]) were substantially reduced. Vascular hyperemia (1.0[1.0–2.0]) and collagen/glycogen accumulation (1.0[0.0–2.0]) were also milder. (Figure 2, Table I).

In the PAS-stained sections, the Cis group showed a marked thickening of the glomerular and tubular basement membranes, as well as an increase in glycogen accumulation, indicated by prominent staining in the tubular epithelium. In contrast, the Cis+Exo group exhibited basement membrane thickness and glycogen content comparable to the control groups (Figure 2)

Statistical analysis confirmed that these differences were significant ($p<0.001$ for all parameters). Post-hoc comparisons (b-values) indicated particularly strong significance for tubular degeneration (b = 0.022) and dilation (b = 0.03), while vascular hyperemia (b = 0.352) and collagen/glycogen accumulation (b = 0.054) showed trends toward significance (Table I).

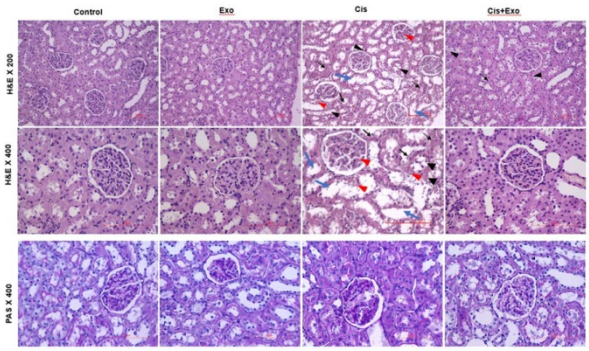


Figure 2.
Representative hematoxylin and eosin (H&E)-stained sections demonstrating the effects of exosome administration on renal tubules and glomeruli (Nikon Eclipse Si, Tokyo, Japan; scale bar: 100 µm). Black arrows indicate necrotic tubular epithelial cells, while arrowheads highlight hydropic degeneration. Blue arrows denote tubular dilatation, and red arrowheads indicate hyperemia observed in both glomerular and intertubular regions. In the lower panel, a representative periodic acid–Schiff (PAS)-stained section (scale bar: 100 µm) is shown.

Table I. Histopathological scoring of kidney tissue.

Group (n=8)	Tubular Degeneration (H&E)	Tubular Necrosis (H&E)	Tubular Dilatation (H&E)	Vascular Hyperemia (H&E)	Collagen/ Glycogen Increase (PAS)
Control	0.0 [0.0–0.0]	0.0 [0.0–1.0]	0.0 [0.0–0.0]	0.0 [0.0–1.0]	0.0 [0.0–1.0]
Exo	0.0 [0.0–1.0]	0.0 [0.0–1.0]	1.0 [0.0–2.0]	0.0 [0.0–1.0]	0.0 [0.0–1.0]
Cis	4.0 [3.0–4.0]	4.0 [3.0–4.0]	4.0 [3.0–4.0]	3.0 [2.0–4.0]	3.0 [2.0–4.0]
Cis+Exo	1.0 [0.0–2.0]	0.0 [0.0–1.0]	1.0 [0.0–2.0]	1.0 [1.0–2.0]	1.0 [0.0–2.0]
p-value	<0.001	<0.001 (b=0.022)	<0.001 (b=0.03)	<0.001 (b=0.352)	<0.001 (b=0.054)

Cis: Cisplatin group; **Exo:** Exosome grup. **b:** Comparison between Cis and Cis+Exo group.

Statistical test: Kruskal–Wallis with Dunn's post hoc test.

Immunohistochemical Results

The aim was to determine the positivity of TGF-β1, α-SMA, and TLR-4, which serve as key markers of fibrosis, in kidney tissues from all experimental groups. Accordingly, areas stained with primary antibodies were photographed. Upon evaluation of the obtained data, the Cis group showed an increased expression of fibrosis markers compared to all other groups. TGF-β1 was strongly expressed in the glomeruli, tubulointerstitial areas, and around blood vessels. α-SMA expression was predominantly observed in the tubulointerstitial regions and around

the glomeruli. TLR-4 expression was mainly detected in the proximal tubules, tubulointerstitial areas, and podocytes of the glomeruli. In the Cis+Exo group, the staining intensity of TGF-β1, α-SMA, and TLR-4 was significantly reduced, indicating decreased expression levels (Figure 3).

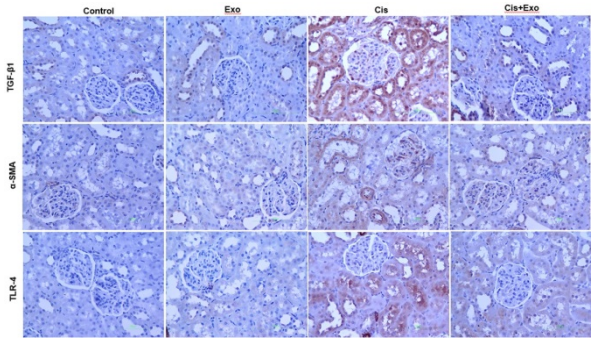


Figure 3.
Immunohistochemical analysis of kidney sections (Nikon Eclipse Si, Tokyo, Japan; X40, scale bar: 100 µm). *Expression patterns of fibrosis-related markers in the kidneys of control and treatment groups. Exosome treatment reduced the expression levels of TGF-β1, α-SMA, and TLR-4 in the kidneys of cisplatin-induced rats. Immunolabeling was performed using the avidin-biotin peroxidase method. TGF-β1: Transforming growth factor beta 1; α-SMA: Alpha-smooth muscle actin; TLR-4: Toll-like receptor 4.*

TGF-β immunoreactivity, showed a significant increase in the Cis group compared to both the Control and Exo groups ($p<0.001$). In the Cis+Exo group, which received Exo treatment after Cis administration, a significant reduction in TGF-β immunoreactivity was observed compared to the Cis group ($p<0.001$). TGF-β promotes the differentiation of fibroblasts into myofibroblasts by increasing α-SMA expression. The expression of α-SMA, was significantly higher in the Cis group compared to the Control and Exo groups ($p<0.001$). Although a significant decrease in α-SMA expression was observed in the Cis+Exo group compared to the Cis group ($p<0.05$), the comparison with the Control group still showed a significant difference ($p<0.001$), indicating that the reduction was not sufficient to reach baseline levels. The TLR-4 marker, also showed a significant increase in the Cis group compared to the Control group ($p<0.001$). A significant decrease in TLR-4 expression was detected in the Cis+Exo group compared to the Cis group (**$p<0.01$). (Figure 4)

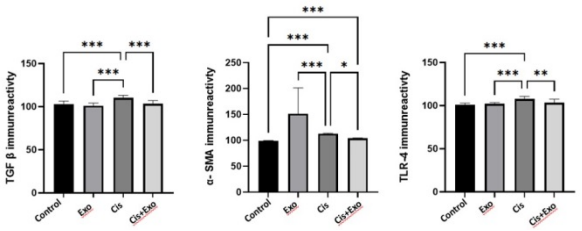


Figure 4.
Immunoreactivity intensity of fibrosis-related proteins. *Effect of Exo on TGF-β, α-SMA, and TLR-4 expression in renal tissue of cisplatin-treated rats. *$p<0.05$, **$p<0.01$, ***$p<0.001$.*

Discussion and Conclusion

Although Cis exhibits strong antitumoral activity and is widely used in the treatment of various types of cancer, it also causes nephrotoxicity[27,28]. Nephrotoxicity is the most significant side effect of cisplatin, observed in 30–40% of treated patients[29]. The histopathological and immunohistochemical findings of this study clearly demonstrated the harmful effects of Cis on kidney tissue through the mechanism of fibrosis. These findings are consistent with previous studies reporting that cisplatin activates apoptosis, autophagy, and inflammatory pathways, leading to irreversible damage in renal cells[27–32]. Commonly reported histological indicators of cisplatin-induced nephrotoxicity include tubular dilatation, cellular necrosis, intraluminal cellular debris, interstitial inflammatory infiltration, and fibrosis.

Numerous studies have demonstrated that bone marrow-derived MSC Exo possesses therapeutic potential for tissue damage and degenerative diseases[33,34]. Depending on their source, Exo vary in content and act as extracellular organelles playing paracrine/endocrine roles by transferring proteins, microRNAs, and enzymes to target cells, thus mediating cell-to-cell communication. MSC-derived Exo have been characterized in many studies for their anti-apoptotic, anti-inflammatory, and regenerative properties[33,35–37].

The chemotherapeutic agent Cis disrupted the kidney histoarchitecture and induced fibrosis. Nephrotoxicity observed in the Cis group was characterized by degenerative changes in the tubular epithelium, necrotic cells, hydropic degeneration, tubular dilatation, and vasculer hyperemia in both glomerular and intertubular regions. Similar histopathological alterations have been reported in other studies investigating nephrotoxicity[27,38,39]. Following a single dose of Exo treatment, a distinct regenerative effect was observed, significantly alleviating nephrotoxicity. Cisplatin-induced fibrosis was associated with the activation of genes promoting fibrosis, leading to an

increase in fibrosis-related proteins. Exosome administration modulated these fibrotic pathways at the genetic level, resulting in a significant reduction in fibrosis protein expression. Furthermore, the findings reported in histopathological scoring, including histopathological improvements in tubular structure, reduction in necrosis and decrease in collagen/glycogen accumulation, support the antifibrotic and protective effects of Exo treatment.

Exo are highly effective paracrine components with significant potential for repairing damaged tissues, playing a critical role in cell-to-cell communication[2-5,24,26]. Exo facilitate intercellular communication by transferring their contents proteins, microRNAs, and enzymes to target cells. Thus, as extracellular organelles, Exo perform paracrine and endocrine functions[40]. Exosomal proteins and microRNAs are functionally complex and are involved in various biochemical and cellular processes, including communication, structural and mechanical functions, inflammation, exosome biogenesis, tissue repair and regeneration, and metabolism[41]. In the present study, Exo treatment substantially reduced tissue damage associated with Cis-induced nephrotoxicity. Since exosome uptake is dependent on intracellular and microenvironmental acidity and since tissue injury is often associated with tissue acidosis Exo are preferentially endocytosed by cells within injured tissues. This suggests that damaged tissues may selectively host Exo[37]. Moreover, the capacity of Exo to trigger appropriate cellular responses during tissue injury is likely influenced by the biochemical potential of their protein and RNA cargo and the maintenance of tissue microenvironment homeostasis[38]. Exo that reach the site of injury have been shown to reduce oxidative stress through their anti-apoptotic, anti-cancer, and antioxidant properties[41,42].

Oxidative stress and tissue acidosis induced by Cis in the kidney tissue activated apoptotic mechanisms. During apoptosis, as cell death occurs, signals released from fragmented cells activate immune cells and fibroblasts, initiating the tissue repair process. However, if cell death becomes chronic or if the inflammatory response is excessive, fibroblasts may overproduce collagen, leading to the replacement of normal tissue with rigid and dysfunctional fibrotic tissue[43]. Previous studies have shown that antitumor agents, including cisplatin, can induce apoptosis and subsequently initiate fibrotic responses through persistent inflammation and tissue remodeling processes[44,45]. In this study, cisplatin-induced fibrosis in the kidney was identified by the increased expression of fibrosis-related proteins such as TGF-β, α-SMA, and TLR-4. A single dose of Exo treatment significantly contributed to tissue repair by modulating the fibrosis induced by Cis. This suggests that Exo can modulate profibrotic pathways, possibly by interrupting TGF-β–mediated signaling cascades, which not only drive myofibroblast activation and ECM accumulation but are also amplified by inflammatory responses that further enhance TGF-β expression[13]. Interestingly, α-SMA immunoreactivity in the Exo-only group was higher than in both the Cis and Cis+Exo groups, as seen in Figure 4. While unexpected, this elevation may reflect a physiological myofibroblast presence involved in homeostatic repair or cytoskeletal remodeling rather than pathological fibrosis. Previous reports suggest that α-SMA can transiently increase during normal tissue regeneration processes, especially under the influence of extracellular vesicles containing growth factors or RNA species[46]. Therefore, the elevated α-SMA in the Exo group might indicate regenerative activation, distinct from the sustained fibrotic remodeling seen in the Cis group. The elevated expression of TLR-4 in the Cis group supports its established role in mediating inflammation-driven fibrosis. By activating downstream NF-κB and TGF-β signaling pathways, TLR-4 amplifies fibrotic responses in the kidney[47], a process that appears to be significantly modulated by Exo treatment in our study.

These observations are consistent with earlier studies demonstrating that MSC-derived exosomes can reduce oxidative stress, suppress proinflammatory cytokines, and modulate fibrotic mediators in kidney injury models[45,48-50]. However, the current study provides direct molecular and histological evidence for the antifibrotic effects of exosomes in a cisplatin-induced nephrotoxicity model. Taken together, these findings highlight the dual role of Exo in mitigating both oxidative and fibrotic damage. Nevertheless, the complex regulatory behavior of markers like α-SMA warrants further investigation to distinguish between regenerative and pathological myofibroblast activation.

This study provides novel and compelling evidence that cisplatin-induced nephrotoxicity extends beyond acute tubular injury, initiating a cascade of events that culminate in progressive fibrotic remodeling a dimension often overlooked in previous in vivo models. Specifically, Exo treatment significantly suppressed oxidative stress-induced tissue damage and attenuated key fibrotic markers such as TGF-β, α-SMA, and TLR-4, indicating a dual mechanism of protection and fibrosis modulation. These results highlight MSC-Exo not only as cytoprotective agents but also as promising antifibrotic therapeutics with potential applications in chronic kidney injury and fibrotic diseases. However, the study is limited by the use of a single animal model and a one-time Exo administration, which may not fully capture the long-term therapeutic potential or dosing dynamics required for clinical translation. Future research should focus on elucidating the molecular pathways underlying the

antifibrotic effects of Exos, optimizing dosing strategies, and evaluating their efficacy in chronic and diverse models of renal fibrosis to better inform their translational potential.

Researcher Contribution Statement:
Idea and design: A.Y., H.T.Y.; Data collection and processing: Ö.C.M, E.K., N.B., Z.B.G., G.Ö.Ö.; Analysis and interpretation of data: H.T.Y., K.T.K.; Writing of significant parts of the article: H.T.Y.
Support and Acknowledgement Statement:
The studies included in this article were supported by Kırşehir Ahi Evran University Scientific Research Project. Project no: TIP.A2.24.006.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Kırşehir Ahi Evran Animal Experiments Local Ethics Committee
Approval Date: 07.02.2024
Decision No: 24/030

References

1. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018;19:213–28. https://doi.org/10.1038/nrm.2017.125.
2. Liu S, Wu X, Chandra S, et al. Extracellular vesicles: Emerging tools as therapeutic agent carriers. Acta Pharm Sin B. 2022;12(10):3822–42. https://doi.org/10.1016/j.apsb.2022.05.002.
3. Chen YF, Luh F, Ho YS, et al. Exosomes: a review of biologic function, diagnostic and targeted therapy applications, and clinical trials. J Biomed Sci. 2024;31:67. https://doi.org/10.1186/s12929-024-01055-0 .
4. Yu H, Feng H, Zeng H, et al. Exosomes: The emerging mechanisms and potential clinical applications in dermatology. Int J Biol Sci. 2024;20(5):1778–95. https://doi.org/10.7150/ijbs.92897.
5. Dai J, Su Y, Zhong S, et al. Exosomes: key players in cancer and potential therapeutic strategy. Signal Transduct Target Ther. 2020;5(1):145. https://doi.org/10.1038/s41392-020-00261-0 .
6. Hall JE. Guyton & Hall Textbook of Medical Physiology. 14th ed. Philadelphia: Elsevier; 2021.
7. Qi L, Luo Q, Zhang Y, et al. Advances in toxicological research of the anticancer drug cisplatin. Chem Res Toxicol. 2019;32(8):1469–86.
8. Gheorghe-Cetean S, Cainap C, Oprean L, et al. Platinum derivatives: a multidisciplinary approach. J Balkan Union Oncol. 2017;22:568–77.
9. Chabner BA, Ryan DP, Paz-Ares L, et al. Antineoplastic agents. In: Hardman JG, Limbird LE, editors. The Pharmacological Basis of Therapeutics. 10th ed. New York: McGraw-Hill; 2001. p. 1432–4.
10. Crona DJ, Faso A, Nishijima TF, et al. A systematic review of strategies to prevent cisplatin-induced nephrotoxicity. Oncologist. 2017;22(5):609–19.
11. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: a review. Am J Med Sci. 2007;334(2):115–24.
12. Zhou D, Fu H, Zhang L, et al. Tubule-derived Wnts are required for fibroblast activation and kidney fibrosis. J Am Soc Nephrol. 2017;28:2322–36. https://doi.org/10.1681/ASN.2016080902 .
13. Webster AC, Nagler EV, Morton RL, et al. Chronic kidney disease. Lancet. 2017;389:1238–52. https://doi.org/10.1016/S0140-6736(16)32064-5.
14. Sarıbaş GS, Tozak Yıldız H, Gorgulu O. Ellagic acid inhibits TGFβ1/smad-induced renal fibrosis in diabetic kidney injury. Duzce Med J. 2022;24(3):321–7. https://doi.org/10.18678/dtfd.1198021.
15. Wang Y, Xu R, Yan Y, et al. Exosomes-mediated signaling pathway: a new direction for treatment of organ ischemia-reperfusion injury. Biomedicines. 2024;12(2):353. doi:10.3390/biomedicines12020353.
16. Yoon SY, Yoon JA, Park M, et al. Recovery of ovarian function by human embryonic stem cell derived mesenchymal stem cells in cisplatin-induced premature ovarian failure in mice. Stem Cell Res Ther. 2020;11(1):255.
17. Li P, Ou Q, Shi S, Shao C. Immunomodulatory properties of mesenchymal stem cells/dental stem cells and their therapeutic applications. In: Cellular and Molecular Immunology. Vol. 20. Springer Nature; 2023. p. 558–69.
18. Zhang M, Zang X, Wang M, et al. Exosome-based nanocarriers as bio-inspired and versatile vehicles for drug delivery: recent advances and challenges. J Mater Chem B. 2019;7:2421–33. https://doi.org/10.1039/C9TB00170KZ.
19. Fan Z, Jiang C, Wang Y, et al. Engineering exosomes for targeted drug delivery. Nanoscale Horiz. 2022;7:682–714. https://doi.org/10.1039/D2NH00070A.
20. Helwa I, Cai J, Drewry MD, et al. A comparative study of serum exosome isolation using differential ultracentrifugation and three commercial reagents. PLoS One. 2017;12(1):e0170628.
21. Abdel-Latif R, Fathy M, Anwar HA, et al. Cisplatin-induced reproductive toxicity and oxidative stress: ameliorative effect of kinetin. Antioxidants (Basel). 2022;11(5):863. doi:10.3390/antiox11050863.
22. Sezer G, Yay AH, Sarica ZS, Gonen ZB, Onder GO, Alan A, Yilmaz S, Saraymen B, Bahar D. Bone marrow-derived mesenchymal stem cells alleviate paclitaxel-induced mechanical allodynia in rats. J Biochem Mol Toxicol. 2022 Dec;36(12):e23207. doi: 10.1002/jbt.23207. Epub 2022 Sep 2. PMID: 36052563.
23. Antes T, et al. Methods for microvesicle isolation and selective removal. Patent No.: US 9,005,888 B.
24. Bahar D, Gonen ZB, Gumusderelioglu M, et al. Repair of rat calvarial bone defect by using exosomes of umbilical cord-derived mesenchymal stromal cells embedded in chitosan/hydroxyapatite scaffolds. Int J Oral Maxillofac Implants. 2022;37(5):943–50.
25. Gibson-Corley KN, Olivier AK, Meyerholz DK. Principles for valid histopathologic scoring in research. Vet Pathol. 2013;50(6):1007–15. https://doi.org/10.1177/0300985813485099.
26. Kalkan KT, Tozak Yıldız H, Yalçın B, et al. Therapeutic effects of apilarnil, a bee product, on apoptosis and autophagy in cisplatin-induced rat ovarian toxicity. Food Chem Toxicol. 2025;115594. doi:10.1016/j.fct.2025.115594.
27. McSweeney KR, Gadanec LK, Qaradakhi T, et al. Mechanisms of cisplatin-induced acute kidney injury: pathological mechanisms, pharmacological interventions, and genetic mitigations. Cancers. 2021;13(7):1572. https://doi.org/10.3390/cancers13071572.
28. Sueishi K, Mishima K, Makino K, et al. Protection by a radical scavenger edaravone against cisplatin-induced nephrotoxicity in rats. Eur J Pharmacol. 2002;451(3):203–8. https://doi.org/10.1016/S0014-2999(02)02183-1 .
29. Volarevic V, Djokovic B, Jankovic MG, et al. Molecular mechanisms of cisplatin-induced nephrotoxicity: a balance on the knife edge between renoprotection and tumor toxicity. J Biomed Sci. 2019;26(1):25. doi:10.1186/s12929-019-0518-9.

30. Dasari S, Njiki S, Mbemi A, et al. Pharmacological effects of cisplatin combination with natural products in cancer chemotherapy. Int J Mol Sci. 2022;23(3):1532. https://doi.org/10.3390/ijms23031532.
31. Sallam AO, Rizk HA, Emam MA, et al. The ameliorative effects of L-carnitine against cisplatin-induced gonadal toxicity in rats. Pak Vet J. 2021;41(1):147–51. https://doi.org/10.29261/pakvetj/2020.082.
32. Moradi M, Goodarzi N, Faramarzi A, et al. Melatonin protects rats' testes against bleomycin, etoposide, and cisplatin-induced toxicity via mitigating nitro-oxidative stress and apoptosis. Biomed Pharmacother. 2021;138:111481. https://doi.org/10.1016/j.biopha.2021.111481.
33. Lai RC, Yeo RW, Lim SK. Mesenchymal stem cell exosomes. Semin Cell Dev Biol. 2015;40:82–8. https://doi.org/10.1016/j.semcdb.2015.03.001.
34. Chen B, Li Q, Zhao B, et al. Stem cell-derived extracellular vesicles as a novel potential therapeutic tool for tissue repair. Stem Cells Transl Med. 2017;6(9):1753–8. https://doi.org/10.1002/sctm.17-0094 .
35. Xie Y, Chen Y, Zhang L, et al. The roles of bone-derived exosomes and exosomal microRNAs in regulating bone remodelling. J Cell Mol Med. 2017;21(5):1033–41. https://doi.org/10.1111/jcmm.13037.
36. Lai RC, Tan SS, Teh BJ, et al. Proteolytic potential of the MSC exosome proteome: Implications for an exosome-mediated delivery of therapeutic proteasome. Int J Proteomics. 2012;2012:971907. https://doi.org/10.1155/2012/971907.
37. Parolini I, Federici C, Raggi C, et al. Microenvironmental pH is a key factor for exosome traffic in tumor cells. J Biol Chem. 2009;284(49):34211–22. https://doi.org/10.1074/jbc.M109.041152.
38. Sung CY, Hayase N, Yuen PS, et al. Macrophage depletion protects against cisplatin-induced ototoxicity and nephrotoxicity. Sci Adv. 2024;10(30):eadk9878. https://doi.org/10.1126/sciadv.adk9878.
39. Lee B, Kim YY, Jeong S, et al. Oleanolic acid acetate alleviates cisplatin-induced nephrotoxicity via inhibition of apoptosis and necroptosis in vitro and in vivo. Toxics. 2024;12(4):301. https://doi.org/10.3390/toxics12040301.
40. Xie Y, Chen Y, Zhang L, et al. The roles of bone-derived exosomes and exosomal microRNAs in regulating bone remodelling. J Cell Mol Med. 2017;21(5):1033–41. https://doi.org/10.1111/jcmm.13037.
41. Arslan F, Lai RC, Smeets MB, et al. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress, and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. Stem Cell Res. 2013;10(3):301–12. https://doi.org/10.1016/j.scr.2013.01.002.
42. Guo X, Zhai J, Xia H, et al. Protective effect of bone marrow mesenchymal stem cell-derived exosomes against the reproductive toxicity of cyclophosphamide is associated with the p38MAPK/ERK and AKT signaling pathways. Asian J Androl. 2021;23(4):386–91. https://doi.org/10.4103/aja.aja_98_20.
43. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity. 2016;44(3):450–62. https://doi.org/10.1016/j.immuni.2016.02.015.
44. Ye L, Wang D, Yang M, et al. Chemotherapy effect on myocardial fibrosis markers in patients with gynecologic cancer and low cardiovascular risk. Front Oncol. 2023;13:1173838. https://doi.org/10.3389/fonc.2023.1173838.
45. Zhang S, Liu Q, Chang M, et al. Chemotherapy impairs ovarian function through excessive ROS-induced ferroptosis. Cell Death Dis. 2023;14(5):340. https://doi.org/10.1038/s41419-023-05859-0.
46. Brennan MÁ, Layrolle P, Mooney DJ. Biomaterials functionalized with MSC secreted extracellular vesicles and soluble factors for tissue regeneration. Adv Funct Mater. 2020;30(37):1909125. doi:10.1002/adfm.201909125.
47. Vijayakumar G, Latha A, Anil AP, et al. Cell autonomous TLR4 signaling modulates TGF-β induced activation of human cardiac fibroblasts. Heliyon. 2025;11(4): e42452.
48. Yang X, Liu J, Yin Y, et al. MSC-EXs inhibits uranium nephrotoxicity by competitively binding key proteins and inhibiting ROS production. Ecotoxicol Environ Saf. 2025;289:117654. https://doi.org/10.1016/j.ecoenv.2024.117654.
49. Oz Oyar E, Aciksari A, Azak Pazarlar B, et al. The therapeutical effects of damage-specific stress induced exosomes on the cisplatin nephrotoxicity in vivo. Mol Cell Probes. 2022;66:101861. https://doi.org/10.1016/j.mcp.2022.101861.
50. Kang X, Chen Y, Xin X, et al. Human amniotic epithelial cells and their derived exosomes protect against cisplatin-induced acute kidney injury without compromising its antitumor activity in mice. Front Cell Dev Biol. 2022;9:752053. https://doi.org/10.3389/fcell.2021.752053.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 233-237, 2025
DOI: https://doi.org/10.32708/uutfd.1701735

ORIGINAL RESEARCH

Assessment of Sexual Dysfunction in Female Kidney Transplant Patients Using the Arizona Sexual Experiences Scale

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ABSTRACT

This study aimed to investigate the prevalence of sexual dysfunction (SD) and its subcategories using the Arizona Sexual Experiences Scale (ASEX) in women undergoing kidney transplantation (KTx) and to compare the prevalence of SD before and after KTx. This study included 70 female KTx patients. Patient characteristics, chronic diseases, and medications were obtained from electronic records. ASEX was used to assess SD. The median age of our patients was 39 (23-66) years. Diabetes mellitus was diagnosed in 11.4% of our patients. In total, 97% of patients were receiving prednisolone therapy. Among patients receiving immunosuppressive therapy, 58.6% were receiving mycophenolic acid, 20% mycophenolate mofetil, 18.6% azathioprine, 57.1% tacrolimus, 34.3% cyclosporine, 2.9% sirolimus, and 4.3% everolimus. The SD rate as assessed by the ASEX was 24.3%. No significant difference was found between the current and previous ASEX subgroup and total scores when the scores of our patients were compared. We observed a decrease in the SD rate of 27.4% to 24.3% after KTx in our patients. In our study, sexual dysfunction showed a slight, non-significant decrease following kidney transplantation. In conclusion, kidney transplantation may reduce the incidence of SD and improve patient well-being among renal replacement therapy patients.

Keywords: ASEX. Kidney transplantation. Sexual dysfunction.

Böbrek Nakli Yapılan Kadın Hastalarda Cinsel işlev Bozukluğu'nun Arizona Cinsel Yaşantılar Ölçeği ile Değerlendirilmesi

ÖZET

Bu çalışma böbrek nakli (KTx) yapılan kadınlarda Arizona Sexual Experiences Scale (ASEX) ile cinsel işlev bozukluğu sıklığı (SD), SD alt başlıklarını araştırmayı ve KTx öncesi ve sonrası SD sıklığını karşılaştırmayı amaçlamıştır. Çalışmaya 70 KTx yapılan kadın hasta dahil edildi. Hastaların karakteristik özellikleri, kronik hastalıkları, kullandıkları ilaçlar elektronik kayıtlardan elde edildi. SD değerlendirilmesi için ASEX kullanıldı. Hastalarımızın median yaş değeri 39 (23-66) yıldı. Hastalarımızın %11.4'inde diabetes mellitus (DM) tanısı vardı. Hastaların %97'si prednisolon tedavisi alıyordu. İmmünsupresif tedavi alan hastaların %58.6'i mikofenolik asit, %20'si mikofenolat mofetil, %18.6'sı azathioprin, %57.1'i takrolimus, %34.3'ü siklosporin, %2.9'u sirolimus, %4.3'ü everolimus tedavisi alıyordu. ASEX ile değerlendirilen SD oranı, %24.3 olarak saptandı. Hastalarımızın güncel ve önceki döneme ait ASEX alt grup skorları karşılaştırıldığında, alt grup skorları ve toplam skorlar arasında anlamlı fark bulunamadı. Hastalarımızda SD oranının KTx sonrası %27.4'den %24.3'e düştüğü gözlemlendi. Çalışmamızda böbrek nakli sonrası cinsel işlev bozukluğunda hafif, anlamlı olmayan bir azalma görüldü. Sonuç olarak böbrek nakli böbrek replasman tedavisi hastalarında SD insidansını azaltabilir ve hastanın iyilik halini arttırabilir.

Anahtar Kelimeler: ASEX. Böbrek nakli. Cinsel işlev bozukluğu.

Female sexual dysfunction (SD) is a disorder that results from disruptions to any of the physiological processes involved in desire, arousal, and orgasm during the sexual response cycle. SD develops in relation to biological, medical, and psychological factors, and is strongly influenced by a woman's psychological and relational status[1,2]. The prevalence of SD ranges from 26% to 63%[3]. Chronic kidney disease (CKD) can also affect patients' social, economic, and psychological well-being. Sexual dysfunction has been reported in between 20% and 100% of patients with end-stage kidney disease (ESKD)[4,5]. Female CKD patients experience decreased libido, difficulty reaching orgasm, a lack of vaginal lubrication, pain during intercourse, and infertility[6]. In female hemodialysis patients, decreased plasma estrogen levels due to hyperprolactinemia, as well as associated atrophic vaginitis and renal anemia,

Date Received: 26.May.2025
Date Accepted: 2.July.2025

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also contribute to sexual dysfunction[7–12]. Following a successful kidney transplant, the sexual desire of many female patients with ESKD increases significantly alongside an improvement in their serum hormone profile. However, the frequency of sexual activity and sexual satisfaction do not improve as significantly as sexual desire[13]. Many studies have examined the frequency of SD in women receiving renal replacement therapy (RRT) using different scales[14-21]. Previously, in 115 premenopausal female patients receiving RRT, we observed the lowest SD rate with the Arizona Sexual Experiences Scale (ASEX) in kidney transplant (KTx) patients compared with hemodialysis and peritoneal dialysis patients[22]. In the current study, we aimed to investigate the frequency of SD with the ASEX and SD subheadings and to compare the frequency of SD before and after RRT in female patients undergoing KTx.

Materials and Methods

This study was conducted on female patients over 18 years of age who underwent KTx between December 2012 and June 2014 at the Nephrology and Kidney Transplantation Clinic—Polyclinic Units of Bursa Uludağ University Faculty of Medicine Hospital, with the approval of the Bursa Uludağ University Faculty of Medicine Ethics Committee, dated 05/10/2012 and numbered 2012-21/10. This study included patients with a history of kidney transplantation for at least three months and no active psychiatric illness. This study was organized in accordance with Good Clinical Practice and the Declaration of Helsinki. Informed consent was obtained from all participants. A total of 135 KTx patients were screened. Of these, 70 women with KTx who met the inclusion criteria and volunteered were included in this study. The following characteristics of the patients were recorded: age, systolic blood pressure, diastolic blood pressure, weight, body mass index, waist circumference, and hip circumference. Medical information, details of chronic diseases and medications, and creatinine values from the last visit were obtained from patient files and electronic records.

Scales

Sexual function was evaluated using the ASEX[23]. The ASEX is a brief, five-item Likert-type scale developed to efficiently assess sexual dysfunction in clinical populations. The female version of the scale examines sex drive, psychological arousal, physiological arousal (vaginal lubrication), the capacity to reach orgasm, and satisfaction after orgasm. Low scores indicate a strong and satisfying sexual response, while high scores suggest SD. The total score ranges from 5 to 30, with each question scored from 1 to 6. A total score of 19 or above, a score of 5 or above for any individual question, or a score of 4 or above for any three individual questions indicates SD. Soykan[24] conducted a validity and reliability study of the scale in Türkiye. This study compared the frequency of SD in patients with the ASEX, the subheadings of the ASEX, and the frequency of SD before and after renal transplantation.

Statistical analysis

The analysis was performed using the SPSS 13.0 software package. Continuous variables were expressed as median (minimum–maximum) values, and categorical variables were expressed as frequency and related percentage values. The Shapiro–Wilk test was used to evaluate the normality of the data distribution. The Wilcoxon signed-rank test was used to compare changes in the ASEX and subgroups before and after in the KTx groups. A p-value of less than 0.05 was considered significant.

Findings

Characteristic features of patients

The characteristics of our patients are shown in Table I.

Table I. Demographic and clinical characteristics of KTx patients.

Variable	KTx (n= 70) Median (minimum–maximum)
Age, years	39 (23-66)
Systolic BP, mmHg	120 (90-160)
Diastolic BP, mmHg	80 (60-90)
Weight, kg	65 (39-105)
BMI, kg/m²	26.6 (16.6-39.5)
Waist circumference, cm	89 (63-115)
Hip circumference, cm	103.5 (80-160)

KTx: kidney transplantation; BP: blood pressure; BMI: body mass index.

Seventy female kidney transplant patients participated in this study. The median age of our patients was 39 years[23–66]. Patients with KTx and a median CKD history of 156 months had a pre-transplant hemodialysis duration of 30 (1–240) months, a peritoneal dialysis (PD) duration of 42 (6–138) months, and a post-transplant follow-up duration of 33.5 (4–180) months.

Eight patients (11.4%) had diabetes mellitus (DM), ten patients (7%) had hyperlipidemia, and forty-four patients (62.9%) had hypertension (HT). The median creatinine value at the last visit was 1.1 (0.69–3.7) mg/dL.

A total of 68 patients (97.1%) were receiving prednisolone treatment. Of the immunosuppressive drugs, 41 (58.6%) received mycophenolic acid, 14 (20%) received mycophenolate mofetil, 13 (18.6%) received azathioprine, 1 (1.4%) received cyclophosphamide, 40 (57.1%) received tacrolimus, 24 (34.3%) received cyclosporine, 2 (2.9%) received sirolimus, and 3 (4.3%) received everolimus. Patients with HT and DM received appropriate antihypertensive and antidiabetic treatments (51 patients received antihypertensive treatment, and 7 patients received antidiabetic treatment).

ASEX results for the current and previous periods

SD was detected in 17 (24.3%) of our patients with ASEX-current. The subgroup scores for our patients in the current period are provided in Table II. SD was detected in 17 (27.4%) of our patients with ASEX-previous. The subgroup scores of our patients belonging to the previous period are noted in Table II.

Comparison of ASEX results for the current and previous periods

Although the SD rate decreased from 27.4% to 24.3% in our patients, this decrease was not statistically significant ($p > 0.05$).

We compared the current and previous ASEX subgroup scores of our patients. No statistically significant differences were observed in ASEX subscale scores before and after transplantation. (Table II).

Discussion and Conclusion

We evaluated the frequency of SD in KTx patients using the ASEX scale. Subgroup scores on the ASEX scale were determined, and current scores were compared with those in the pre-KTx period. The SD rate assessed by ASEX was found to be 24.3% in our patients. While not statistically significant, we observed a decrease in the SD rate from 27.4% to 24.3% after KTx.

A successful kidney transplant may reduce the incidence of sexual dysfunction in female patients with CKD by improving the effects on the hypothalamic-pituitary axis.[25-27]. In a study by Kurdoğlu et al., in which SD was evaluated using the ASEX in female patients undergoing hemodialysis (HD) or predialysis (PreD), it was shown that the total ASEX scores and scores indicating the capacity to reach orgasm were significantly higher in the PreD and HD groups than in the control group[20]. A previous study conducted at our center reported an SD rate of 18.2% in the KTx group when evaluated using the ASEX; this rate was found to be lower than in HD and PD patients[22]. In a study conducted by Vranjes et al. employing another scale used to assess SD, the Female Sexual Function Index (FSFI), the SD rate was found to be 44.4% in the KTx group[28]. Another study by Pyrgidis et al., also using the FSFI, reported an SD frequency of 63% in KTx patients and 80% in HD patients. Additionally, KTx patients were found to have increased their total FSFI scores by 7.5 points[29]. Higher scores on the FSFI scale indicate better sexual function and a decreased risk of sexual dysfunction[30]. That study emphasized that advanced age and menopause are factors associated with SD; consequently, it was concluded that SD is prevalent among women with end-stage renal disease (ESRD) and that sexual function improves in patients who have undergone kidney transplantation[29]. In a study by Kurtulus et al. involving 23 KTx patients, the SD rate was reported as 73.9%. The same study showed that FSFI scores improved significantly in the KTx group, suggesting that successful KTx can have a positive effect on the sexual lives of women with chronic renal failure. In our study, the SD rate, as determined using the ASEX, was found to be 24.3%. No significant differences were found when comparing the current and previous ASEX subgroup scores of our patients in terms of sexual drive, psychological arousal, physiological arousal, capacity to reach orgasm, satisfaction scores, and total scores. However, the SD rate was found to decrease from 27.4% to 24.3% after KTx. SD showed a slight, non-significant decrease following kidney transplantation.

In their review, Pertuz et al. reported that SD affected between 60% and 80% of female patients. Of the patients in the reviewed studies, 40% to 78% reported an improvement in their overall sexual function after KTx. The reviewed literature suggests that there is a significant improvement in sexual function following

Table II. Comparison of ASEX subscale scores between ASEX-current and ASEX-previous assessments.

KTx/Scores	Sexual Drive	Psychological Arousal	Physiological Arousal	Ability to Reach Orgasm	Satisfaction Feeling	Total Score
ASEX-current (n=70)	3 (1-6)*	3 (1-6)*	3 (1-6)*	3 (1-6)*	2 (1-6)*	13 (5-30)*
ASEX-previous (n=62)	3 (1-6)*	3 (1-6)*	3 (1-6)*	3 (1-6)*	2 (1-6)*	14 (5-30)*
Within-group p-value	p=987	p=0.388	p=0.948	p=0.804	p=0.855	p=0.928
Difference (median, range)	0 ((-4)-4)	0 ((-4)-5)	0 ((-4)-5)	0 ((-4)-5)	0 ((-5)-5)	0 ((-16)-24)

ASEX: Arizona Sexual Experiences Scale; KTx: kidney transplantation; * median (minimum–maximum).

KTx. Kidney transplantation has been reported to have a positive effect on sexual function, particularly by increasing sexual desire and general sexual satisfaction. However, it should be noted that individual factors such as age-related end-stage kidney disease (ESKD), neuroendocrine or metabolic disorders, and immunosuppressive therapies administered after transplantation may affect patients who do not report improvement in sexual function[32]. In addition, the literature emphasizes that psychosocial variables such as quality of life, treatment compliance, relationship satisfaction, and depression also affect sexual dysfunction after renal transplantation[33–35]. In a study by Xiao et al. involving 154 female kidney transplant patients, the prevalence of SD measured by the ASEX was found to be 61.6%, which is higher than the rates reported in the literature. The same study emphasized that avoidance of activity due to graft anxiety after transplantation was one of the factors associated with SD. This study also highlighted that sexual health is often overlooked by healthcare professionals, including physicians, nurses, and nephrologists, particularly during the post-transplant period[36]. Laguerre et al. evaluated SD in female patients undergoing KTx using the FSFI and compared the results with data from the pre-KTx period and at the 6-month, 12-month, and final visits. Although a significant increase in FSFI subgroup scores was reported at six months post-transplant, no significant increase was observed at 12 months post-transplant or at the final visit. This study emphasized that the effect of kidney transplantation on sexual dysfunction was significant in the early period, but that this improvement decreased over time due to confounding factors affecting sexual function, such as age. The median age of our patients was 39 years[23–66]. DM was diagnosed in 11.4% of our patients. Our patients were receiving prednisolone and immunosuppressive treatments (mycophenolic acid, mycophenolate mofetil, azathioprine, tacrolimus, cyclosporine, sirolimus, and everolimus).

This study was limited to single-center data, which is considered an important limitation, as is the heterogeneity of the patients' transplantation times, which limits the generalizability of the findings.

However, our study is valuable in providing real-life data on SD, a topic that is generally ignored in our society and not discussed by patients or prioritized by physicians.

In this study, the ASEX was used to evaluate the frequency of SD, and the SD rate was determined in our patient group. SD showed a slight, non-significant decrease following kidney transplantation. Kidney transplantation may reduce the incidence of SD and improve patient well-being among RRT patients.

Researcher Contribution Statement:
Idea and design: A.E., N.L; Data collection and processing: N.L.; Analysis and interpretation of data: A.E., N.L.; Writing of significant parts of the article: N.L., A.E.
Support and Acknowledgement Statement:
This study received no financial support
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Bursa Uludag University Faculty of Medicine Clinical Research Ethics Committee
Approval Date: 05.10.2012
Decision No: 2012–21/10

References

1. Dalpiaz O, Kerschbaumer A, Mitterberger M, et al. Female sexual dysfunction: a new urogynaecological research field. BJU Int 2008;101(6):717-21.
2. İncesu C. Cinsel islevler ve cinsel işlev bozuklukları. Klinik Psikiyatri 2004;3:3-13.
3. Lewis RW, Fugl-Meyer KS, Corona G, et al. Definitions/epidemiology/risk factors for sexual dysfunction. J Sex Med 2010;7:1598–607.
4. Registry of the Nephrology, Dialysis and Transplantation in Turkey report, 2003.
5. Burgos FJ, Pascual J, Gomez V, et al. Effect of kidney transplantation and cyclosporine treatment on male sexual performance and hormone profile: a prospective study. Transplant Proc 1997;29:227-8.
6. Finkelstein SH, Finkelstein FO. Evaluation of sexual dysfunction in dialysis patients. In: Nissenson AR, Fine RN, editors. Dialysis Therapy. 3rd ed. Philadelphia: Hanley and Belfus; 2002. 368-73.
7. Michaelides N, Humke W. Erfahrungen bei der gynäkologischen Betrteuung von Patientinnen mit chronischer Niereninsuffizienz. Nieren-und Hochdruckkrankheiten 1993;22:187-92.
8. Schaefer RM, Kokot F, Wernze H, Geiger H, Heidland A. Improved sexual function in hemodialysis patients on recombinant erythropoietin: A possible role for prolactin. Clin Nephrol 1989;31:1-5.
9. Fearing MO. Case management of anemic patient. Epoetin alfa: Focus on sexual dysfunction. ANNA J 1992;19:570-1.
10. Imagawa A, Kawanishi Y, Numata A. Is eryhropoetin effective for impotance in dialysis patients. Nephron 1990;54:95-6.
11. Lawrence IG, Price DE, Howlett TA, et al. Erytropoetin and Sexual dysfunction. Nephrol Dial Transplant 1997;12:741-7.
12. Zarifian A. Case study of anemic patient: Epoetin anfa-focus on sexual function. ANNA J 1994;21:368-71.
13. Diemont WL, Vruggink PA, Meuleman EJ, et al. Sexual dysfunction after renal replacement therapy. Am J Kidney Dis 2000;35:845-51.
14. Aslan E, Beji NK, Gungor I, Kadioglu A, Dikencik BK. Prevalence and Risk Factors for Low Sexual Function in Women: A Study of 1009 Women in an Outpatient Clinic of a University Hospital in İstanbul. J Sex Med 2008;5:2044-52.
15. Cayan S, Akbay E, Bozlu M, Canpolat B, Acar D, Ulusoy E. The Prevalance of Female Dysfunction and Potential Risk Factors That May Impair Sexual Function in Turkish Women. Urol Int 2004;72:52-7.
16. Amidu N, Owiredu WK, Woode E, et al. Incidence of sexual dysfunction: a prospective survey in Ghanaian females. Reprod Biol Endocrinol 2010;8:106-11.

17. Lew-Starowicz M, Gellert R. The sexuality and quality of life of hemodialyzed patients--ASED multicenter study. J Sex Med 2009;6:1062-71.
18. Pontiroli AE, Cortelazzi D, Morabito A. Female sexual dysfunction and diabetes: a systematic review and meta-analysis. J Sex Med 2013;10:1044-51.
19. Basok EK, Atsu N, Rifaioglu MM, et al. Assessment of female sexual function and quality of life in predialysis, peritoneal dialysis, hemodialysis, and renal transplant patients. Int Urol Nephrol 2009;41:473-81.
20. Kurdoglu Z, Usul Soyoral Y, Tasdemir M, et al. Evaluation of the relationship between endogenous gonadotropins and female sexual function and psychological status in predialysis and hemodialysis patients. Gynecol Endocrinol 2012 ;28:336-9.
21. Strippoli GF1; Collaborative Depression and Sexual Dysfunction (CDS) in Hemodialysis Working Group, Vecchio M, et al. Sexual dysfunction in women with ESRD requiring hemodialysis. Clin J Am Soc Nephrol 2012;7:974-81.
22. Koca TG, Koca N, Ersoy A. The comparison of the relationship between sociocultural-economic features and sexual dysfunction frequency in sexually active premenopausal female patients on renal replacement therapy. J Sex Med 2012;9:3171-9.
23. McGahuey CA, Gelenberg AJ, Laukes CA, et al. The Arizona Sexual Experience Scale: Validity and reliability, in New Research Program and Abstracts, 150th Annual Meeting of the American Psychiatric Association. Washington, DC, APA, 1997:116.
24. Soykan A. The reliability and validity of Arizona Sexual Experiences Scale in Turkish ESRD patients undergoing hemodialysis. Int J Impot Res 2004;16:531–4
25. Ali, S., & Dave, N. N. (2020). Sexual dysfunction in women with kidney disease. *Advances in Chronic Kidney Disease*, *27*(6), 506-515.
26. Filocamo, M. T., Zanazzi, M., Li Marzi, V., Lombardi, G., Del Popolo, G., Mancini, G., ... & Nicita, G. (2009). Sexual dysfunction in women during dialysis and after renal transplantation. *The journal of sexual medicine*, *6*(11), 3125-3131.
27. Wang, G. C., Zheng, J. H., Xu, L. G., Min, Z. L., Zhu, Y. H., Qi, J., & Duan, Q. L. (2010). Measurements of serum pituitary-gonadal hormones and investigation of sexual and reproductive functions in kidney transplant recipients. *International journal of nephrology*, *2010*(1), 612126.
28. Vranješ, I. M., Školka, I., Jakab, J., Krajina, I., Krajina, V., Šantić, A., & Zibar, L. (2022). Sexual function in hemodialysis and post-renal transplant women in a relationship: a cross-sectional study. *International Urology and Nephrology*, *54*(8), 2037-2046.
29. Pyrgidis, N., Mykoniatis, I., Tishukov, M., Sokolakis, I., Nigdelis, M. P., Sountoulides, P., ... & Hatzichristou, D. (2021). Sexual dysfunction in women with end-stage renal disease: a systematic review and meta-analysis. *The journal of sexual medicine*, *18*(5), 936-945.
30. Rosen R, Brown C, Heiman J, et al. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther 2000;26:191-208.
31. Kurtulus, F. O., Salman, M. Y., Fazlioglu, A., & Fazlioglu, B. (2017, November). Effects of renal transplantation on female sexual dysfunction: comparative study with hemodialysis and a control group. In *Transplantation proceedings* (Vol. 49, No. 9, pp. 2099-2104). Elsevier.
32. Pertuz, W., Castaneda, D. A., Rincon, O., & Lozano, E. (2014, November). Sexual dysfunction in patients with chronic renal disease: does it improve with renal transplantation?. In *Transplantation proceedings* (Vol. 46, No. 9, pp. 3021-3026). Elsevier.
33. Einollahi, B., Tavallaii, S. A., Bahaeloo-Horeh, S., Omranifard, V., Salehi-Rad, S., & Khoddami-Vishteh, H. R. (2009). Marital relationship and its correlates in kidney recipients. *Psychology, health & medicine*, *14*(2), 162-169.
34. Kenawy, A. S., Gheith, O., Al-Otaibi, T., Othman, N., Abo Atya, H., Al-Otaibi, M., & Nagy, M. S. (2019). Medication compliance and lifestyle adherence in renal transplant recipients in Kuwait. *Patient preference and adherence*, 1477-1486.
35. Mota, R. L., Fonseca, R., Santos, J. C., Covita, A. M., Marques, N., Matias, P., ... & Cardoso, J. (2019). Sexual dysfunction and satisfaction in kidney transplant patients. *The journal of sexual medicine*, *16*(7), 1018-1028.
36. Xiao, P., Liu, M., Cui, L., Ding, S., Xie, J., & Cheng, A. S. (2021). Sexual dysfunction and activity avoidance in female kidney transplant patients. *Clinical Transplantation*, *35*(8), e14363.
37. Laguerre, M., Bouvier, N., Guleryuz, K., Doerfler, A., Parienti, J. J., Ait Said, K., & Tillou, X. (2021). Sexual Dysfunction Improvement after Kidney Transplantation: A Prospective Study in Men and Women. *International Journal of Sexual Health*, *33*(1), 1-8.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 239-246, 2025
DOI: https://doi.org/10.32708/uutfd.1718121

ORIGINAL RESEARCH

Performance of Generative AI Models on Cardiology Practice in Emergency Service: A Pilot Evaluation of GPT-4.o and Gemini-1.5-Flash

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ABSTRACT

In healthcare, emergent clinical decision-making is complex and large language models (LLMs) may enhance both the quality and efficiency of care by aiding physicians. Case scenario-based multiple choice questions (CS-MCQs) are valuable for testing analytical skills and knowledge integration. Moreover, readability is as important as content accuracy. This study aims to compare the diagnostic and treatment capabilities of GPT-4.o and Gemini-1.5-Flash and to evaluate the readability of the responses for cardiac emergencies. A total of 70 single-answer MCQs were randomly selected from the Medscape Case Challenges and ECG Challenges series. The questions were about cardiac emergencies and were further categorized into four subgroups according to whether the question included a case presentation or an image, or not. ChatGPT and Gemini platforms were used to assess the selected questions. The Flesch–Kincaid Grade Level (FKGL) and Flesch Reading Ease (FRE) scores were utilized to evaluate the readability of the responses. GPT-4.o had a correct response rate of 65.7%, outperforming Gemini-1.5-Flash, which had a 58.6% correct response rate (p=0.010). When comparing by question type, GPT-4.o was inferior to Gemini-1.5-Flash only for non-case questions (52.5% vs. 62.5%, p=0.011). For all other question types, there were no significant performance differences between the two models (p>0.05). Both models performed better on easy questions compared to difficult ones, and on questions without images compared to those with images. Additionally, while GPT-4.o performed better on case questions than non-case questions. Gemini-1.5-Flash's FRE score was higher than GPT-4.o's (median [min-max], 23.75 [0-64.60] vs. 17.0 [0-56.60], p<0.001). Although on the whole GPT-4.o outperformed Gemini-1.5-Flash, both models demonstrated an ability to comprehend the case scenarios and provided reasonable answers.

Keywords: Cardiology. Decision making. Artificial intelligence. GPT-4.o. Gemini-1.5-Flash.

Kardiyak Acil Durumların Yönetiminde ChatGPT ve Gemini

ÖZET

Sağlık hizmetlerinde, acil klinik karar alma karmaşıktır ve büyük dil modelleri (LLM'ler) hekimlere yardımcı olarak hem bakımın kalitesini hem de verimliliğini artırabilir. Vaka senaryosuna dayalı çoktan seçmeli sorular (VS-ÇSS), analitik becerileri ve bilgi bütünleştirmeyi test etmek için değerlidir. Ayrıca, okunabilirlik, içerik doğruluğu kadar önemlidir. Bu çalışma, GPT-4.o ve Gemini-1.5-Flash'ın tanı ve tedavi yeteneklerini karşılaştırmayı ve kardiyak acil durumlar için yanıtların okunabilirliğini değerlendirmeyi amaçlamaktadır. Medscape Vaka Zorlukları ve EKG Zorlukları serilerinden toplam 70 tek cevaplı ÇSS rastgele seçildi. Sorular kardiyak acil durumlarla ilgiliydi ve sorunun bir vaka sunumu veya bir görüntü içerip içermemesine göre dört alt gruba ayrıldı. Seçilen soruları değerlendirmek için CahtGPT ve Gemini platformları kullanıldı. Yanıtların okunabilirliğini değerlendirmek için Flesch-Kincaid Sınıf Düzeyi (FKGL) ve Flesch Okuma Kolaylığı (FRE) puanları kullanıldı. GPT-4.o'nun doğru yanıt oranı %65,7'ydi ve %58,6 doğru yanıt oranına sahip Gemini-1.5-Flash'ı geride bıraktı (p=0,010). Soru türüne göre karşılaştırıldığında, GPT-4.o yalnızca vaka dışı sorularda Gemini-1.5-Flash'tan daha düşüktü (%52,5'e karşı %62,5, p=0,011). Diğer tüm soru türleri için, iki model arasında önemli bir performans farkı yoktu (p>0,05). Her iki model de kolay sorularda zor sorulara göre ve resimsiz sorularda resimli sorulara göre daha iyi performans gösterdi. Ek olarak, GPT-4.o vaka dışı sorulara göre vaka sorularında daha iyi performans gösterdi. Gemini-1.5-Flash'ın FRE puanı GPT-4.o'dan daha yüksekti (ortanca [min-maks], 23.75 [0-64.60] - 17.0 [0-56.60], p<0.001). Her ne kadar toplamda GPT-4.o, Gemini-1.5-Flash'tan daha iyi performans gösterse de, her iki model de durum senaryolarını anlama becerisi gösterdi ve makul yanıtlar sağladı.

Anahtar Kelimeler: Kardiyoloji. Karar verme. Yapay zeka. GPT-4.o. Gemini-1.5-Flash.

Date Received: 12.June.2025
Date Accepted: 2.July.2025

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The advancement of digital technologies has significantly impacted various aspects of daily life, including how people access health information globally. Generative Pre-trained Transformer (GPT) models, designed for deep learning tasks like text generation, language modeling, and text completion, have become essential in this context[1-3]. The integration of deep learning (DL) with natural language processing (NLP) and the availability of large datasets have led to the emergence of large language models (LLMs)[4]. In healthcare, where clinical decision-making is becoming increasingly complex, LLMs have the potential to enhance both the quality and efficiency of care by aiding physicians[5]. Accurate evaluation in emergency departments (EDs) depends on prompt disease diagnosis and treatment. In order to treat patients whose symptoms closely match those of a certain specialty, emergency physicians (EPs) may need the assistance of an attending physician. The consultation procedure can be a significant burden for the medical community due to the 24 hours a day unavailability of cardiologists and other specialists in emergency departments. In addition to needing help reading a patient's ECG, emergency department doctors may occasionally need to consult in order to diagnose and treat a patient.

AI-powered conversational agents can simulate human-like interactions and are useful for delivering medical information. OpenAI introduced ChatGPT, powered by GPT-3.5, in 2022 as a general-purpose AI chatbot[6,7], followed by GPT-4.0 in March 2023 and the more advanced GPT-4.o, which can handle both image and text inputs, in May 2024[8,9]. Google's Gemini, previously known as Bard, is another generative AI chatbot, with studies comparing the performance of ChatGPT and Gemini across various medical specialties[10-16].

Multiple-choice question (MCQ) exams are widely used in educational assessments across many disciplines, including medicine. These questions can either be stand-alone (questioning knowledge, not including a case presentation; named as non-case questions in this study) or based on patient scenarios, which include laboratory results, vital signs, and diagnostic tests (named as case questions in this study). Case scenario-based MCQs (CS-MCQs) are particularly valuable for testing analytical skills, problem-solving abilities and knowledge integration, making them ideal for problem-based learning (PBL)[17]. When evaluating the performance of AI in answering such questions, readability is also as important as content accuracy. Readability can be assessed using objective, quantitative formulas like the Flesch-Kincaid Grade Level (FKGL) and the Flesch Reading Ease (FRE) score[18].

The purpose of this study was to assess the success of using LLMs rather than cardiology consultations for cardiology cases and/or ECG interpretation comparing the diagnostic and treatment capabilities of GPT-4.o and Gemini-1.5-Flash using cardiology MCQs sourced from Medscape which is one of the leading online global destination for physicians and healthcare professionals worldwide providing quick access to medical information in daily practice. Medscape website includes both stand-alone questions and case presentations with and without an ECG image. Additionally, since the readability and verbosity of the responses are important in daily practice, the responses generated by both models were evaluated using the FKGL and FRE metrics[18].

Material and Method

Study design

Medscape provides comprehensive medical information for healthcare professionals, including cardiology-related content. For this study, all cardiology questions from the Medscape Case Challenges[19] and ECG Challenges[20] series were screened to select cardiac emergent issues. Any questions that contained visual elements other than ECG images, such as radiological or clinical images were excluded. A total of 70 freely accessible single-answer MCQs were randomly selected from these sources[19,20]. Alongside the correct answer, real life data regarding the percentage of human respondents who answered correctly were also available. This data allowed us to classify each question as either difficult (correct response rate below 60%) or easy (correct response rate 60% or higher). Questions that included patient history, physical examination, and laboratory findings were categorized as case questions, while others were labeled non-case questions. The questions were further classified as image or non-image based on the inclusion of an ECG image.

The questions were further categorized into four subgroups: those included neither a case presentation nor an image (group 1), those with an image but no case presentation (group 2), those with a case presentation but without an image (group 3), and those containing both a case presentation and an image (group 4).

Evaluation tools:

Both OpenAI's GPT-4.o and Google's Gemini-1.5-Flash were used to assess the selected questions. Each question was inputted identically into both platforms only once, and their responses were categorized as correct or incorrect based on Medscape's answer key. Additionally, the percentage of human respondents who answered correctly was noted for each question.

The Flesch–Kincaid Grade Level (FKGL) and Flesch Reading Ease (FRE) scores were utilized to evaluate the readability and verbosity of the responses generated by GPT-4.o and Gemini-1.5-Flash. The FKGL estimates the reading grade level of a text, while the FRE assigns a score between 1 and 100, with higher scores indicating easier readability (Table I). Both metrics account for the number of sentences, words, and syllables in the text to measure verbosity. The FKGL is calculated using the formula: FKGL = (0.39 × [Total Words / Total Sentences] + 11.8 × [Total Syllables / Total Words] − 15.59), and the FRE score is determined using the equation: FRE = (206.835 − 1.015 × [Total Words / Total Sentences] − 84.6 × [Total Syllables / Total Words]). The number of words, syllables, and sentences in each text was automatically calculated using the website https://readability-score.com/18, 21, 22.

Table I. Interpretation of Flesch Reading Ease Score (22)

Flesch Reading Ease Score	Readability Level	Estimated Reading Grade
0-30	very difficult	college graduate
30-50	difficult	college
50-60	fairly difficult	10th to 12th grade
60-70	standard	8th or 9th grader
70-80	fairly easy	7th grader
80-90	easy	6th grader
90-100	very easy	5th grader

Statistical Analyses:

The normality of the data was tested using the Shapiro-Wilk test. Normally distributed data were presented with mean±standard deviation while non-normal data presented with median (minimum-maximum) values. For non-normally distributed variables, the Mann-Whitney U test was employed to compare two independent groups. To compare two depedendent groups paired t test was used for normally disributed data, while the Wilcoxon test was used for non-normal data. Categorical variables were analyzed using Pearson chi-square and Fisher's exact chi-square tests, with the data reported as n(%). A significance level of 0.05 was considered for two-sided hypothesis tests. All statistical analyses were conducted using IBM SPSS Statistics (Version 28.0, IBM Corp, Armonk, NY).

Results

The study included 70 randomly selected questions from the Medscape Case Challenges and ECG Challenges[19,20]. The questions represented various types: some included a case presentation or image, while others did not. Additionally, they were categorized by difficulty, with 36 hard and 34 easy questions, 30 case questions, 40 non-case questions, 31 image questions, and 39 non-image questions. GPT-4.o had a correct response rate of 65.7%, outperforming Gemini-1.5-Flash, which had a 58.6% correct response rate—a significant difference of 7.1% (p=0.010, Table II). When comparing by question type, GPT-4.o was inferior to Gemini-1.5-Flash only for non-case questions (52.5% vs. 62.5%, p=0.011). For all other question types, there were no significant performance differences between the two models (p>0.05, Table III).

Table II. Comparison of GPT-4.o and Gemini-1.5-Flash for all of the questions

	GPT-4.o (n,%)	Gemini-1.5-Flash (n,%)	p value
Correct	46 (65.7)	41 (58.6)	0.010
Incorrect	24 (34.3)	29 (41.4)	

Table III. Distribution of correct response percentage of GPT-4.o and Gemini-1.5-Flash according to questions types

Question type		GPT-4.o n (%)	Gemini-1.5-Flash n (%)	p-value
Difficulty level	Hard	17 (47.2)	16 (44.4)	0.332
	Easy	29 (85.3)	25 (73.5)	0.102
Case presentation	Case	25 (83.3)	16 (53.3)	0.157
	Non-case	21 (52.5)	25 (62.5)	**0.011**
Image presence	Image	11 (35.5)	14 (45.2)	0.153
	Non-image	35 (89.7)	27 (69.2)	0.573

Both models performed better on easy questions compared to difficult ones, and on questions without images compared to those with images. Additionally, while GPT-4.o performed better on case questions than non-case questions, the presence of a case presentation had no impact on Gemini's performance. Detailed comparison results for each question type are displayed in Figures 1 and 2 for GPT-4.o and Gemini-1.5-Flash, respectively.

When the questions were divided into subgroups based on the presence of a case or image, the group 1 (without a case presentation and an image) contained 18 questions, group 2 (with an image, without a case presentation) contained 22 questions, group 3 (with a case presentation, without an image) contained 21 questions and group 4 (with a case presentation and an image) contained 9 questions. GPT-4.o provided 46 correct answers, while Gemini-1.5-Flash provided 41 correct answers. These responses are distributed across the subgroups, as shown in Figure 3. A significant difference was found in GPT-4.o's correct response rate across the subgroups (p<0.001), but no

significant difference was found for Gemini-1.5-Flash (p=0.076, Table IV). Pairwise comparisons revealed that groups 1 vs. 2, 2 vs. 3, and 2 vs. 4 had significant differences (p<0.001, p<0.001, and p=0.038, respectively). Group 2 (with an image, without a case presentation) showed the lowest performance from GPT-4.o.

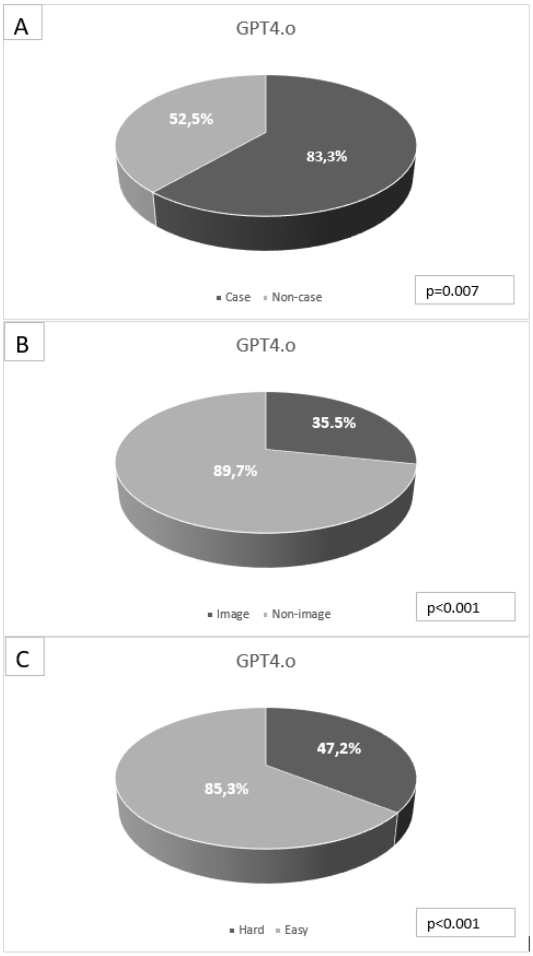

Figure 1:
Comparisons of correct response percentages of GPT-4.o for each question type

Table IV. Comparison of GPT-4.o and Gemini-1.5-Flash among question subgroups regarding correct response percentage

		GPT-4.o n (%)	Gemini-1.5-Flash n (%)
Question subgroup	Group 1	16 (34.8)	15 (36.6)
	Group 2	5 (10.9%)	10 (24.4%)
	Group 3	19 (41.3%)	12 (29.3%)
	Group 4	6 (13.0%)	4 (9.8%)
p-value		<0.001	0.076

Group 1; without a case presentation and an image, Group 2; with an image, without a case presentation, Group 3; with a case presentaion, without an image, Group 4; with an image and a case presentation

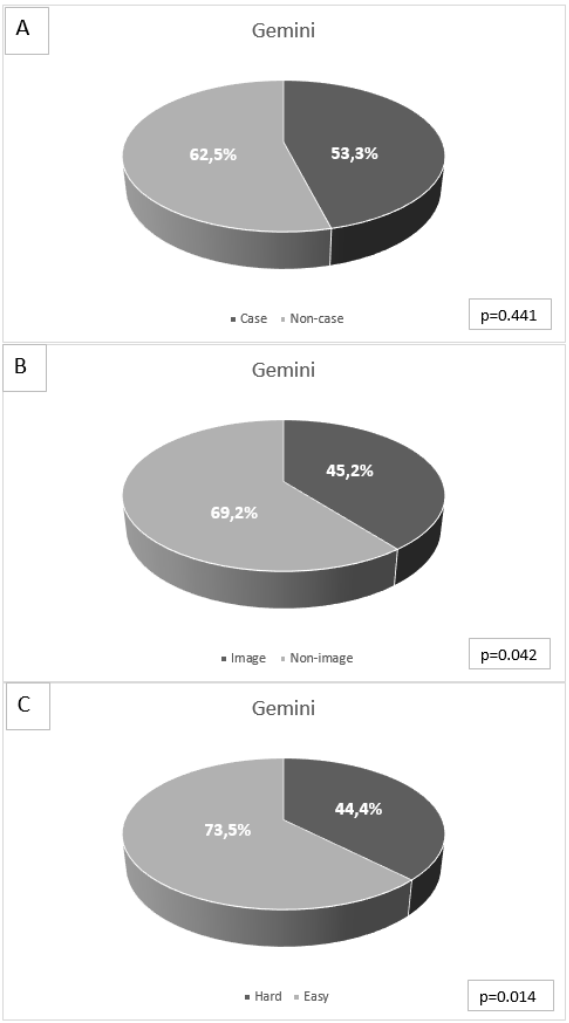

Figure 2:
Comparisons of correct response rates of Gemini-1.5-Flash for each question type

None of the questions answered correctly by GPT-4.o or Gemini-1.5-Flash were answered correctly by all human participants. However, some human respondents answered questions correctly that both AI models answered incorrectly. The median (minimum-maximum) correct response rate for human participants across the 70 questions was 58.50% (15.00-94.00). Human participants performed better on questions where GPT-4.o gave the correct answer (median [min-max], 63.50% [15.00-94.00]) compared to those where GPT-4.o answered incorrectly (median [min-max], 47.50% [17.00-74.00], p<0.001). A similar difference was found for Gemini-1.5-Flash's correct and incorrect answers in terms of human performance (p=0.001, Table V).

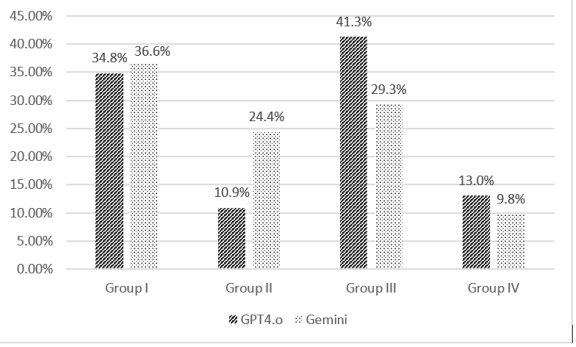

Figure 3:
The distribution of correct responses given by GPT-4.o (n=46) and Gemini-1.5-Flash (n=41) into subgroups (Group 1; without a case presentation and an image, Group 2; with an image, without a case presentation, Group 3; with a case presentaion, without an image, Group 4; with an image and a case presentation)

Table V. Comparison of the correct response percentage of human participants in questions answered correctly and incorrectly by GPT 4.o and Gemini-1.5-Flash

	correct	incorrect	p- value
GPT-4.o	63.5 (15-94)	47.5 (17-74)	<0.001
Gemini-1.5-Flash	63 (38-94)	47 (15-92)	0.001

Data presented as median (minimum-maximum)

Regarding readability and verbosity, GPT-4.o had a higher FKGL than Gemini-1.5-Flash (mean ± SD, 14.79 ± 2.76 vs. 13.87 ± 3.56, p=0.023). Both models produced responses with a FRE score below 30, indicating that their texts were at the college graduate level. However, Gemini-1.5-Flash's FRE score was higher than GPT-4.o's (median [min-max], 23.75 [0-64.60] vs. 17.0 [0-56.60], p<0.001, Table VI).

Table VI. Comparison of readability and verbosity of responses given by GPT-4.o and Gemini-1.5-Flash

	GPT-4.o	Gemini-1.5-Flash	p value
FKGL	14.79 ± 2.76	13.87 ±3.56	0.023
FRE score*	17.00 (0-56.60)	23.75 (0-64.60)	<0.001

*Data presented as median (minimum-maximum),
FKGL: Flesch–Kincaid Grade Level, FRE: Flesch Reading Ease

Discussion and Conclusion

This study compared the performance of GPT-4.o and Gemini-1.5-Flash in answering cardiology-related multiple-choice questions. GPT-4.o demonstrated a superior performance, particularly with a correct response rate exceeding the 60% threshold that is typically considered a passing grade for many exams. In contrast, Gemini-1.5-Flash's correct response rate of 58.6% indicated a failure to meet this standard.

In terms of human performance, it is well-documented that CS-MCQs are designed to assess higher-order thinking skills such as analysis, problem-solving, and knowledge integration. These questions challenge students to think beyond isolated medical facts, instead encouraging a holistic view of the patient[23,24]. Case and Swanson (2002) have noted the particular importance of case-based clusters in PBL settings, as they test the practical application of knowledge[25].

In our study, both CS-MCQs and stand-alone questions (non-case questions) were used to assess the models' current level of learning. Stand-alone questions, which do not involve case presentations, mainly test basic recall of medical knowledge. Bhayana et al. found that LLMs performed well on questions requiring lower levels of cognitive processing, such as basic knowledge recall[26]. In our findings, Gemini significantly outperformed GPT-4.o in non-case questions, which could suggest that Gemini's training may have been based on a similar dataset and also that Gemini may not have been able to effectively apply the information it learned to more complex formats such as case-based scenarios.

However, GPT-4.o excelled in case-based questions, indicating its superior ability to apply knowledge beyond mere recall. When analyzing question subgroups, Gemini-1.5-Flash performed consistently across all categories, while GPT-4.o struggled with questions that included images but lacked case details. This suggests that GPT-4.o's training may have emphasized textual over visual elements, with additional textual details facilitating better performance. In contrast, previous studies have shown ChatGPT-4's effectiveness in responding to clinical image-based questions. ChatGPT has been particularly useful in diagnostic decision-making within radiology[27,28], although in our study only ECG images were used, rather than a broader range of radiological images. Group 2 (with an image, without a case presentation) showed the lowest performance from GPT-4.o, indicating that the presence of an image without a case presentation hindered correct responses.

The FKGL and the FRE scores consider the number of sentences and words to determine a text's reading level. Our study results revealed that the responses of both GPT-4.o and Gemini-1.5-Flash were at the level of college graduate. Consistent with our results, in a previous study, ChatGPT's FKGL and FRE scores indicated a hard reading level appropriate for only 33% of adults and those with a college education[29]. Furthermore, the texts produced by ChatGPT were

harder than those from Bard, Gemini's predecessor[30]. Our results show similar characteristics, as Gemini-1.5-Flash's FKGL was significantly lower than GPT-4.o's, and Gemini-1.5-Flash's score was higher than GPT-4.o. Conversely, Atkinson et al. identified that although ChatGPT's responses were consistently accurate, they were somewhat superficial and corresponded to the knowledge level of a trainee[31]. Rizwan et al. reported that if healthcare information was not sufficient, reaching into consistent conclusion based on ChatGPT has proved to be an efficient and effective tool both academically and in clinical setups[32].

AI models like GPT-4.o and Gemini-1.5-Flash generate responses based on patterns in their training data, which means that their answers are probabilistic rather than absolute. While their output can seem authoritative, AI models can produce misinformation or misunderstanding due to their lack of deep, principle-based medical reasoning.

In the field of cardiology, AI has made strides, including more accurate predictions of myocardial infarction (MI) risk than traditional methods and successfully passing the European Exam in Core Cardiology (EECC)[33,34]. However, it remains unclear to what extent these models base on specific medical guidelines such as American College of Cardiology (ACC), American Heart Association (AHA) and European Society of Cardiology (ESC) standards. Also, studies about ECG interpretation performance of AI are present[35-37]. In a previous study it was found that the limited accuracy and consistency of GPT-4 and Gemini suggest that their current use in clinical ECG interpretation is risky[36]. Further research is needed to quantitatively assess AI variability in clinical settings to better understand its reliability.

The ethical implications of LLMs in medicine are significant. In cases where AI provides erroneous advice leading to negative outcomes, questions of liability arise. Notably, Gemini-1.5-Flash warns users that it cannot offer medical advice, while GPT-4.o provides direct responses. Additionally, concerns over data privacy and security are critical, as current LLMs store information on their servers. Therefore, AI applications must warn their users that personal information may be uploaded anonymously. For AI to be fully integrated into clinical practice, it must be able to handle personal patient data securely[38]. AI systems are also subject to biases from their training data, potentially leading to outdated information, unequal care, or even discrimination[39,40].

On the other hand, the integration of artificial intelligence into emergency room practice is of critical importance in many countries due to reasons such as the long waiting times of patients in emergency rooms, the lack of doctors from all departments in emergency rooms 24 hours a day, physician shortage and extraordinary conditions such as the COVID-19 pandemic or earthquakes, where emergency rooms will be visited far beyond their capacity. In the future, as artificial intelligence technology develops, the initial evaluation can be made more comprehensive by using artificial intelligence applications, at least while waiting to reach the relevant branch physician in emergency room consultations.

Study Limitations

This study evaluated GPT-4.o and Gemini-1.5-Flash solely in the English language. While both models are capable of communicating in multiple languages, their performance depends on the quality and amount of training data available in a specific language. Since English is the most common language in AI training, performance in other languages may be lower. Further research is required to evaluate LLM performance across different languages. Additionally, the number of questions in this study was limited. Future studies should include much more questions with diverse question types. Also, in our study, questions were posed to GPT-4o and Gemini-1.5-Flash only once. Therefore, the study does not reflect how performance might vary in repeated questioning. It also does not allow for evaluation of the models' consistency in their responses.

Conclusion:

Both models demonstrated an ability to comprehend the scenarios presented and provided reasonable answers. Despite the limitations and ethical concerns surrounding the use of AI in medicine, it is essential for physicians to remain engaged with ongoing AI research and support its responsible development. The integration of AI could potentially elevate the standards of medical care, education, research, and clinical decision-making in the future. However, AI should not be seen as a replacement for critical thinking, creativity, and innovation—skills that remain uniquely human and are crucial in the medical field.

Acknowledgements: I would like to thank Medscape executives and the editorial coordinator Kennedy Maklos (Schaff), MHCDS for allowing us to use case presentations and ECG questions that are available to healthcare professionals on Medscape's online platform. The manuscript was prepared entirely with no direct assistance from generative artificial intelligence or similar technologies.

Researcher Contribution Statement:
Idea and design: S.G.P., D.S..; Data collection and processing: S.G.P, V.A.D., C.A., I.I.K. Analysis and interpretation of data: D.S., S.G.P.; Writing of significant parts of the article: S.G.P., D.S, V.A.D.

Support and Acknowledgement Statement:
No financial support

Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
This study does not include any human or animal participants. Ethics committee approval is not applicable like the similar studies in literature.

References

1. Labadze L, Grigolia M, Machaidze L. Role of AI chatbots in education: systematic literature review. Int J Educ Technol High Educ. 2023;20(56). doi:10.1186/s41239-023-00416-7
2. Dwivedi YK, Kshetri N, Hughes L, et al. Opinion paper: "So what if ChatGPT wrote it?" Multidisciplinary perspectives on opportunities, challenges and implications of generative conversational AI for research, practice and policy. Int J Inform Manag. 2023;71:102642. doi:10.1016/j.ijinfomgt.2023.102642
3. Yenduri G. GPT (Generative Pre-Trained Transformer)—A comprehensive review on enabling technologies, potential applications, emerging challenges, and future directions. IEEE Access. 2024;12:1-36. doi:10.1109/ACCESS.2024.3389497
4. Hadi MU, Al-Tashi Q, Qureshi R, et al. Large language models: a comprehensive survey of applications, challenges, limitations, and future prospects. Authorea. Preprint. 2023.
5. Johnson D, Goodman R, Patrinely J, et al. Assessing the accuracy and reliability of AI-generated medical responses: an evaluation of the Chat-GPT model. Res Sq. 2023. doi:10.21203/rs.3.rs-2924050/v1
6. Saka A, Taiwo R, Saka N, et al. GPT models in construction industry: opportunities, limitations, and a use case validation. Dev Built Environ. 2024;17:100300. doi:10.1016/j.dibe.2023.100300
7. Urbina F, Lentzos F, Invernizzi C, Ekins S. Dual use of artificial intelligence-powered drug discovery. Nat Mach Intell. 2022;4(3):189-191. doi:10.1038/s42256-022-00480-0
8. OpenAI. GPT-4 Technical Report. 2023. Available at: https://cdn.openai.com/papers/gpt-4.pdf. Accessed June 16, 2025.
9. OpenAI. Introducing GPT-4o and more tools to ChatGPT free users. 2024. Available at: https://openai.com/index/gpt-4o-and-more-tools-to-chatgpt-free/. Accessed June 16, 2025.
10. Chen CH, Hsieh KY, Huang KE, Lai HY. Comparing vision-capable models, GPT-4 and Gemini, with GPT-3.5 on Taiwan's pulmonologist exam. Cureus. 2024;16(8):e67641. doi:10.7759/cureus.67641
11. Masanneck L, Schmidt L, Seifert A, et al. Triage performance across large language models, ChatGPT, and untrained doctors in emergency medicine: comparative study. J Med Internet Res. 2024;26:e53297. doi:10.2196/53297
12. Builoff V, Shanbhag A, Miller RJ, et al. Evaluating AI proficiency in nuclear cardiology: large language models take on the board preparation exam. medRxiv. Preprint. 2024. doi:10.1101/2024.07.16.24310297
13. Botross M, Mohammadi SO, Montgomery K, Crawford C. Performance of Google's artificial intelligence chatbot "Bard" (now "Gemini") on ophthalmology board exam practice questions. Cureus. 2024;16(3):e57348. doi:10.7759/cureus.57348
14. Khan MP, O'Sullivan ED. A comparison of the diagnostic ability of large language models in challenging clinical cases. Front Artif Intell. 2024;7:1379297. doi:10.3389/frai.2024.1379297
15. Hirosawa T, Harada Y, Mizuta K, et al. Evaluating ChatGPT-4's accuracy in identifying final diagnoses within differential diagnoses compared with those of physicians: experimental study for diagnostic cases. JMIR Form Res. 2024;8:e59267. doi:10.2196/59267
16. Gomez-Cabello CA, Borna S, Pressman SM, Haider SA, Forte AJ. Large language models for intraoperative decision support in plastic surgery: a comparison between ChatGPT-4 and Gemini. Medicina (Kaunas). 2024;60(6):957. doi:10.3390/medicina60060957
17. Rush R. Assessing readability: formulas and alternatives. Read Teach. 1984;39(3):274-283.
18. Flesch R. A new readability yardstick. J Appl Psychol. 1948;32(3):221-233. doi:10.1037/h0057532
19. Medscape. Case Challenges. Available at: https://reference.medscape.com/features/casechallenges?icd=login_success_email_match_norm. Accessed September 6, 2024.
20. Medscape. Home Page. Available at: https://www.medscape.com/index/section_60_0. Accessed September 6, 2024.
21. Readable. Flesch Reading Ease and the Flesch Kincaid Grade Level. Available at: https://readable.com/readability/flesch-reading-ease-flesch-kincaid-grade-level/. Accessed June 16, 2025.
22. Klare GR. The measurement of readability: useful information for communicators. ACM J Comput Doc. 2000;24:107-121.
23. Palmer EJ, Devitt PG. Assessment of higher order cognitive skills in undergraduate education: modified essay or multiple choice questions? BMC Med Educ. 2007;7:49. doi:10.1186/1472-6920-7-49
24. Hays RB, Coventry P, Wilcock D, Hartley K. Short and long multiple-choice question stems in a primary care oriented undergraduate medical curriculum. Educ Prim Care. 2009;20(3):173-177.
25. Case SM, Swanson DB. Constructing Written Test Questions for the Basic and Clinical Sciences. 3rd ed. Philadelphia: National Board of Medical Examiners; 2002.
26. Bhayana R, Krishna S, Bleakney RR. Performance of ChatGPT on a radiology board-style examination: insights into current strengths and limitations. Radiology. 2023;307(5):e230582. doi:10.1148/radiol.230582
27. Rao A, Kim J, Kamineni M, et al. Evaluating ChatGPT as an adjunct for radiologic decision-making. medRxiv. 2023. doi:10.1101/2023.02.02.23285399
28. Kitamura FC. ChatGPT is shaping the future of medical writing but still requires human judgment. Radiology. 2023;307:e230171.
29. Momenaei B, Wakabayashi T, Shahlaee A, et al. Appropriateness and readability of ChatGPT-4-generated responses for surgical treatment of retinal diseases. Ophthalmol Retina. 2023;7:862-868.
30. Al-Sharif EM, Penteado RC, Dib El Jalbout N, et al. Evaluating the accuracy of ChatGPT and Google Bard in fielding oculoplastic patient queries: a comparative study on artificial versus human intelligence. Ophthalmic Plast Reconstr Surg. 2024;40:303-311.
31. Atkinson CJ, Seth I, Xie Y, et al. Artificial intelligence language model performance for rapid intraoperative queries in plastic surgery: ChatGPT and the deep inferior epigastric perforator flap. J Clin Med. 2024;13:900.
32. Rizwan A, Sadiq T. The use of AI in diagnosing diseases and providing management plans: a consultation on cardiovascular disorders with ChatGPT. Cureus. 2023;15(8):e43106. doi:10.7759/cureus.43106
33. Mahendiran T, Thanou D, Senouf O, et al. Deep learning-based prediction of future myocardial infarction using invasive coronary angiography: a feasibility study. Open Heart. 2023;10:e002237.
34. Skalidis I, Cagnina A, Luangphiphat W, et al. ChatGPT takes on the European Exam in Core Cardiology: an artificial

intelligence success story? Eur Heart J Digit Health. 2023;4:279-281.

35. Herman R, Kisova T, Belmonte M, et al. Artificial intelligence-powered electrocardiogram detecting culprit vessel blood flow abnormality: AI-ECG TIMI study design and rationale. J Soc Cardiovasc Angiogr Interv. 2025;4(3Part B):102494. doi:10.1016/j.jscai.2024.102494
36. Günay S, Öztürk A, Yiğit Y. The accuracy of Gemini, GPT-4, and GPT-4o in ECG analysis: a comparison with cardiologists and emergency medicine specialists. Am J Emerg Med. 2024;84:68-73. doi:10.1016/j.ajem.2024.07.043
37. Martínez-Sellés M, Marina-Breysse M. Current and future use of artificial intelligence in electrocardiography. J Cardiovasc Dev Dis. 2023;10(4):175. doi:10.3390/jcdd10040175
38. Yuan J, Tang R, Jiang X, Hu H. Large language models for healthcare data augmentation: an example on patient-trial matching. AMIA Annu Symp Proc. 2023;2023:1324-1333.
39. Leslie D, Mazumder A, Peppin A, Wolters MK, Hagerty A. Does "AI" stand for augmenting inequality in the era of COVID-19 healthcare? BMJ. 2021;372:n304.
40. Zaidi D, Miller T. Implicit bias and machine learning in health care. South Med J. 2023;116:62-64.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 247-253, 2025
DOI: https://doi.org/10.32708/uutfd.1694098

ORIGINAL RESEARCH

Assessment of Metabolic, Clinical and Radiological Risk Factors for Nephrolithiasis in Autosomal Dominant Polycystic Kidney Disease: A Single-Center Retrospective Study

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is a common monogenic disorder associated with an increased risk of nephrolithiasis. This study aimed to evaluate the clinical, metabolic, and radiological risk factors contributing to kidney stone formation in patients with ADPKD. A total of 55 patients followed in the nephrology outpatient setting at Bursa City Hospital between January 2022 and January 2025, with available non-contrast abdominopelvic CT scans, were retrospectively analyzed. Demographic data, laboratory values, radiological characteristics, and 24-hour urine analyses were recorded. Kidney stones were detected in 58.2% of patients based on CT reports. Macroscopic hematuria was observed exclusively in the stone-positive group. In multivariate logistic regression analysis, the presence of hepatic cysts (OR: 4.34) and increased 24-hour urinary calcium excretion (OR: 1.01) were identified as independent risk factors for nephrolithiasis. No significant association was found between stone formation and urinary citrate or oxalate levels. The number of patients receiving Tolvaptan therapy was equal between the two groups, limiting the assessment of its potential effect on stone formation. Although the prevalence of hypertension was higher in the stone-positive group, the difference was not statistically significant. The higher prevalence of nephrolithiasis observed in our cohort compared to the literature suggests that even asymptomatic ADPKD patients may benefit from non-contrast CT screening. Evaluating parameters such as hypercalciuria and hepatic cysts may aid in the development of individualized monitoring and management strategies.

Keywords: Autosomal dominant polycystic kidney disease. Nephrolithiasis. Risk factors. Radiological assessment.

Otozomal Dominant Polikistik Böbrek Hastalığında Nefrolitiazis İçin Metabolik, Klinik ve Radyolojik Risk Faktörlerinin Değerlendirilmesi: Tek Merkezli Retrospektif Bir Çalışma

ÖZET

Otozomal dominant polikistik böbrek hastalığı (ODPKBH), böbrek taşı gelişimi açısından artmış risk barındıran bir monogenik hastalıktır. Bu çalışmada, ODPKBH tanılı bireylerde böbrek taşı oluşumuna katkıda bulunabilecek klinik, metabolik ve radyolojik risk faktörlerinin değerlendirilmesi amaçlanmıştır. Ocak 2022 - Ocak 2025 tarihleri arasında Bursa Şehir Hastanesi Ayaktan Nefroloji Polikliniği'nde takip edilen ve non-kontrast batın BT görüntülemesi mevcut olan, 55 ODPKBH hastasının verileri retrospektif olarak incelendi. Demografik, laboratuvar ve radyolojik özellikler ile 24 saatlik idrar analizleri kaydedildi. Hastaların %58,2'sinde BT raporlarında taş varlığı tespit edildi. Makroskopik hematüri sadece taş saptanan grupta gözlendi. Çok değişkenli lojistik regresyon analizinde, karaciğer kisti varlığı (OR: 4,34) ve 24 saatlik idrarda artmış kalsiyum atılımı (OR: 1,01) bağımsız risk faktörleri olarak belirlendi. Sitrat ve oksalat düzeyleri ile taş varlığı arasında anlamlı ilişki saptanmadı. Tolvaptan tedavisi alan hasta sayısı eşit olduğundan bu ilacın taş üzerine etkisi değerlendirilemedi. Taş saptanan grupta hipertansiyon oranı yüksek olmakla birlikte istatistiksel anlamlılık izlenmedi. Çalışmamızda literatüre kıyasla daha yüksek taş prevalansı tespit edilmesi, asemptomatik ODPKBH hastalarında dahi non-kontrast BT ile taş taramasının değerli olabileceğini düşündürmektedir. Hiperkalsiüri ve karaciğer kisti varlığı gibi parametrelerin değerlendirilmesi, kişiye özgü takip ve tedavi planlarının oluşturulmasına katkı sağlayabilir.

Anahtar Kelimeler: Otozomal dominant polikistik böbrek hastalığı. Nefrolitiyazis. Risk faktörleri. Radyolojik değerlendirme.

Date Received: 7.May.2025
Date Accepted: 20.July.2025

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Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder characterized by bilateral renal cysts and affects approximately 1 in 400 to 1,000 individuals, making it one of the most common monogenic kidney diseases[1]. The diagnosis is typically made in adulthood, and approximately 50% of patients progress to end-stage renal disease (ESRD) around the age of 60[2]. The disease is not

limited to the kidneys; it also affects multiple organ systems such as the liver, pancreas, cerebral vasculature, and cardiac valves. The most common extrarenal manifestation is hepatic cysts[3]. Nephrolithiasis is one of the important complications in the course of ADPKD. The prevalence of kidney stone formation in ADPKD patients is higher than in the general population and has been reported to range from 20% to 36%[4–5]. The increased risk of stone formation is associated with anatomical distortion due to cystic structures, urinary stasis, recurrent infections, and metabolic abnormalities such as hypercalciuria, hypocitraturia, and hyperuricosuria[6–7]. Kidney stones in this patient group may be linked to recurrent pain, hematuria, infections, and a more rapid decline in renal function[8–9]. However, the clinical, metabolic, and radiological risk factors contributing to stone formation in ADPKD patients have not yet been fully clarified. This study aims to evaluate retrospectively the potential risk factors for nephrolithiasis in patients diagnosed with ADPKD and to determine the differences between individuals with and without kidney stones.

Material and Method

This retrospective study was conducted in the Nephrology Department of Bursa City Hospital and aimed to evaluate clinical and metabolic factors associated with kidney stone formation in patients diagnosed with autosomal dominant polycystic kidney disease (ADPKD) between January 2022 and January 2025. The study was approved by the local ethics committee with decision number 2025-3/7, dated February 5, 2025. A total of 55 patients aged 18 years and older, with a confirmed diagnosis of ADPKD based on imaging (ultrasonography, CT, or MRI) and/or family history, and who had available non-contrast abdominopelvic CT scans, were included in the study. The presence of kidney stones was determined based on CT reports that included the statement “millimetric stones are present” and/or provided the stone size in millimeters. Patients were classified as either “stone present” or “stone absent” accordingly. Hepatic cysts were considered present only if explicitly reported in radiology reports. Renal volumes were calculated by multiplying sagittal length, width, and depth measurements by the ellipsoid formula constant (0.523). These volume values were then evaluated according to the Mayo Clinic classification system, taking into account the patient’s age and height. Demographic (age, sex, body mass index, smoking history), clinical (hypertension, comorbidities, use of antihypertensive drugs, Tolvaptan use, and family history), and laboratory data were obtained from the hospital’s electronic medical records. Laboratory parameters included complete blood count, serum creatinine, eGFR, uric acid, albumin, fasting blood glucose, parathyroid hormone (PTH), AST, ALT, lipid profile, urine specific gravity, and pH. Proteinuria was evaluated using either spot urine samples or 24-hour urine collections. Additionally, 24-hour urinary parameters including volume, calcium, phosphorus, uric acid, oxalate, potassium, and citrate levels were analyzed.

Statistical analysis

The normality of continuous variables was assessed using the Shapiro-Wilk test. According to the results of the normality test, variables that conform to normal distribution were expressed as mean ± standard deviation and variables that do not conform to normal distribution were expressed as median (minimum: maximum) values; categorical variables were expressed as n (%). In the comparisons between two groups, Independent Samples t test was used in case of conformity to normal distribution and Mann Whitney U test was used in case of non-conformity to normal distribution. Categorical variables were compared between groups using Pearson Chi-Square test, Fisher's Exact Chi-Square test and Fisher Freeman Halton test. Logistic regression analysis was used to determine the risk factors thought to be effective on the development of kidney stones. For statistical analyses, SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) programme was used and type I error rate was accepted as 5%.

Results

A total of 55 patients with ADPKD were included in the study, of whom 32 (58.2%) had kidney stones and 23 (41.8%) did not. In terms of demographic and clinical characteristics, there were no statistically significant differences between the stone-positive and stone-negative groups in age, sex, body mass index (BMI), smoking status, family history, or hypertension ($p>0.05$; Table I). No significant differences were observed between the groups regarding the use of antihypertensive medications including RAS blockers, calcium channel blockers, beta-blockers, diuretics, alpha-blockers, or Tolvaptan therapy ($p>0.05$; Tables II and IV). Although hepatic cysts were more frequently observed in the stone-positive group, the difference did not reach statistical significance ($p=0.134$; Table II). Macroscopic hematuria was present in 22.6% of the stone-positive patients, while none of the stone-negative patients had this finding. This difference was statistically significant ($p=0.033$; Table II). Regarding laboratory parameters, no significant differences were found between the groups in hemoglobin, serum creatinine, eGFR, uric acid, parathyroid hormone, fasting glucose, lipid profile, electrolytes, albumin, or proteinuria ($p>0.05$; Table III). In the 24-hour urine analysis, parameters including urine volume, citrate, oxalate, calcium,

phosphorus, uric acid, potassium, urine density, and pH showed no significant differences between the groups (p>0.05; Table IV). Similarly, total renal volume and Mayo Clinic imaging classification stages did not differ significantly between groups (Table IV). Logistic regression analysis revealed that, in the multivariate model, the presence of hepatic cysts was identified as an independent risk factor for kidney stone formation (OR: 4.34; 95% CI: 1.05–17.93; p=0.042). In addition, each 1 mg/day increase in 24-hour urinary calcium excretion was associated with a 1.01-fold increased risk of nephrolithiasis (OR: 1.01; 95% CI: 1.00–1.02; p=0.041; Table V). The final multivariate logistic regression model was found to be statistically significant (model p=0.022), with acceptable goodness of fit (Hosmer-Lemeshow test, p=0.244).

Table I. Comparison of demographic and clinical characteristics between patients with and without kidney stones

Variable	Total (n=55)	Stone Present (n=32)	Stone Absent (n=23)	p-value
Age (years)	45.5±12.5	47.4±9.9	42.8±15.3	0.216[a]
Sex				
• Male	28 (50.9%)	18 (56.3%)	10 (43.5%)	0.350[b]
• Female	27 (49.1%)	14 (43.8%)	13 (56.5%)	
BMI (kg/m²)	26.8±3.4	27.4±3.1	26.1±3.7	0.156[a]
Smoking				
• Yes	20 (36.4%)	11 (34.4%)	9 (39.1%)	0.718[b]
• No	35 (63.6%)	21 (65.6%)	14 (60.9%)	
Family history				
• Yes	45 (81.8%)	25 (78.1%)	20 (87%)	0.494[c]
• No	10 (18.2%)	7 (21.9%)	3 (13%)	
Hypertension				
• Yes	33 (60%)	22 (68.8%)	11 (47.8%)	0.118[b]
• No	22 (40%)	10 (31.3%)	12 (52.2%)	

Data are expressed as mean ± standard deviation, median (minimum: maximum) and n%.
a: Independent Sample t test, b: Pearson Chi-Square test, c: Fisher's Exact Chi-Square test
BMI: Body Mass Index

Table II. Comparison of antihypertensive medication use, urinary complications, and radiological findings (Liver Cyst) between patients with and without kidney stones

	Total (n=55)	Stone Present (n=32)	Stone Absent (n=23)	p-value
Renin–Angiotensin System Blocker (RAS Blocker)				
• Yes	30 (54.5%)	19 (59.4%)	11 (47.8%)	0.396[b]
• No	25 (45.5%)	13 (40.6%)	12 (52.2%)	
Calcium Channel Blocker (CCB)				
• Yes	9 (16.4%)	8 (25.0%)	1 (4.3%)	0.064[c]
• No	46 (83.6%)	24 (75.0%)	22 (95.7%)	
Beta Blocker				
• Yes	8 (14.8%)	5 (16.1%)	3 (13.0%)	>0.99[c]
• No	46 (85.2%)	26 (83.9%)	20 (87.0%)	
Diuretic				
• Yes	13 (23.6%)	8 (25.0%)	5 (21.7%)	0.779[b]
• No	42 (76.4%)	24 (75.0%)	18 (78.3%)	
Alpha Blocker				
• Yes	8 (14.5%)	5 (15.6%)	3 (13.0%)	>0.99[c]
• No	47 (85.5%)	27 (84.4%)	20 (87.0%)	
Urinary Tract Infection (UTI)				
• Yes	2 (3.8%)	0 (0.0%)	2 (9.5%)	0.158[c]
• No	50 (96.2%)	31 (100.0%)	19 (90.5%)	
Macroscopic Hematuria				
• Yes	7 (13.5%)	7 (22.6%)	0 (0.0%)	0.033[c]
• No	45 (86.5%)	24 (77.4%)	21 (100.0%)	
Hepatic Cyst				
• Yes	35 (63.6%)	23 (71.9%)	12 (52.2%)	0.134[b]
• No	20 (36.4%)	9 (28.1%)	11 (47.8%)	

Data are expressed as n(%)
b: Pearson Chi-Square test, c: Fisher's Exact Chi-Square test

Table III. Comparison of biochemical and hematological parameters between patients with and without kidney stones

Parameter	Total (n=55)	Stone Present (n=32)	Stone Absent (n=23)	p-value
Hemoglobin (g/dL)	13.6±1.8	13.5±1.8	13.5±2.1	0.926[a]
Creatinine (mg/dL)	1 (0.6-6.1)	1 (0.6-6.1)	0.9 (0.6-4.4)	0.298[d]
eGFR (mL/min)	72.4±32.1	67.5±30.3	77.1±36.7	0.293[a]
Uric Acid (mg/dL)	5.1 (2.5-9.5)	5.9 (2.5-8.5)	5.1 (3-38)	0.544[d]
Parathyroid Hormone (pg/mL)	51.1 (13.5-287)	51.2 (20.4-287)	52.1 (13.5-243)	0.850[d]
Fasting Glucose (mg/dL)	93.5 (71-201)	94.5 (71-141)	89.5 (76-201)	0.679[d]
Total Cholesterol (mg/dL)	191.5±36.2	208.7±34.1	189±37.8	0.055[a]
LDL (mg/dL)	125.5±31	131.3±28.4	115.3±32.5	0.064[a]
Triglycerides (mg/dL)	123 (51-330)	119.5 (51-330)	120.5 (54-270)	0.912[d]
HDL (mg/dL)	48.4±12.9	49.9±13.5	46.3±12.1	0.334[a]
Sodium (mmol/L)	140.7±2.3	141.2±2.3	140.2±2.1	0.092[a]
Potassium (mmol/L)	4.4 (3-6.7)	4.4 (3-6.7)	4.3 (3.8-5.2)	0.114[d]
Calcium (mg/dL)	9.3 (8.4-10.5)	9.3 (7.1-10.5)	9.3 (8.4-10.1)	0.712[d]
Phosphorus (mg/dL)	3.3 (2.5-5.1)	3.2 (2.4-5.1)	3.6 (2-4.9)	0.206[d]
Albumin (g/L)	44.7±2.6	44.9±2.4	44.5±2.9	0.522[a]
Proteinuria (mg/day)	130 (30-4152)	128.2 (30-4152)	130 (30-3134)	0.791[d]

Data are expressed as mean ± standard deviation and median (minimum: maximum)
a: Independent Sample t test, d: Mann-Whitney U test

Table IV. Comparison of radiological and 24-hour urinary parameters between patients with and without kidney stones

Parameter	Total (n=55)	Stone Present (n=32)	Stone Absent (n=23)	p-value
Total Kidney Volume (mL)	1412 (273–6902)	1344 (399–5698)	1412 (273–6902)	0.682[d]
Mayo Stage				0.688[e]
• 1A	1 (1.8%)	0	1 (4.3%)	
• 1B	17 (30.9%)	10 (31.3%)	7 (30.4%)	
• 1C	14 (25.5%)	8 (25%)	6 (26.1%)	
• 1D	18 (32.7%)	12 (37.5%)	6 (26.1%)	
• 1E	5 (9.1%)	2 (6.3%)	3 (13%)	
Urine Volume (mL/day)	2300 (900–8900)	2600 (800–6000)	2500 (1300–8900)	0.925[d]
Urine Specific Gravity	1014.8 ± 7.4	1011.9 ± 6	1015.4 ± 7.6	0.073[a]
Urine pH	5.5 (5–6.5)	5.5 (5–6.5)	5.5 (5–7)	0.754[d]
24h Urinary Citrate (mg/day)	309 (15–1615)	240 (13.5–1615)	249 (6–708)	0.709[d]
24h Urinary Oxalate (mg/day)	22.4 (4.2–168.6)	18.7 (3.5–66.3)	16.5 (0.8–168.6)	0.853[d]
24h Urinary Uric Acid (mg/day)	403.2 (181.8–1097)	355 (100.5–1097)	420 (147.2–873.6)	0.428[d]
24h Urinary Calcium (mg/day)	102.7 (29.2–321.5)	95.7 (8.8–321.5)	108.9 (19.6–245.3)	0.900[d]
24h Urinary Phosphorus (mg/day)	690.8 (228–1650.5)	567.6 (87.1–1650.5)	624.9 (266.2–1528.8)	0.759[d]
24h Urinary Potassium (mmol/day)	56.6 (17.6–111.4)	53.5 (8.3–111.4)	47.9 (26.7–140.6)	0.479[d]
Tolvaptan				0.707[c]
• Receiving	8 (14.5%)	4 (12.5%)	4 (17.4%)	
• Not Receiving	47 (85.5%)	28 (87.5%)	19 (82.6%)	

Data are expressed as mean ± standard deviation and median (minimum: maximum)
a: Independent Sample t test, c: Fisher's Exact Chi-Square test, d: Mann-Whitney U test,
e: Fisher-Freeman-Halton test

Table V. Univariate and multivariate logistic regression analysis of risk factors for kidney stone formation

	OR (95% CI)	p	OR (95% CI)	p
Age	1.03 (0.99–1.08)	0.184		
Sex (Male)	0.59 (0.20–1.76)	0.351		
BMI	1.13 (0.95–1.34)	0.157		
Family history	0.54 (0.12–2.34)	0.407		
Hypertension history	2.4 (0.79–7.27)	0.122		
Smoking	0.82 (0.27–2.47)	0.718		
UTI	-	0.999		
Macroscopic hematuria	-	0.999		
Hepatic cyst (Present)	2.34 (0.76–7.21)	0.138	4.34 (1.05–17.93)	0.042
Hb	1.01 (0.77–1.34)	0.924		
Creatinine	1.23 (0.71–2.13)	0.467		
GFR (ml/min)	0.99 (0.98–1.01)	0.288		
Uric acid	0.95 (0.82–1.09)	0.446		
PTH	0.99 (0.99–1.01)	0.872		
Fasting glucose	0.99 (0.96–1.01)	0.336		
Total cholesterol	1.02 (0.99–1.03)	0.062		
LDL	1.02 (0.99–1.04)	0.072		
TG	1.00 (0.99–1.01)	0.978		
HDL	1.02 (0.98–1.07)	0.329		
Sodium	1.25 (0.96–1.62)	0.097		
Potassium	2.06 (0.59–7.19)	0.258		
Calcium	1.11 (0.38–3.18)	0.853		
Phosphorus	0.59 (0.25–1.39)	0.227		
Albumin	1.07 (0.87–1.32)	0.515		
Proteinuria (mg/day)	1.00 (0.99–1.00)	0.750		
Total Kidney Volume	1.00 (1.00–1.00)	0.800		
Urine Volume	1.00 (1.00–1.00)	0.456		
Urine Density	0.93 (0.85–1.01)	0.078		
Urine pH	0.80 (0.28–2.28)	0.676		
24h Urine Citrate (mg/day)	1.00 (0.99–1.00)	0.918		
24h Urine Oxalate (mg/day)	0.99 (0.97–1.02)	0.526	0.98 (0.95–1.01)	0.122
24h Urine Uric Acid (mg/day)	0.99 (0.99–1.00)	0.512		
24h Urine Calcium (mg/day)	1.00 (0.99–1.02)	0.625	1.01 (1.00–1.02)	0.041
24h Urine Phosphorus (mg/day)	1.00 (0.99–1.00)	0.580		
24h Urine Potassium (mmol/day)	1.01 (0.98–1.03)	0.582		
Tolvaptan	0.68 (0.15–3.05)	0.613		

OR: Odds Ratio, CI: Confidence Interval
For the variables, the following reference categories were used: "Female" for sex, "Absent" for family history, "Absent" for history of hypertension, "Non-smoker" for smoking status, "Absent" for urinary tract infection (UTI), "Absent" for macroscopic hematuria, "Absent" for liver cyst, and "Not receiving" for tolvaptan use.

Discussion and Conclusion

In this study, the prevalence of kidney stones was found to be higher (58.2%) compared to the rates reported in the literature for patients with ADPKD (20–36%). This difference may be attributed to several factors. Given the high sensitivity of CT in detecting stones—especially small or asymptomatic ones—this imaging modality may have contributed to the higher observed prevalence. Furthermore, in this study, stone presence was not limited to large or clinically significant calculi; phrases such as "millimetric stones are present" in CT reports were also included in the assessment. This broader approach may have resulted in a higher reported prevalence by encompassing a wider spectrum of stone burden.

In the current study, macroscopic hematuria was observed only in patients with kidney stones (22.6%) and was not detected in the stone-free group. This finding suggests that nephrolithiasis may be one of the causes of hematuria in patients with ADPKD. In ADPKD, hematuria typically arises from cyst rupture, infection, papillary necrosis, or nephrolithiasis. The association between stone disease and hematuria has been reported in the literature. As noted by Torres et al. in *UpToDate*, kidney stones are considered one of the common causes of hematuria in ADPKD patients[9]. Therefore, when hematuria is present in these patients, nephrolithiasis should be evaluated in addition to cyst-related causes.

In the present study, the presence of liver cysts was identified as an independent risk factor for kidney stone formation in ADPKD patients. This relationship may be explained by the distortion of anatomical structures and the subsequent urinary stasis caused by the extensive cyst burden. Although data on this topic are limited in the literature, the study by Onur Kaygısız et al. also reported a significantly higher prevalence of kidney stones in patients with liver cysts [10]. These findings support the results of our study and suggest that liver cysts, although typically considered benign extrarenal manifestations, may contribute to nephrolithiasis through anatomical or physiological changes.

Twenty-four-hour urinary calcium excretion was found to be a statistically significant independent risk factor for stone formation in multivariate logistic regression analysis. This finding supports the established role of hypercalciuria in the pathogenesis of calcium oxalate stones. Similarly, numerous studies have reported that hypercalciuria increases urinary supersaturation, thereby promoting crystallization and facilitating stone formation[11,12]. Furthermore, studies by Worcester and Coe emphasized that hypercalciuria is a key predisposing factor in both sporadic and familial stone disease[13]. Although the use of diuretics did not differ significantly between the stone-positive and stone-negative groups in our study, the specific types of diuretics were not analyzed separately. This may be relevant, as thiazide diuretics are known to reduce urinary calcium excretion and potentially lower the risk of calcium-based stones, whereas loop diuretics may increase calciuria and promote stone formation. The absence of detailed diuretic subclass data constitutes a minor limitation in evaluating their potential impact on nephrolithiasis risk.

However, in the current study, no statistically significant association was found between 24-hour urinary citrate or oxalate levels and the presence of stones. In contrast, the literature highlights hypocitraturia (low 24-hour urinary citrate) and hyperoxaluria (elevated oxalate excretion) as major metabolic abnormalities predisposing to stone formation[14,15]. Citrate inhibits crystallization by forming soluble complexes with urinary calcium, while oxalate is a major contributor to calcium oxalate stone formation. Although some studies have reported more frequent hypocitraturia in patients with stones, this difference has not always reached statistical significance[6,16].

Another known stone inhibitor is magnesium, which impedes calcium oxalate crystallization by competing with calcium to form soluble oxalate complexes. Several studies have identified low 24-hour urinary magnesium levels as a significant risk factor for calcium oxalate stones[17,18]. In the current study, 24-hour urinary magnesium was not measured due to laboratory requirements for acidified collection containers, which constitutes one of the limitations of this study.

In the current cohort, the mean total kidney volumes of patients with and without stones were similar (1344 mL vs. 1412 mL), and most patients in both groups were classified as Mayo Clinic imaging classes 1B and 1D. Similarly, a cross-sectional study conducted in Türkiye also reported no significant difference in total kidney volume between ADPKD patients with and without stones[19]. Conversely, the study by Grampsas et al. found that patients with stone disease had a greater number of renal cysts and larger dominant cysts[6]. Likewise, in another study by Nishiura et al., ADPKD patients with stones had significantly larger total kidney volumes[16]. These findings suggest that, in addition to total kidney volume, the distribution of cyst burden and the size of dominant cysts may play a role in stone pathophysiology.

Another limitation of the current study was the small number of patients receiving tolvaptan therapy, which precluded a comparative analysis with untreated patients. Tolvaptan, a vasopressin V2 receptor antagonist, is the first and only approved pharmacological treatment to slow disease progression

in autosomal dominant polycystic kidney disease (ADPKD). It reduces cyst expansion and kidney growth by increasing urine output through decreased water reabsorption in the renal tubules[20]. Theoretically, increased urine volume may reduce urinary supersaturation and thereby lower the risk of nephrolithiasis. However, clinical evidence regarding the preventive effect of tolvaptan on stone formation remains limited. In our study, the number of patients treated with tolvaptan was equal in both groups (4 vs. 4).

Hypertension is another factor worth considering in the pathogenesis of nephrolithiasis in ADPKD. In our study, the prevalence of hypertension was higher among patients with stones compared to those without (68.8% vs. 48.8%), although this difference did not reach statistical significance. Some studies have explored the association between hypertension and nephrolithiasis. For instance, Borghi et al. reported a significantly increased risk of stone formation in individuals with hypertension, especially when accompanied by obesity, which may be related to increased oxaluria, calciuria, and urinary supersaturation of calcium oxalate and uric acid [21]. Madore et al. similarly found a higher prevalence of hypertension in individuals with a history of nephrolithiasis[22]. However, both studies were conducted in the general population, not specifically among ADPKD patients.

Other limitations of this study include its single-center design and relatively small sample size, which may limit the generalizability of our findings and reduce statistical power in subgroup analyses. Nevertheless, our study also possesses notable strengths. The comprehensive evaluation of clinical, laboratory, and radiological data; the inclusion of 24-hour urine analyses in a substantial portion of patients; and the use of the updated Mayo Clinic imaging classification system to assess kidney volumes enhance the methodological rigor of this research. Furthermore, the investigation of extrarenal findings such as liver cysts in relation to nephrolithiasis represents a relatively novel approach in the literature.

In conclusion, this study provides a multidimensional analysis of clinical, metabolic, and radiological risk factors contributing to nephrolithiasis in ADPKD patients. It emphasizes that hypercalciuria and the presence of liver cysts may be particularly important parameters warranting close attention. The higher prevalence of kidney stones observed in our cohort compared to the literature suggests that non-contrast abdominal CT may be a valuable tool for detecting stones, even in asymptomatic patients. These findings underscore the importance of early recognition of stone risk in ADPKD and support the need for individualized patient management strategies.

Researcher Contribution Statement:
Idea and design: M.S.
Data collection and processing: M.S., S.A.
Analysis and interpretation of data: M.S.
Writing of significant parts of the article: M.S.
Support and Acknowledgement Statement:
Support and Acknowledgement Statement: There is no financial support related to this study. The authors would like to thank the staff of the Nephrology Outpatient Clinic at Bursa City Hospital for their assistance in patient follow-up and data access.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Bursa City Hospital Clinical Research Ethics Committee
Approval Date: February 5, 2025
Decision No: 2025-3/7

References

1. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. *Lancet*. 2007;369(9569):1287-301.
2. Grantham JJ. Clinical practice. Autosomal dominant polycystic kidney disease. *N Engl J Med*. 2008;359(14):1477-85.
3. Chapman AB, Devuyst O, Eckardt KU, et al. Autosomal-dominant polycystic kidney disease (ADPKD): Executive summary from a KDIGO Controversies Conference. *Kidney Int*. 2015;88(1):17–27.
4. Torres VE, Erickson SB, Smith LH, Wilson DM, Hattery RR, Segura JW. The association of nephrolithiasis and autosomal dominant polycystic kidney disease. Am J Kidney Dis. 1988 Apr;11(4):318-25.
5. Levine E, Grantham JJ. Calcified renal stones and cyst calcifications in autosomal dominant polycystic kidney disease: clinical and CT study in 84 patients. AJR Am J Roentgenol. 1992 Jul;159(1):77-81.
6. Grampsas SA, Chandhoke PS, Fan J, Glass MA, Townsend R, Johnson AM, Gabow P. Anatomic and metabolic risk factors for nephrolithiasis in patients with autosomal dominant polycystic kidney disease. Am J Kidney Dis. 2000 Jul;36(1):53-7.
7. Romero V, Akpinar H, Assimos DG. "Kidney stones: a global picture of prevalence, incidence, and associated risk factors." *Rev Urol*. 2010;12(2-3):e86–e96.
8. Shastri S, Patel J, Sambandam KK, Lederer ED. Kidney Stone Pathophysiology, Evaluation and Management: Core Curriculum 2023. Am J Kidney Dis. 2023 Nov;82(5):617-634.
9. Torres VE, Harris PC. Autosomal dominant polycystic kidney disease (ADPKD): Kidney manifestations. UpToDate. Waltham, MA: UpToDate Inc.; Accessed April 30, 2025. Available from: https://www.uptodate.com/contents/autosomal-dominant-polycystic-kidney-disease-adpkd-kidney-manifestations
10. Kaygısız O, Coşkun B, Oruç A, Gedik CC, Ersoy A, Kordan Y, Kılıçarslan H, Yıldız A. Evaluation of nephrolithiasis risk factors in autosomal dominant polycystic kidney disease (ADPKD): a single center experience. Okmeydanı Tıp Dergisi. 2018;34(2):87-91.
11. Pak CY, Sakhaee K, Moe OW, Poindexter JR, Adams-Huet B, Pearle MS. Biochemical profile of stone-forming patients with diabetes mellitus. *Urology*. 2003;61(3):523–527.
12. Sakhaee K, Capolongo G, Maalouf NM, Pasch A, Moe OW, Poindexter J, Adams-Huet B. Metabolic syndrome and the risk of calcium stones. Nephrol Dial Transplant. 2012 Aug;27(8):3201-9.
13. Worcester EM, Coe FL. Clinical practice. Calcium kidney stones. N Engl J Med. 2010 Sep 2;363(10):954-63.

14. Taylor EN, Curhan GC. Oxalate intake and the risk for nephrolithiasis. J Am Soc Nephrol. 2007 Jul;18(7):2198-204.
15. Coe FL, Evan A, Worcester E. Kidney stone disease. *J Clin Invest.* 2005;115(10):2598–2608.
16. Nishiura JL, Neves RF, Eloi SR, Cintra SM, Ajzen SA, Heilberg IP. Evaluation of nephrolithiasis in autosomal dominant polycystic kidney disease patients. Clin J Am Soc Nephrol. 2009 Apr;4(4):838-44.
17. Resnick MI, Munday D, Boyce WH. Magnesium excretion and calcium oxalate urolithiasis. Urology. 1982 Oct;20(4):385-9. Ettinger B, Citron JT, Livermore B, Dolman LI. Chronic potassium-magnesium citrate therapy for calcium oxalate nephrolithiasis. J Urol. 1997;158(6):2069–2073.
18. Ferraro PM, Taylor EN, Curhan GC. 24-Hour Urinary Chemistries and Kidney Stone Risk. Am J Kidney Dis. 2024 Aug;84(2):164-169.
19. Chasan O, Mirioglu S, Artan AS, Gursu M, Kazancioglu R, Elcioglu OC. Assessment of metabolic risk factors for nephrolithiasis in patients with autosomal dominant polycystic kidney disease: a cross-sectional study. Clin Exp Nephrol. 2023 Nov;27(11):912-918.
20. Torres VE, Chapman AB, Devuyst O, Gansevoort RT, Grantham JJ, Higashihara E, Perrone RD, Krasa HB, Ouyang J, Czerwiec FS. Tolvaptan in Patients with Autosomal Dominant Polycystic Kidney Disease. *N Engl J Med.* 2012 Dec 20;367(25):2407-18.
21. Borghi L, Meschi T, Guerra A, Briganti A, Schianchi T, Allegri F, Novarini A. Essential arterial hypertension and stone disease. Kidney Int. 1999 Jun;55(6):2397-406
22. Madore F, Stampfer MJ, Rimm EB, Curhan GC. Nephrolithiasis and risk of hypertension. Am J Hypertens. 1998 Jan;11(1 Pt 1):46-53.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 255-261, 2025
DOI: https://doi.org/10.32708/uutfd.1704426

ORIGINAL RESEARCH

Impact of Nephrology Follow-Up on Survival and Clinical Outcomes in Patients Initiating Emergency Dialysis

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ABSTRACT

This study aimed to evaluate the clinical and laboratory characteristics of patients presenting with emergency dialysis requirements and to assess the impact of nephrology follow-up on patient outcomes. In this retrospective study. patients who were given an emergency hemodialysis indication in the emergency department were divided into two groups according to the presence or absence of nephrology follow-up within the past year. Dialysis indications. laboratory findings. mortality rates. and transition to routine dialysis were analyzed. The most common indications for emergency dialysis were severe metabolic acidosis and elevated creatinine levels. The 28-day mortality rate was significantly lower in patients with nephrology follow-up (16.2% vs. 36.2%. p=0.001). The transition rate to routine hemodialysis was also higher in this group (p=0.024). However, there was no statistically significant difference between the groups regarding 90-day mortality or hospital readmission rates. Regular nephrology follow-up reduces short-term mortality and increases the transition to planned dialysis in patients requiring emergency dialysis. However, more effective monitoring and early intervention strategies are needed to improve long-term outcomes.

Keywords: Emergency dialysis. Hemodialysis. Acute kidney injury. Chronic kidney disease. Nephrology follow-up. Mortality. Clinical outcomes.

Acil Diyaliz Başlangıcında Nefroloji İzleminin Sağkalım ve Klinik Sonuçlar Üzerindeki Rolü

ÖZET

Bu çalışma, acil diyaliz gereksinimi ile başvuran hastaların klinik ve laboratuvar özelliklerini. nefroloji takibinin hasta sonuçları üzerindeki etkisini değerlendirmeyi amaçlamaktadır. Bu retrospektif çalışmada. acil serviste acil hemodiyaliz endikasyonu konulan hastalar. Son bir yıl içinde nefroloji takibi bulunup bulunmamasına göre iki gruba ayrılarak karşılaştırılmıştır. Diyaliz endikasyonları, laboratuvar bulguları, mortalite oranları ve rutin diyaliz geçişleri analiz edilmiştir. Acil diyaliz endikasyonları arasında en sık ağır metabolik asidoz ve kreatinin yüksekliği saptanmıştır. Nefroloji takibi olan hastalarda 28 günlük mortalite oranı anlamlı olarak daha düşük bulunmuştur (%16,2 vs. %36,2. p=0,001). Rutin hemodiyaliz programına geçiş oranı nefroloji takibi olan grupta daha yüksek bulunmuştur (p=0,024). Ancak 90 günlük mortalite ve yeniden hastaneye yatış oranlarında gruplar arasında anlamlı fark izlenmemiştir. Düzenli nefroloji takibi, acil diyaliz gereksinimi olan hastalarda kısa dönem mortaliteyi azaltmakta ve planlı diyaliz geçişini artırmaktadır. Ancak uzun dönem sonuçlar için daha etkin izlem ve erken müdahale stratejilerine ihtiyaç vardır.

Anahtar Kelimeler: Acil diyaliz. Hemodiyaliz. Akut böbrek hasarı. Kronik böbrek hastalığı. Nefroloji takibi. Mortalite. Klinik sonuçlar.

Acute kidney injury (AKI) is a clinical syndrome characterized by a rapid decline in renal function occurring within hours or days across a broad etiological spectrum. leading to a decrease in glomerular filtration rate (GFR) and accumulation of nitrogenous waste products in the blood[1]. In AKI. as in chronic kidney disease (CKD). renal replacement therapy (RRT) becomes vital in specific clinical conditions. Hemodialysis indications include volume overload resistant to diuretics. Hyperkalemia unresponsive to medical therapy. Refractory metabolic acidosis. encephalopathy. pericarditis. or bleeding diathesis[2].

Hemodialysis is a treatment method based on the exchange of fluids and solutes between the patient's blood and a suitable dialysis solution via a semipermeable membrane. Aiming to restore fluid and solute balance to physiological limits[3]. According to the European Renal Association–European Dialysis

Date Received: 22.May.2025
Date Accepted: 20.July.2025

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and Transplant Association (ERA-EDTA). RRT was initiated in 81.327 end-stage renal disease (ESRD) cases in 2015. corresponding to an incidence rate of 119 per million populations[4].

Although hospitalization rates among ESRD patients have declined. A significant increase in emergency department visits has been observed. Individuals with renal failure are approximately eight times more likely to present to the emergency department compared to the general population[5]. Repeated emergency visits by dialysis patients not only impose an additional financial burden on the healthcare system but also negatively affect service planning in emergency departments[6]. According to a study conducted in the United States. healthcare expenditures for dialysis patients amount to approximately $36 billion annually. accounting for 7.2% of total healthcare costs[7].

Emergency departments are the primary units where patients diagnosed with AKI receive intensive treatment. Patients not receiving regular dialysis or nephrology follow-up and presenting with AKI constitute a critical patient group.

Among RRT modalities. approximately 85% of patients undergo hemodialysis. 10% peritoneal dialysis. and 5% kidney transplantation. Mortality rates are particularly high in patients requiring emergency medical intervention with hemodialysis. reaching around 50%[8].

Limited studies in the existing literature indicate that this patient group is associated with intensive utilization of medical resources and high healthcare costs. To minimize this burden. identifying the clinical characteristics of patients presenting to the emergency department and determining potential preventive measures are of great importance. This study aims to evaluate the demographic. clinical. and laboratory findings of patients who presented to the emergency department and were determined to require emergency dialysis. It also analyzes the outcomes of their treatment processes. The literature shows a lack of descriptive studies focusing on patients requiring emergency hemodialysis due to AKI or exacerbations of CKD. This study aims to contribute to the literature by providing a detailed analysis of the clinical characteristics of this specific patient group.

Material and Method

This retrospective cohort study was conducted to examine the demographic and clinical characteristics of patients who presented to our emergency department between January 2022 and September 2023 and were diagnosed with an emergency hemodialysis indication following nephrology consultation. Ethical approval was obtained from the local Ethics Committee (KAEK-2023-21/11).

Our hospital is a tertiary-level training and research hospital. receiving approximately 700.000 emergency admissions annually. It includes a 24-hour operational hemodialysis center. and bedside dialysis can also be performed in emergency and intensive care units.

All patients who underwent dialysis via central venous catheterization in the emergency department during the specified period were retrospectively reviewed. Data on patients' age. gender. history of chronic diseases. and dialysis indications were retrieved from the hospital's digital records. Patients were divided into two groups based on the presence or absence of nephrology follow-up and a known diagnosis of CKD: Group 1: Patients with nephrology follow-up within the past year. whose renal function was monitored by nephrology or internal medicine outpatient clinics and who were planned for RRT but had not yet started. Group 2: Patients without nephrology follow-up or not considered RRT candidates prior to emergency admission.

Dialysis indications identified in the emergency department and laboratory results requested by the attending physician were recorded for both groups. Differences between the groups were evaluated in terms of dialysis indications. laboratory findings. Hospitalization requirements in hospital 28-day and 90-day mortality rates as well as adverse cerebrovascular and cardiac events. Additionally, the inclusion of patients in routine hemodialysis programs and the need for recurrent hospitalization were assessed.

Inclusion criteria: Patients aged 18 years or older who presented to the emergency department during the specified period were evaluated by emergency medicine specialists as requiring hemodialysis, underwent nephrology consultation, received an emergency hemodialysis indication from a nephrologist and underwent dialysis during their emergency follow-up. Patients lacking sufficient clinical information and those already receiving routine hemodialysis were excluded from the study.

Statistical Analysis

Statistical analyses were performed using SPSS 15.0 for Windows. Descriptive statistics were presented as frequency and percentage for categorical variables and as mean. standard deviation, minimum, maximum and median for numerical variables. Since the assumption of normal distribution was not met, the Mann-Whitney U test was used to compare numerical variables between groups. The chi-square test was used for comparisons of categorical variables. A p-value of <0.05 was considered statistically significant

Results

Among the 278 patients included in the study, 53.6% (n=149) were male and 46.4% (n=129) were female, with a mean age of 66.6 ± 15.8 years (range: 1–100 years). Diabetes mellitus was observed in 13.7% (n=38), hypertension in 16.5% (n=46) and malignancy in 2.1% (n=6) of patients. The proportion of patients with nephrology follow-up within the last year was 21.8% (n=67). The most common indications for emergency dialysis were severe metabolic acidosis (40.7%, n=113) and elevated creatinine (26.7%, n=74). Table I summarizes the patients' demographic characteristics and distribution of dialysis indications.

Table I. Demographic Characteristics and Dialysis Indications of Patients Presenting with Emergency Dialysis Requirement

Gender n (%)	Male	149 (53.6)
	Female	129 (46.4)
Age Mean.±SD (Min-Max)		66.6±15.8 (1-100)
Comorbidities n (%)	Diabetes mellitus	38 (13.7)
	Hypertension	46 (16.5)
	Coronary artery disease	17 (6.1)
	Chronic Renal Failure	209(75.2)
	Congestive Heart Failure	16 (5.7)
	Asthma/COPD	4 (1.4)
	Malignancy	6 (2.1)

Although patients with nephrology follow-up constituted a smaller portion of all dialysis indications, the majority of those requiring emergency dialysis did not have any nephrology follow-up. Refractory metabolic acidosis was identified as the most common dialysis indication. present in 15.9% of patients with nephrology follow-up and in 84.1% of those without. Elevated creatinine was the second most frequent indication. observed in 32.4% of patients with nephrology follow-up and in 67.6% of those without. All patients who underwent dialysis due to uremic symptoms belonged to the group without nephrology follow-up (Table II).

Uremic symptoms were only identified in patients without a history of chronic kidney disease (CKD) (4.2%, $p<0.001$). Refractory metabolic acidosis was the most frequent indication in both groups with rates of 24.3% in patients with known CKD and 35.8% in those without. When comparing laboratory results between patients with and without nephrology follow-up, the significant differences were observed. Patients with nephrology follow-up had significantly higher white blood cell counts, neutrophil counts and IG values ($p<0.01$). Furthermore, eGFI levels were significantly lower and creatinine levels were notably higher in this group ($p<0.001$).

Although hemoglobin (Hb) and hematocrit (HCT) levels were lower in patients with nephrology follow-up, the difference approached but did not reach statistical significance. There were no significant differences between the groups in serum sodium, potassium or calcium levels. In terms of blood gas parameters, patients with nephrology follow-up had significantly lower PO_2 levels ($p=0.001$), while there were no significant differences in pH and HCO_3 levels. Lactate levels were significantly lower in patients with nephrology follow-up ($p<0.001$). Laboratory parameter distributions are presented in Table III.

Significant differences were identified in mortality and enrollment in routine dialysis programs between the two groups. The 28-day mortality rate was 14.9% in the nephrology follow-up group and 39.8% in the non-follow-up group, with this difference being statistically significant ($p=0.001$). However, 90-day mortality rates were 40.3% and 54% respectively, which did not reach statistical significance ($p=0.127$) (Table IV).

Table II. Association Between Emergency Dialysis Indications. Nephrology Follow-Up. and Known CKD History

Emergency Dialysis Indication	Nephrology Follow-Up		Known chronic kidney disease history		Previous Dialysis History	
	Yes n (%)	No n (%)	Yes n (%)	No n (%)	Yes n (%)	No n (%)
Uremic symptoms (encephalopathy. bleeding. pericarditis)	0 (0.0%)	9 (100%)	1 (11.1%)	8 (88.9%)	0 (0.0%)	9 (100%)
Volume overload (dyspnea. distension)	13 (31.7%)	28 (68.3%)	13 (31.7%)	28 (68.3%)	2 (4.9%)	39 (95.1%)
Refractory metabolic acidosis	18 (15.9%)	95 (84.1%)	19 (16.8%)	94 (83.2%)	1 (0.9%)	112 (99.1%)
Hyperkalemia	10 (27.8%)	26 (72.2%)	9 (25.0%)	27 (75.0%)	0 (0.0%)	36 (100%)
Hypertensive urgency	2 (40.0%)	3 (60.0%)	3 (60.0%)	2 (40.0%)	0 (0.0%)	5 (100%)
Elevated creatinine	24 (32.4%)	50 (67.6%)	22 (29.7%)	52 (70.3%)	2 (2.7%)	72 (97.3%)

Table III. Relationship Between Nephrology Follow-Up Within the Last Year and Laboratory Parameters

	Nephrology Follow-Up (Past Year)						
	Yes			No			
	Median	IQR		Median	IQR		p
WBC (×10³/μL)	10.09	7.48	13.47	12.95	9.055	18.03	0.001
NEUT (×10³/μL)	7.93	5.43	11.65	10.33	6.26	15.285	0.006
NEUT%	79.8	74.6	88.7	82.55	72.88	88.2	0.650
LYMPH (×10³/μL)	0.9	0.67	1.32	1.17	0.68	2.03	0.012
LUMPH%	9.4	5.5	15	9.15	5.425	17.1	0.764
HgB (g/dL)	9.6	8.1	11.2	9.95	8.6	12.5	0.064
HCT (%)	29.5	24.2	33.4	30.7	26.2	37.7	0.061
PLT (×10³/μL)	256	195	323	238	176.75	325.75	0.645
IG%	0.65	0.4	1.375	1	0.6	1.775	0.002
IG (×10³/μL)	0.07	0.03	0.14	0.13	0.07	0.31	0.001
Urea (mg/dL)	164.6	117.1	247.6	147.35	79.98	226.7	0.211
BUN (mg/dL)	76.9	54.7	115.7	68.6	37.3	105.1	0.175
Creatinine (mg/dL)	6.32	4.135	9.39	3.97	1.5	8	<0.001
eGFR	7.9	5	11.675	12.4	5.1	37.125	0.001
Calcium (mg/dL)	8.4	7.6	9.1	8.5	7.7	9.1	0.885
Sodium (mmol/L)	137	132	139	136.5	131	141	0.656
Potassium (mmol/L)	5.2	4.5	6.2	5	4.1	6	0.152
PH	7.28	7.19	7.34	7.25	7.12	7.34	0.082
PO2 (mmHg)	31.4	24.9	45	42	28.9	69.1	0.001
pCO2 (mmHg)	37.6	30.4	43.9	35.9	28.1	42.5	0.362
HCO3 (mmol/L)	17.2	13	22.8	16	10.375	21.7	0.091
Base excess (mmol/L)	-9.1	-14.2	-3.3	-10.7	-18.2	-4.15	0.083
Lactate (mmol/L)	1	0.5	1.6	1.8	0.8	4.7	<0.001

WBC = White Blood Cell. NEUT – neutrophils; NEUT% – neutrophil percentage; LYMPH – lymphocytes; LYMPH% – lymphocyte percentage; HgB – hemoglobin; HCT – hematocrit; PLT – platelets; IG – immature granulocytes; IG% – immature granulocyte percentage; BUN – blood urea nitrogen; eGFR – estimated glomerular filtration rate; PO_2 – partial pressure of oxygen; PCO_2 – partial pressure of carbon dioxide; HCO_3 – bicarbonate;

Table IV. Association Between Nephrology Follow-Up (Past Year) and Mortality and Patient Outcomes

	Nephrology Follow-Up				
	Yes		No		
	n	%	n	%	p
28 day mortality	10	14.9%	84	39.8%	0.003
90 day mortality	27	40.3%	114	54.0%	0.069
Readmission within 90 days	15	22.4%	38	18%	0.537
Enrollment in routine HD program	11	16.4%	20	9.5%	0.177

Chi-square Test

The rate of enrollment in routine hemodialysis programs was 16.4% in the nephrology follow-up group versus 9.5% in the other group and this difference was statistically significant (p=0.024). In contrast, 90-day readmission rates were 22.4% and 18% respectively. With no significant difference (p=0.618).

There were statistically significant differences in 28-day and 90-day mortality rates based on the type of emergency dialysis indication (p<0.001 and p=0.001, respectively). The 28-day mortality rate was highest in cases of severe metabolic acidosis and hyperkalemia (Table V).

Table VI. Association Between Emergency Dialysis Indications and Mortality. Readmission. and Routine Hemodialysis

Emergency Dialysis Indication	28 day mortality		90 day mortality		90 day readmission		Routine Hemodialysis Program	
	n	%	n	%	n	%	n	%
Uremic symptoms	3	33.3%	3	33.3%	2	22.2%	0	0.0%
Volume overload	9	22.0%	21	51.2%	5	12.2%	7	17.1%
Severe metabolic acidosis	59	52.2%	72	63.7%	23	20.4%	12	10.6%
Hyperkalemia	9	25.0%	19	52.8%	8	22.2%	2	5.6%
Hypertensive urgency	0	0.0%	0	0.0%	1	20.0%	0	0.0%
Elevated creatinine	14	189%	26	35.1%	14	18.9%	10	13.5%
	<0.001		0.001		0.911		0.064	

Among the 278 patients included in the study. 24.1% (n=67) had a known history of CKD. Although 85% (n=57) of these patients had received nephrology follow-up in the past year, only 7.4% (n=5) had a planned dialysis schedule. The remaining 62 patients (92.6%) were not enrolled in a dialysis program despite nephrology follow-up and had to initiate dialysis in the emergency department. As a result, all

67 patients with a CKD history received their first dialysis in the emergency department.

Discussion and Conclusion

Acute kidney injury and exacerbations of chronic kidney disease (CKD) account for a significant portion of emergency department visits and frequently necessitate the initiation of hemodialysis under urgent rather than planned conditions. This scenario directly impacts both patient survival and the utilization of healthcare resources. Recent studies have shown that early nephrology follow-up and planned dialysis transitions can reduce mortality and decrease complication rates. However, evidence regarding the effectiveness of this process in current clinical practice remains limited. In this study, we compared the demographic characteristics. clinical findings. and outcomes of patients who presented with emergency dialysis needs to assess the role and effectiveness of nephrology follow-up.

The average age of patients presenting with emergency hemodialysis needs was 66.6 years. indicating a higher demand for such interventions among the elderly population. Similarly, Han et al. reported in their systematic review that age is a determining factor in emergency department use among chronic hemodialysis patients. with a mean age above 65 years[5]. The predominance of male patients in our study is consistent with U.S. database analyses. which also identify male gender as more common in CKD[9,10]. The prevalence of diabetes and hypertension as comorbidities aligns with known etiologies of CKD and is also supported by European dialysis reports[4]. However, the fact that only 21.8% of patients were under nephrology follow-up in the past year suggests either delayed diagnosis or insufficient regular monitoring.

Severe metabolic acidosis was the most common indication for emergency dialysis in our study, consistent with findings in the literature[11,12]. Nevertheless, some studies have identified hyperkalemia and volume overload as more frequent reasons for dialysis initiation in emergency settings[13]. This discrepancy may reflect the patient profile of our institution and possibly systemic gaps in monitoring, which allow metabolic imbalances to progress to critical levels. These findings indicate areas for improvement not only at the individual but also at the organizational level.

It was noted that patients under nephrology follow-up represented a minority of emergency dialysis cases, while patients without follow-up frequently presented with severe metabolic derangements. The dominance of life-threatening conditions such as refractory metabolic acidosis in the non-follow-up group supports the view that regular nephrology follow-up can prevent critical clinical consequences. not just biochemical imbalances. Previous studies have also shown that nephrology follow-up is associated with lower hospital admission and complication rates[7]. Similarly, systematic reviews have indicated that patients under regular dialysis follow-up present to emergency departments mainly due to technical issues rather than metabolic complications[14]. Our finding that all patients undergoing dialysis for uremic symptoms were from the non-follow-up group supports this observation.

Interestingly, uremic signs and symptoms were observed only in patients without a history of CKD. This suggests that dialysis needs in these individuals become apparent only after reaching a symptomatic stage, resulting in delayed intervention. This observation highlights the importance of early diagnosis. As AKI and CKD exacerbations can trigger systemic inflammation and organ dysfunction[15]. The fact that refractory metabolic acidosis was the most frequent dialysis indication in both groups suggests that follow-up alone may not suffice in preventing metabolic deterioration under current healthcare practices.

Higher levels of WBC, neutrophils and IG in patients under follow-up may indicate a higher incidence of inflammatory or infectious conditions. Moreover, the lower eGFR and higher creatinine levels in this group suggest that nephrology follow-up is more commonly performed in patients with advanced disease stages.

Although hemoglobin and hematocrit levels were lower in patients under nephrology follow-up, the differences were not statistically significant. This may reflect the more advanced CKD stage in this group. The lack of significant differences in electrolyte levels between the groups indicates a comparable metabolic burden at presentation. It has been previously noted that electrolyte imbalances support the decision to initiate dialysis but are not sufficient as standalone indicators[16,17].

Blood gas analysis showed that PO_2 levels were significantly lower in patients with nephrology follow-up, possibly reflecting greater pulmonary dysfunction or anemia. Singbartl and Joannidis have emphasized that tissue oxygenation capacity declines in progressive kidney disease, contributing to systemic complications[1]. Conversely, higher lactate levels in patients without follow-up may indicate more pronounced circulatory stress and hypo perfusion. Han et al. have reported that lactate levels have prognostic value in emergency admissions and are associated with increased mortality[18–20].

The significantly lower 28-day mortality in patients with nephrology follow-up highlights the protective effect of early nephrology intervention on short-term survival. This finding is supported by a study by Fang

et al. which demonstrated that fluid overload prior to continuous RRT increased 28-day mortality in sepsis-associated AKI[21]. The importance of early intervention and appropriate fluid management is thus underscored. Additionally, the elevated 28-day mortality observed in our patients who started dialysis emergently aligns with findings from other studies, which report a 28-day mortality rate of 22.7% in such cases[22].

However, the lack of significant difference in 90-day mortality suggests that mid-term outcomes are influenced not only by follow-up but also by comorbidities and continuity of care. This highlights the need for a comprehensive patient management strategy to improve long-term survival.

The significantly higher rate of enrollment in routine dialysis programs among patients with nephrology follow-up demonstrates the role of monitoring in facilitating planned dialysis transitions. Previous reports also emphasize the positive effect of planned dialysis initiation on survival[4]. Nonetheless, the absence of significant differences in 90-day hospital readmission rates suggests that long-term outcomes depend on more complex factors. Cardiovascular and infectious complications are among the most common causes of readmissions in dialysis patients. Approximately 34% of dialysis patients hospitalized for cardiovascular events are readmitted within 30 days. and only 43% of these readmissions are due to cardiovascular causes, indicating a multifactorial etiology[22].

Finally, it was observed that most patients with a known history of CKD who were under nephrology follow-up had not been enrolled in a routine dialysis program and underwent their first dialysis session in the emergency department. This finding implies that the mere presence of nephrology follow-up is insufficient; rather, the effectiveness of the follow-up process is what matters. Without a proactive approach to dialysis planning in high-risk individuals, emergency admissions and complication rates are likely to increase. Therefore, prioritizing not only outpatient monitoring but also proactive dialysis planning is essential in the management of CKD.

Regular nephrology follow-up plays a critical role in reducing short-term mortality and promoting planned transition to dialysis in patients requiring emergency dialysis. Our study shows that patients without nephrology monitoring present with more severe clinical conditions. particularly life-threatening metabolic derangements. However, the mere presence of follow-up is not sufficient; what is essential is the effectiveness of the monitoring process and proactive planning. To improve long-term outcomes, comprehensive patient management strategies, including early nephrology engagement and timely RRT planning, must be integrated into clinical practice.

Researcher Contribution Statement:
Idea and design: S.E.; Data collection and processing: E.D.; Analysis and interpretation of data: S.E., E.D.; Writing of significant parts of the article: E.D.
Support and Acknowledgement Statement:
None apliciable.
Conflict of Interest Statement:
There is no conflict of interest between authors
Ethics Committee Approval Information:
Approving Committee: UNIVERCİTY OF HEALTH SCIENCE TURKEY, BURSA SEHİR TRAINING AND RESEARCH HOSPITAL ETHİCALS COMİTEE
Approval Date: 11/2023
Decision No: KAEK-2023-21/11

References

1. Singbartl K. Joannidis M. Short-term effects of acute kidney injury. Crit Care Clin. 2015;31(4):751–62.
2. Li PK. Burdmann EA. Mehta RL. Acute kidney injury: global health alert. Kidney Int. 2013;83(3):372–6.
3. Murdeshwar HN. Anjum F. Hemodialysis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–. [cited 2025 Jun 3]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK563296/
4. Kramer A. Pippias M. Noordzij M. Stel VS. Afentakis N. Ambühl PM. et al. The ERA-EDTA Registry Annual Report 2015: a summary. Clin Kidney J. 2018;11(1):108–22.
5. Han G. Bohmart A. Shaaban H. Mages K. Jedlicka C. Zhang Y. et al. Emergency department utilization among maintenance hemodialysis patients: a systematic review. Kidney Med. 2021;4(2):100391.
6. Loran MJ. McErlean M. Eisele G. et al. The emergency department care of hemodialysis patients. Clin Nephrol. 2002;57(6):439–43.
7. Saran R. Robinson B. Abbott KC. et al. US Renal Data System 2019 Annual Data Report: epidemiology of kidney disease in the United States. Am J Kidney Dis. 2020;75(1 Suppl 1):S1–S64.
8. Baugh CW. Wang TJ. Caterino JM. et al. Emergency department management of patients with febrile neutropenia: guideline concordant or overly aggressive? Acad Emerg Med. 2017;24(1):83–91.
9. Johansen KL. Gilbertson DT. Li S. Liu J. Roetker NS. Ku E. et al. US Renal Data System 2023 Annual Data Report: Epidemiology of kidney disease in the United States. Am J Kidney Dis. 2024;83(4 Suppl 1):A8–A13.
10. Pippias M. Kramer A. Noordzij M. Afentakis N. Alonso de la Torre R. Ambühl PM. et al. The ERA-EDTA Registry Annual Report 2014: a summary. Clin Kidney J. 2017;10(2):154–69.
11. Morales KJM. Mondragon AR. Most frequent cause of dialysis emergency and precipitating factor in patients with chronic kidney disease attending the emergency department of HGZ 50 in San Luis Potosí. Mexico. Int J Crit Care Emerg Med. 2023;9:153.
12. Ruiz-Mejía R. Ortega-Olivares LM. Naranjo-Carmona CA. Suárez-Otero R. Treatment of hyperkalemia in patients with chronic kidney disease on dialysis therapy. Internal Med Mex. 2017;33:778–96.
13. Aktepe M. Akdeniz YS. İpekci A. Çakmak F. Altıparmak MR. İkizceli İ. Acil serviste hemodiyaliz endikasyonu konulan hastaların analizi. Phnx Med J. 2020;2(3):145–51.
14. Özpolat Ç. Altunbaş E. Acil serviste acil hemodiyaliz endikasyonu konulan hastaların retrospektif analizi. Anatolian J Emerg Med. 2022;5(3):124–7.

15. Faubel S. Shah PB. Immediate consequences of acute kidney injury: the impact of traditional and nontraditional complications on mortality. Adv Chronic Kidney Dis. 2016;23(3):179–85.
16. Kovesdy CP. Lott EH. Lu JL. Malakauskas SM. Ma JZ. Molnar MZ. et al. Hyponatremia. hypernatremia. and mortality in patients with chronic kidney disease with and without congestive heart failure. Circulation. 2012;125(5):677–84.
17. Madan VD. Novak E. Rich MW. Impact of change in serum sodium concentration on mortality in patients hospitalized with heart failure and hyponatremia. Circ Heart Fail. 2011;4(5):637–43.
18. Kim SG. Lee J. Yun D. et al. Hyperlactatemia is a predictor of mortality in patients undergoing continuous renal replacement therapy for acute kidney injury. BMC Nephrol. 2023;24:11.
19. Chen M. Lv D. Prognostic value of serum lactate level for mortality in patients with acute kidney injury. Eur J Med Res. 2024;29:295.
20. Zhu X. Xue J. Liu Z. Dai W. Xu H. Zhou Q. et al. The lactate/albumin ratio predicts mortality in critically ill patients with acute kidney injury: an observational multicenter study on the eICU database. Int J Gen Med. 2021;14:10511–25.
21. Fang J. Wang M. Gong S. Cui N. Xu L. Increased 28-day mortality due to fluid overload prior to continuous renal replacement in sepsis associated acute kidney injury. Ther Apher Dial. 2022;26(2):288–96.
22. Kim IY. Kim S. Ye BM. Kim MJ. Kim SR. Lee DW. et al. Effect of fluid overload on survival in patients with sepsis-induced acute kidney injury receiving continuous renal replacement therapy. Sci Rep. 2023;13(1):2796.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 263-269, 2025
DOI: https://doi.org/10.32708/uutfd.1711705

ORIGINAL RESEARCH

A Novel Quantitative Index for Objective MRI Grading of Gastrocnemius Muscle Strain

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ABSTRACT

This study presents a quantitative MRI-based method to improve the objectivity and diagnostic accuracy of grading calf muscle strains. Ninety-six patients with MRI-confirmed medial gastrocnemius injuries who underwent bilateral lower leg MRI between January 2018 and December 2023 were retrospectively analyzed. Injuries were graded using the Dai classification (Grades I–III). Axial T2-weighted SPAIR images were used to measure the medial gastrocnemius cross-sectional area (CSA) at the distal one-third between the knee joint and the musculotendinous junction. The Gastrocnemius Medial Head Ratio (GCM ratio) was calculated by dividing the CSA of the injured side by that of the uninjured side. Two experienced radiologists independently performed the measurements twice. Intra- and interobserver reliability were assessed using intraclass correlation coefficients (ICC) and Cohen's kappa. A one-way ANOVA with the Bonferroni correction was used for group comparisons. ROC analysis evaluated the diagnostic performance of the GCM ratio. The GCM ratio significantly differed among Grade I (1.03 ± 0.02), Grade II (1.18 ± 0.06), and Grade III (1.35 ± 0.05) injuries ($p < 0.001$). Ipsilateral CSA increased with injury severity, whereas contralateral CSA remained consistent. CSA measurements showed excellent intra- and interobserver reliability (ICC > 0.87), and lesion grading demonstrated substantial to almost perfect agreement ($\kappa = 0.75–0.88$). ROC analysis revealed strong diagnostic accuracy (AUC = 0.986 for Grade I vs. II; AUC = 1.000 for Grade II vs. III). The GCM ratio provides a reproducible and objective MRI marker to aid in grading gastrocnemius strains, offering excellent diagnostic performance and clinical applicability.

Keywords: Tennis leg. Gastrocnemius. MRI. Cross-sectional area. Muscle grading. quantitative imaging.

Gastroknemius Kas Yırtıklarının Objektif MRG Sınıflaması için Yeni Bir Kantitatif İndeks

ÖZET

Bu çalışmanın amacı, medial gastroknemius kasının yaralı ve sağlam taraflardaki kesitsel alan (KA) ölçümlerini karşılaştırarak baldır kası yaralanmalarının sınıflamasında nesnelliği ve tanısal doğruluğu artırmak üzere MRG tabanlı kantitatif bir yaklaşım geliştirmektir. Bu retrospektif çalışmaya, Ocak 2018 ile Aralık 2023 tarihleri arasında çift taraflı alt ekstremite MRG'si çekilmiş ve MRG ile baldır kası yırtığı doğrulanmış 96 hasta dahil edildi. Kas yaralanmaları Dai sınıflamasına göre (Grade I–III) sınıflandırıldı. Medial gastroknemius kasının KA ölçümleri, diz eklemi ile muskulo-tendinöz bileşke arasındaki distal üçte birlik seviyede elde edilen aksiyel T2-ağırlıklı SPAIR sekanslar kullanılarak yapıldı. Gastroknemius Medial Baş Oranı (GKM oranı), yaralı tarafın KA'sının sağlam tarafın KA'sına oranlanmasıyla hesaplandı. İki deneyimli radyolog ölçümleri birbirinden bağımsız ve iki kez gerçekleştirdi. İntra ve inter-observer güvenilirlik intraclass correlation coefficient (ICC) ve Cohen's kappa istatistikleri ile değerlendirildi. Gruplar arası karşılaştırmalarda Bonferroni düzeltmeli tek yönlü ANOVA testi kullanıldı. GKM oranının tanısal performansı ROC analizi ile değerlendirildi. GKM oranı Grade I (1,03 ± 0,02), Grade II (1,18 ± 0,06) ve Grade III (1,35 ± 0,05) arasında anlamlı fark gösterdi ($p < 0,001$). Yaralı taraftaki KA, yaralanma şiddetiyle birlikte artış gösterirken; sağlam taraf KA'sı gruplar arasında anlamlı fark göstermedi. KA ölçümlerinin inter- ve intra-observer güvenilirliği mükemmeldi (ICC > 0,87), lezyon derecelendirmesi ise anlamlı düzeyde tutarlıydı ($\kappa = 0,75–0,88$). ROC analizi, GKM oranı için yüksek tanısal doğruluk gösterdi (Grade I ile II için AUC = 0,986; Grade II ile III için AUC = 1,000). Gastroknemius kasının GKM oranına dayalı kantitatif MRG ölçümleri, yaralanma sınıflamasında yüksek tekrarlanabilirlik ve güçlü tanısal performans ile güvenilir ve objektif bir değerlendirme aracı sunmaktadır.

Anahtar Kelimeler: Tenisçi bacağı. Gastroknemius. MRG. Kesitsel alan. Kas sınıflaması. kantitatif görüntüleme.

Date Received: 1.June.2025
Date Accepted: 20.July.2025

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Tennis leg, a common calf muscle injury, typically refers to a partial rupture of the medial gastrocnemius at the level of the musculotendinous junction, often seen in recreational athletes.[1] It is characterized by a sudden onset of sharp pain in the calf, frequently described as a "snapping" sensation during activities involving acceleration or abrupt movements. Clinically, patients present with localized tenderness, swelling, and difficulty with weight-bearing or pushing off on the affected leg. [2,3] Diagnosis of tennis leg primarily relies on clinical history and physical examination, but imaging, particularly magnetic resonance imaging (MRI), plays a pivotal role in confirming the diagnosis, assessing the extent of the injury, and guiding treatment.[4,5] Treatment options range from conservative management, including rest and physiotherapy, to surgical intervention in more severe cases.[3] Accurate MRI grading of calf muscle injuries is not only critical for determining the appropriate treatment strategy, predicting prognosis, and estimating the timeline for return to sports, recreational, or daily activities, but also plays a key role in guiding clinical decisions following diagnosis, such as selecting conservative versus more prolonged treatment approaches, tailoring rehabilitation protocols, identifying patients at risk for delayed recovery or recurrence, and facilitating standardized follow-up by providing a reproducible and objective reference for radiological monitoring.[6] Thus, the classification system is particularly valuable after diagnosis, during treatment decision-making, and follow-up monitoring.

MRI-based grading of calf muscle injuries serves as a standard tool for radiologists and clinicians.[7] However, the current classification systems rely heavily on subjective evaluation, leading to variability in interpretations.[8,9] These methods often lack quantitative measures, challenging the standardization of assessments across radiologists or institutions.[9]

This study aimed to introduce a quantitative approach to refine MRI grading of calf muscle injuries by comparing the cross-sectional area (CSA) of the injured muscle to that of the contralateral, healthy side. We hypothesize that this side-to-side comparison can provide a more accurate and objective framework for classifying injury severity, ultimately enhancing clinical decision-making and improving prognostic predictions.

Material and Method

Patients and Study Design

This retrospective radiological study reviewed the institutional digital database to identify patients who underwent MRI for suspected tennis leg between January 2018 and December 2023. As clinical follow-up data were not required, only the availability and quality of imaging were considered. All MRIs were obtained at a public university-affiliated training and research hospital over a six-year period. One hundred one patients with MRI-confirmed calf muscle strain injury (CMSI) were initially identified. Five patients were excluded because their imaging was unilateral, which prevented a side-to-side comparison. The final study sample consisted of 96 patients with bilateral lower leg MRI examinations deemed adequate for radiological analysis. Clinical informed consent was not required, as this study was based solely on anonymized imaging data. Institutional review board approval was obtained from the Antalya Training and Research Hospital Clinical Research Ethics Committee (Approval date and issue: 2024/15-18). The study adhered to the ethical principles of the Declaration of Helsinki and followed the STROBE guidelines for observational studies.[10]

MR Imaging

All MRI examinations were performed using standardized bilateral lower leg imaging protocols on two different MRI systems: a 1.5 Tesla Achieva DS Advance and a 3.0 Tesla Ingenia unit (both by Philips Healthcare, Eindhoven, The Netherlands). Imaging was conducted with the patient in the supine position using a 16-channel Sense XL Torso coil, which allows for simultaneous coverage of both lower legs. Although MRI examinations for post-traumatic evaluation are commonly requested unilaterally in routine clinical practice based on symptom localization, at our institution, lower extremity MRIs are routinely acquired bilaterally. This institutional protocol is driven by technical considerations and coil configuration, enabling comprehensive visualization of both crura within the same field of view. The bilateral acquisition not only facilitates a more consistent and reproducible imaging workflow but also allows direct side-to-side comparisons, which served as the basis for the quantitative assessment employed in this study. The imaging protocol included axial and coronal T1-weighted turbo spin echo (TSE) and T2-weighted SPAIR sequences. All images were stored and reviewed on a PACS workstation (Sectra IDS7, Version 18.2, Sectra AB, Sweden), which operates on a client-server architecture.

MRI evaluation and measurements

The grading of muscle injuries was based on the classification proposed by Dai et al., which divides muscle injuries into three groups.[4] Grade 1 is characterized by edema with no architectural disruption or macroscopic tear. Grade II is characterized by partial muscle disruption with hematoma or local disruption of muscle architecture. Grade III is characterized by a complete muscle

disruption with local hematoma covering the tear cavity or tendon detachment (Figure 1).

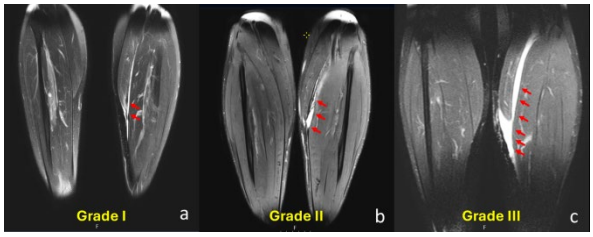

Figure 1.

Representative fat-suppressed T2-weighted coronal MRI images demonstrating the three grades of gastrocnemius muscle injury according to the Dai classification. (a) Grade I: mild intramuscular edema without visible structural disruption (red arrows). (b) Grade II: partial fiber disruption with focal hematoma and architectural distortion (red arrows). (c) Grade III: complete muscle rupture with fluid-filled tear cavity and retraction (red arrows).

Cross-sectional area measurements of the medial head of the gastrocnemius muscle were performed on both the injured and uninjured sides using axial T2-weighted SPAIR sequences. The axial level was selected at the distal one-third of the distance between the knee joint line and the musculotendinous junction. For each measurement, the fascial border of the muscle, characterized by a hypointense signal, was used as the anatomical reference. The measured area values were recorded in square millimeters (mm^2). The gastrocnemius medial head ratio (GMC ratio) was calculated by dividing the cross-sectional area of the injured side by that of the contralateral, uninjured side (Figure 2). The GMC ratio reflected the extent of edema and/or retraction on the injured side, with higher values indicating more pronounced muscle involvement and a higher grade.

Two radiologists with more than 10 years of experience in musculoskeletal imaging and MRI independently performed all evaluations. Each observer conducted the measurements twice, 15 days apart, while being blinded to their previous results and the measurements of the other observer. After completing all individual assessments, a final consensus on the injury grade was reached through a conjoint review meeting.

Statistical Analysis

Descriptive statistics were reported as means ± standard deviation (SD) for continuous variables and as frequencies and percentages for categorical variables. The normality of continuous variables was assessed using both the Kolmogorov–Smirnov and Shapiro–Wilk tests. One-way analysis of variance (ANOVA) was used to compare the Gastrocnemius Medial Head cross-sectional area (CSA) and GMC ratios across the three injury grades. Post-hoc pairwise comparisons were conducted using the Bonferroni correction to control for multiple testing. Receiver Operating Characteristic (ROC) curve analysis was used to evaluate the diagnostic performance of the GMC ratio in distinguishing between muscle injury grades. The area under the curve (AUC), optimal cut-off values, sensitivity, and specificity were calculated using Youden's Index. Intra-observer and inter-observer reliability were evaluated using Intraclass Correlation Coefficients (ICC) with 95% confidence intervals (CIs) for continuous variables, and Cohen's Kappa statistic with 95% CI for categorical variables. ICC values were interpreted as poor (<0.50), moderate (0.50–0.75), good (0.75–0.90), or excellent (>0.90). Kappa values were interpreted as poor (< 0.00), slight (0.00–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), or almost perfect (0.81–1.00). The consensus-based injury grade was used for the final analysis, while the average of all cross-sectional area measurements was applied for ratio calculations. A p-value < 0.05 was considered statistically significant for all analyses.

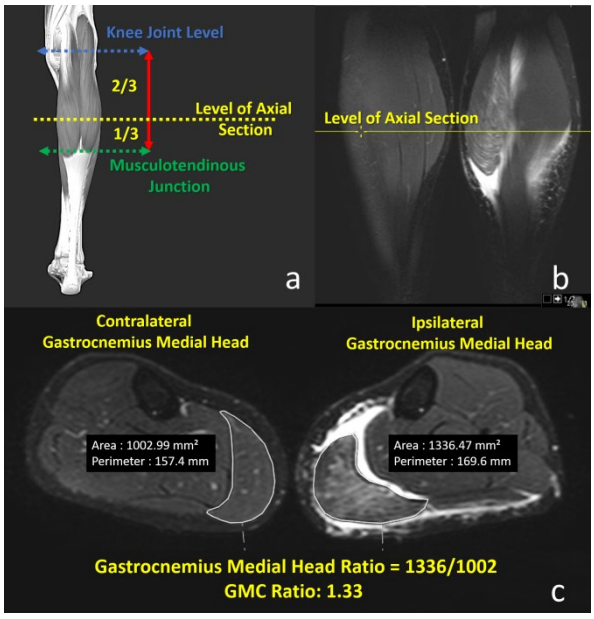

Figure 2.

Measurement of the medial head of the gastrocnemius muscle on axial T2-weighted SPAIR sequences. (a) Schematic illustration of the axial section level, positioned at the distal one-third of the distance between the knee joint and the musculotendinous junction. (b) Coronal MRI showing the selected axial plane. (c) Axial images of the contralateral (left) and ipsilateral (right) medial gastrocnemius heads. Cross-sectional area was measured using the fascial border as a reference. The Gastrocnemius Medial Head Ratio (GMC ratio) was calculated as the area of the injured side divided by the uninjured side.

Results

The final analysis included 96 patients. The mean age was 44.9 ± 10.4 years, with a range of 19 to 74 years. Of the participants, 70 (72.9%) were male, and 26 (27.1%) were female. Additional demographic characteristics are summarized in Table I.

Table I. Demographic characteristics of the patients.

Variables	Data
Number of patients	96
Age (years±SD, range)	44,9±10.4 (19-74)
Sex (n, %)	
Male	70 (72.9%)
Female	26 (27.1%)
Side	
Right	48 (50%)
Left	48 (50%)
Weight (kg±SD, range)	83.4±15.1 (55-125)
Height (cm±SD, range)	172.9±8.9 (150-192)
BMI (kg/m²±SD, range)	27.7±4.0 (20.2-38.5)

Abbreviations: n: number, SD: Standard Deviation, BMI: Body Mass Index.

Reliability analysis showed excellent intra-observer agreement for cross-sectional area measurements, with ICC values ranging from 0.963 to 0.976 for the gastrocnemius medial head and 0.945 to 0.984 for the contralateral side. The inter-observer ICC values also demonstrated high reliability, ranging from 0.869 to 0.937. The agreement for lesion grading was substantial to almost perfect, with intra-observer kappa values of 0.846 and 0.852, and inter-observer kappa values of 0.747 and 0.883 (Table II).

Table II. Results of reliability analysis.

Variables	Intra-observer Reliability, ICC or Kappa (95% CI)	
	A t_1 vs. A t_2	*B t_1 vs. B t_2*
GCM Ipsilateral	0.967 (0.951-0.978)	0.976 (0.964-0.984)
GCM Contralateral	0.963 (0.945-0.975)	0.971 (0.956-0.980)
Lesion Grade	0.846 (0.744-0.948) *	0.852 (0.746-0.958) *
	Interobserver Reliability, ICC or Kappa (95% CI)	
	A t_1 vs. B t_1	*A t_2 vs. B t_2*
GCM Ipsilateral	0.884 (0.831-0.921)	0.937 (0.907-0.958)
GCM Contralateral	0.869 (0.809-0.910)	0.923 (0.886-0.948)
Lesion Grade	0.747 (0.620-0.874) *	0.883 (0.721-0.945) *

*Kappa 95% CI, A represents the first observer, B represents the second observer.

Abbreviations: ICC: Interclass correlation analysis, CI: Confidence interval, GCM: Gastrocnemius medial head. t_1: First time, t_2: Second time

There was a statistically significant difference in the cross-sectional area of the ipsilateral gastrocnemius medial head with increasing injury severity (p = 0.001). No statistically significant difference was found in the contralateral muscle area across the grades (p = 0.324). The gastrocnemius medial head ratio (GCM ratio) also showed a significant difference among the groups, with increasing values corresponding to greater injury severity (p = 0.001). The mean GCM ratio was 1.03 ± 0.02 in Grade 1, 1.18 ± 0.06 in Grade 2, and 1.35 ± 0.05 in Grade 3 injuries (p = 0.001). Post-hoc analysis with the Bonferroni correction revealed that the ipsilateral area and the GCM ratio differed significantly between all grades (Table III and Figure 3).

Table III. Comparison of the Gastrocnemius Medial Head Ratio between grades.

Variable		Grade 1 *(n:17)*	Grade 2 *(n:63)*	Grade 3 *(n:16)*	p-value
GCM Ipsilateral	*(mm²±SD)*	1420.0±300.8	1732.9±362.3	2079.2±377.5	0.001[1]
	Range	796-1866	747-2485	1379-2732	
GCM Contralateral	*(mm²±SD)*	1369.7±303.3	1475.5±336.2	1534.2±284.0	0.324[1]
	Range	742-1851	625-2297	963-1933	
GCM Ratio	*(value ±SD)*	1.03±0.02	1.18±0.06	1.35±0.05	0.001[1]
	Range	1.01-1.10	1.06-1.29	1.3-1.49	

[1] ANOVA. Multiple post-hoc comparisons with the Bonferroni test revealed a significant difference between groups at the 0.005 level for GMC Ipsilateral and GMC Ratio.

Abbreviations: SD: Standard Deviation, GCM: Gastrocnemius medial head.

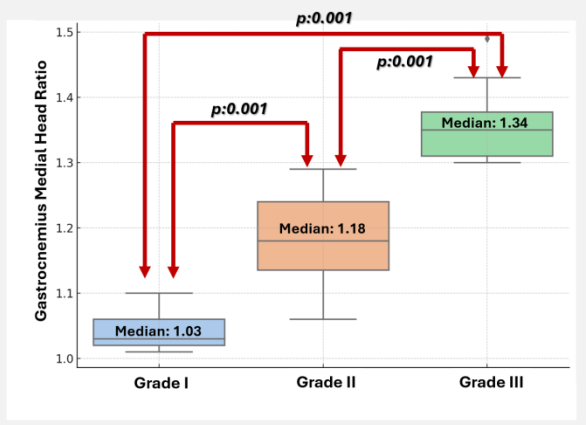

Figure 3.
Boxplot comparing the Gastrocnemius Medial Head Ratio (GMC ratio) across three muscle injury grades.

Receiver operating characteristic (ROC) curve analysis demonstrated excellent diagnostic performance of the GCM ratio (Figure 4). In distinguishing between Grade 1 and Grade 2 injuries, the area under the curve (AUC) was 0.986, with a best threshold of 1.09, yielding a sensitivity of 93.7% and a

specificity of 94.1%. For the differentiation of Grade 2 from Grade 3, the AUC was 1.000, with a best threshold of 1.30 and perfect sensitivity and specificity (100%) (Figure 5).

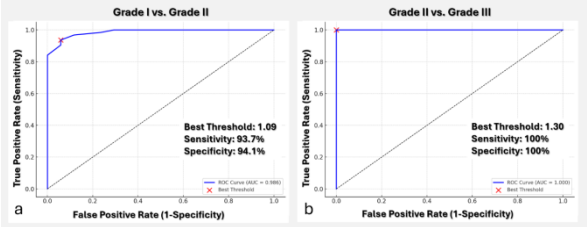

Figure 4.

Receiver Operating Characteristic (ROC) curves illustrating the diagnostic performance of the Gastrocnemius Medial Head Ratio (GMC ratio) for distinguishing between muscle injury grades. (a) ROC curve for Grade I vs. Grade II showing an AUC of 0.986 with a best threshold of 1.09, yielding a sensitivity of 93.7% and specificity of 94.1%. (b) ROC curve for Grade II vs. Grade III demonstrating perfect diagnostic performance with an AUC of 1.000, best threshold of 1.30, and 100% sensitivity and specificity.

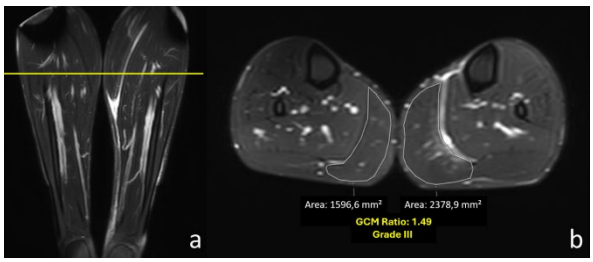

Figure 5.

MRI example of a patient with a Grade III calf muscle injury. (a) Coronal fat-suppressed T2-weighted image showing the level of axial section (yellow line) used for cross-sectional measurement. (b) Axial image at the corresponding level demonstrates medial gastrocnemius muscle measurements on the injured (right) and contralateral (left) sides. The calculated GCM ratio is 1.49, indicating significant edema and retraction consistent with a Grade III injury.

Discussion and Conclusion

This study demonstrates that quantitative analysis of the medial gastrocnemius muscle cross-sectional area (CSA) using MRI can significantly improve the objectivity and diagnostic accuracy of muscle injury grading. We found a strong correlation between ratio values and established injury severity grades by introducing the Gastrocnemius Medial Head Ratio (GCM ratio) as a measurable parameter. The GCM ratio increased consistently across Grades I to III, and ROC curve analysis revealed excellent discriminative performance, particularly between Grades II and III, where the diagnostic accuracy reached 100%. These findings suggest that supplementing conventional MRI-based grading with side-to-side CSA comparisons offers a reproducible and highly sensitive method for refining the assessment of calf muscle injuries.[11–13]

Numerous MRI-based classification systems have been developed to evaluate skeletal muscle injuries, particularly in athletes, ranging from simple clinical grading to anatomically detailed imaging models. The traditional Grade I–III system remains popular due to its simplicity; however, it lacks anatomical specificity and prognostic precision.[13] More advanced approaches, such as the modified Peetrons grading[14] and the British Athletics Muscle Injury Classification[15] (BAMIC), have attempted to incorporate MRI findings, focusing on the severity of structural disruption and the type of tissue involved. The Olympic Park classification, introduced by Prakash et al., [16] emphasized the involvement of connective tissue elements, such as the epimysium, aponeurosis, and tendon, and demonstrated a correlation with return-to-play duration. Additionally, anatomically complex schemes, such as the Chan classification[14] and the FC Barcelona and Munich consensus[17] models, offer detailed injury mapping but are often hindered by their complexity and reduced interobserver agreement.[9]

As highlighted by Hamilton et al.,[9] a common limitation among these systems is their heavy reliance on qualitative descriptors and expert-level anatomical interpretation, resulting in limited reproducibility and poor integration into routine radiological workflows. For instance, in the BAMIC system, categorizing a lesion as "myotendinous" versus "intramuscular" may vary depending on the radiologist's experience and anatomical familiarity.[17,18] Similarly, the Munich model's distinction between "functional" and "structural" injuries is based on subjective clinical assessment rather than reproducible imaging features. Although the Olympic Park classification incorporates MRI findings more directly, it still depends on the reader's ability to discern subtle disruptions in connective tissue structures—findings that may not be consistently visualized across all sequences. Moreover, most systems lack clearly defined, quantifiable thresholds, which reduces standardization and increases interobserver variability. These issues limit their practicality, particularly in time-constrained clinical settings that require rapid, consistent, and reproducible evaluation tools.

To address these shortcomings, the present study introduces the Gastrocnemius Medial Head Ratio (GCM ratio), a novel, quantitative, and objective index based on the comparison of the cross-sectional area of the medial gastrocnemius muscle between

limbs.[16] This measurement can be easily derived from standard axial T2-weighted images without the need for post-processing, advanced segmentation tools, or deep anatomical knowledge, and it demonstrated excellent intra- and interobserver reliability. Unlike classifications that emphasize tissue type or site-specific disruption alone, the GCM ratio reflects the cumulative impact of edema, muscle fiber retraction, and structural damage elements that correlate with clinical severity and functional impairment. Thus, this method provides a reproducible and clinically applicable grading tool suitable for both initial diagnosis and longitudinal follow-up in sports medicine practice, effectively bridging the gap between complex descriptive systems and practical clinical utility.[19]

Furthermore, our findings highlight the diagnostic potential of the GCM ratio, with near-perfect discrimination between injury grades, as demonstrated by AUC values exceeding 0.98. This contrasts with prior studies, such as those validating the BAMIC[15] or Olympic Park systems,[16] where interrater variability and moderate prognostic correlations limited widespread clinical implementation. By integrating a continuous variable into the injury assessment process, our approach aligns with the literature's calls for more quantifiable, MRI-based parameters that support both grading and prognostic stratification.[6,20] Importantly, the GCM ratio does not require advanced software or high-resolution 3D reconstructions, making it feasible for use in standard radiology workflows. As such, this study provides a significant advancement in the objective evaluation of gastrocnemius muscle injuries and may serve as a foundation for future validation studies aimed at standardizing muscle injury classification and prognosis.

The primary strength of this study lies in the introduction of a novel, objective, and easily applicable MRI-based index—the Gastrocnemius Medial Head Ratio (GCM ratio)—for grading calf muscle injuries.[11,19] Unlike existing classification systems that rely predominantly on qualitative assessment or complex anatomical subtyping, the GCM ratio provides a reproducible, quantitative metric that can be integrated into routine clinical imaging protocols.[9] The high intra- and interobserver reliability observed in this study further supports its applicability in various radiological settings. Additionally, the use of bilateral MRIs enabled direct side-to-side comparison, enhancing the precision of CSA-based evaluations and reducing interindividual variability.[6]

However, the study has some limitations. First, its retrospective design may introduce selection bias, although strict imaging inclusion criteria were applied. Second, while the Dai classification was used as a reference standard for injury grading, it remains a descriptive system and lacks histopathological correlation.[15] Third, although the GCM ratio showed excellent diagnostic performance, this study did not include clinical outcome measures such as return-to-sport time or functional recovery, which would be essential for evaluating prognostic utility. Finally, the findings are based on a single-center cohort and may require external validation across broader populations and imaging platforms before generalizability can be confirmed.[12,21]

This study introduces the Gastrocnemius Medial Head Ratio (GCM ratio) as a reliable and objective MRI-based index for grading calf muscle injuries. By quantifying side-to-side differences in cross-sectional area, the GCM ratio enhances the precision and reproducibility of injury assessment beyond conventional qualitative systems. The strong correlation between ratio values and injury severity, along with excellent diagnostic performance, supports its potential as a practical adjunct to existing classification models. Future prospective studies linking this index to clinical outcomes such as recovery time and return-to-play are warranted to further validate its prognostic value and facilitate its integration into standardized muscle injury assessment protocols.

Researcher Contribution Statement:
Idea and design: K.K.K., N.K.; Data collection and processing: M.Y., M.B.E.; Analysis and interpretation of data: O.K., N.K.; Writing of significant parts of the article: K.K.K., M.Y., M.B.E.
Support and Acknowledgement Statement:
It was covered by the investors in the study.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: The Institutional Review Board approved the study protocol
Approval Date: 2024
Decision No: 15-18

References

1. Coffey R, Khan YS. Gastrocnemius Rupture. *Comparative Veterinary Anatomy*. Published online July 24, 2023:1211-1220. doi:10.1016/b978-0-323-91015-6.00167-9
2. Halabchi F, Tavana MM, Seifi V, Zarandi MM. Medial Gastrocnemius Strain: Clinical Aspects and Algorithmic Approach. *Med J Islam Repub Iran*. 2024;38(1). doi:10.47176/MJIRI.38.55,
3. Porrino J, Kent R, Haims A, Kani K, Lee H. "Tennis leg": An update with emphasis on the imaging evaluation. *PM and R*. 2023;15(9):1210-1214. doi:10.1002/PMRJ.12979,
4. Dai M, Liu X, Liu X, Yang L, Duan D. Diagnosis and follow-up MRI evaluation of tennis leg:New understanding of the pathogenesis and imaging. *Injury*. 2021;52(11):3355-3361. doi:10.1016/j.injury.2021.06.035
5. Kilic KK, Yuncu M, Dogruoz F, Buyukarslan V, Ertan MB, Kose O. Correlation between MRI findings and functional outcomes in patients with calf muscle strain injuries: a

retrospective study on 78 patients. *BMC Musculoskelet Disord*. 2024;25(1):1-9. doi:10.1186/S12891-024-08119-0/TABLES/5

6. Sergot L, Kho J, Collins H, Williams J, Murray R, Chakraverty J. MRI classification of calf injuries — a reliability study and correlation with return to play in professional rugby players. *Skeletal Radiol*. 2023;52(1):61-66. doi:10.1007/S00256-022-04108-3,
7. Guermazi A, Roemer FW, Robinson P, Tol JL, Regatte RR, Crema MD. Imaging of muscle injuries in sports medicine: Sports imaging series. *Radiology*. 2017;282(3):646-663. doi:10.1148/RADIOL.2017160267,
8. Hamilton B, Alonso JM, Best TM. Time for a paradigm shift in the classification of muscle injuries. *J Sport Health Sci*. 2017;6(3):255-261. doi:10.1016/j.jshs.2017.04.011
9. Hamilton B, Valle X, Rodas G, et al. Classification and grading of muscle injuries: A narrative review. *Br J Sports Med*. 2015;49(5):306. doi:10.1136/BJSPORTS-2014-093551,
10. Vandenbroucke JP, Von Elm E, Altman DG, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and elaboration. *Epidemiology*. 2007;18(6):805-835. doi:10.1097/EDE.0B013E3181577511,
11. Isern-Kebschull J, Pedret C, García-Diez AI, et al. Magnetic resonance classification proposal for medial gastrocnemius muscle injuries. *Quant Imaging Med Surg*. 2024;14(11):7958-7968. doi:10.21037/QIMS-24-298/COIF
12. Armfield DR, Kim DHM, Towers JD, Bradley JP, Robertson DD. Sports-Related Muscle Injury in the Lower Extremity. *Clin Sports Med*. 2006;25(4):803-842. doi:10.1016/j.csm.2006.06.011
13. McGowan C. Traumatic Muscle Injuries. *Vet Clin North Am Equine Pract*. 2025;41(1):181-192. doi:10.1016/J.CVEQ.2024.11.009
14. Wangensteen A, Tol JL, Roemer FW, et al. Intra- and interrater reliability of three different MRI grading and classification systems after acute hamstring injuries. *Eur J Radiol*. 2017;89:182-190. doi:10.1016/j.ejrad.2017.02.010
15. Pollock N, James SLJ, Lee JC, Chakraverty R. British athletics muscle injury classification: a new grading system. *Br J Sports Med*. 2014;48(18):1347-1351. doi:10.1136/BJSPORTS-2013-093302,
16. Prakash A, Entwisle T, Schneider M, Brukner P, Connell D. Connective tissue injury in calf muscle tears and return to play: MRI correlation. *Br J Sports Med*. 2018;52(14):929-933. doi:10.1136/BJSPORTS-2017-098362,
17. Mueller-Wohlfahrt HW, Haensel L, Mithoefer K, et al. Terminology and classification of muscle injuries in sport: The Munich consensus statement. *Br J Sports Med*. 2013;47(6):342-350. doi:10.1136/BJSPORTS-2012-091448,
18. Chan O, Del Buono A, Best TM, Maffulli N. Acute muscle strain injuries: A proposed new classification system. *Knee Surgery, Sports Traumatology, Arthroscopy*. 2012;20(11):2356-2362. doi:10.1007/S00167-012-2118-Z,
19. Haddouchi I El, Overgård AB, Hansen MS, Weisskirchner Barfod K, El I, Kettegård H. Optimization of MRI measurements of calf muscle atrophy following acute Achilles tendon rupture. *Journal of the Foot & Ankle*. 2025;19(1):1-6. doi:10.30795/JFOOTANKLE.2025.V19.1873
20. Meek WM, Kucharik MP, Eberlin CT, Naessig SA, Rudisill SS, Martin SD. Calf Strain in Athletes. *JBJS Rev*. 2022;10(3). doi:10.2106/JBJS.RVW.21.00183,
21. Kassiano W, Costa B, Kunevaliki G, et al. Greater Gastrocnemius Muscle Hypertrophy after Partial Range of Motion Training Performed at Long Muscle Lengths. *J Strength Cond Res*. 2023;37(9):1746-1753. doi:10.1519/JSC.0000000000004460,

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 271-275, 2025
DOI: https://doi.org/10.32708/uutfd.1621854

ORIGINAL RESEARCH

The Relationship Between Supraspinatus Tendon Moment Arm Length, Shoulder Anatomical Features, and Shoulder Disorders*

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ABSTRACT

This study aimed to evaluate the relationship between the moment arm length of the supraspinatus tendon and shoulder pathologies. The muscle-tendon moment arm represents the mechanical advantage of a muscle and significantly influences its role as a stabilizer or prime mover. In 83 patients, the presence of biceps tendinitis, rotator cuff tendinosis and tears, acromioclavicular joint degeneration, and footprint cysts were assessed. A perpendicular line was drawn from the center of the humeral head to the center of joint motion, and its length was measured (B). The distance from the rotator cuff footprint to the center of joint motion was also calculated (A). Subsequently, the ratio of these two lengths was determined (A/B). The mean moment arm length ratio (A/B) was 1.53 ± 0.11, the mean subacromial distance was 8.56 ± 1.35 mm, and the mean coracohumeral distance was 8.51 ± 1.51 mm. No significant correlation was identified between moment arm length and shoulder pathologies that could impact diagnosis, characterization, or treatment planning. A reduction in coracohumeral distance was associated with an increased incidence of tendinosis and tears in the subscapular tendon. Similarly, a decrease in subacromial distance correlated with a higher incidence of rotator cuff tears. Although moment arm length is important in surgical planning following tendon rupture, it does not serve as a diagnostic guide. The association between subacromial and coracohumeral distances and rotator cuff tendon tears may reflect a bidirectional relationship, where anatomical narrowing and tendon injury may influence each other.

Keywords*: Moment arm. Rotator cuff. Magnetic resonance.*

Supraspinatus Tendonunun Moment Kolu Uzunluğu ve Omuz Anatomik Özelliklerinin Omuz Patolojileri İle İlişkisi

ÖZET

Bu çalışmanın amacı; supraspinatus tendonunun moment kolu uzunluğu ile omuz patolojileri arasındaki ilişkiyi değerlendirmektir. Kas-tendon moment kolu, bir kasın mekanik avantajını temsil eder ve dengeleyici veya ana taşıyıcı olarak rolünü büyük ölçüde belirler. Bu çalışmada, omuz Manyetik Rezonans görüntüleme kaydı bulunan 83 hastanın biseps ile rotator manşet kaslarının tendinozis ve yırtıkları, akromiyoklavikular eklemde dejenerasyon, kemik kisti gibi patolojiler araştırıldı. Humerus başının en geniş olduğu kesitte başın merkezi işaretlenerek eklem hareket merkezine dik bir çizgi çizilmiş ve bu uzunluk (B) ölçülmüştür. Rotator manşet ayak izinin eklem hareket merkezine uzaklığı (A) hesaplanmış ve ardından bu iki uzunluğun oranı (A/B) belirlenmiştir. Ayrıca, akromiyon sınıflandırılmış ve subakromiyal ile korakohumeral mesafeler ölçülmüştür. Elde edilen sonuçlara göre, moment kolu uzunluğu oranının (A/B) ortalaması 1,53 ± 0,11, subakromiyal mesafe ortalaması 8,56 ± 1,35 mm ve korakohumeral mesafe ortalaması 8,51 ± 1,51 mm olarak hesaplanmıştır. Moment kolu uzunluğu ile omuz çevresi patolojileri arasında tanımlayıcı, tanı ve tedavi sürecini etkileyebilecek anlamlı bir ilişki tespit edilmemiştir. Bununla birlikte, Tip 2 ve Tip 3 akromiyonların, Tip 1'e kıyasla daha dar bir subakromiyal mesafe oluşturduğu görülmüştür.Korakohumeral mesafe azaldıkça subskapular tendonda tendinozis ve yırtık görülme sıklığının arttığı, subakromiyal mesafenin azalması ile rotator manşet yırtıklarının görülme sıklığının arttığı belirlenmiştir. Sonuç olarak moment kolu uzunluğu, rüptür sonrası cerrahi onarımda önemli bir faktör olmasına rağmen, tanı için yol göstericiliği yoktur . Subakromiyal ve korakohumeral mesafeler ile rotator manşet tendon yırtıkları arasındaki ilişki, anatomik daralma ile tendon hasarının birbirini karşılıklı olarak etkileyebileceği çift yönlü bir ilişkiyi yansıtıyor olabilir.

Anahtar Kelimeler*: Moment kolu. Rotator manşet. Manyetik rezonans.*

Date Received: 19.January.2025
Date Accepted: 20.July.2025

* Presented as an oral presentation at the "33. Ulusal Türk Ortopedi ve Travmatoloji Kongresi" (November 2024, Antalya).

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Shoulder disorders are prevalent and frequently associated with trauma, sports activity, and aging[1]. Medical imaging technologies, including ultrasonography, X-rays, magnetic resonance imaging (MRI), and MR arthrography, provide valuable diagnostic information for formulating an effective treatment plan[2].

Biomechanical calculations yield information such as joint torque and muscle force, thereby facilitating a more comprehensive understanding of the circumstances under which mechanical overload of the musculoskeletal system can result in disorders[3]. The moment arm is defined as the perpendicular distance between the muscle-tendon line of action and the center of rotation of the joint from which the tendon originates. It plays a pivotal role in translating muscle force and linear displacement into joint torque and angular motion, thereby serving as a fundamental parameter in biomechanical analyses and musculoskeletal modeling[4]. The moment arm is defined as the mechanical advantage of a muscle and plays a crucial role in determining its function as either a stabilizer or a prime mover[5]. The geometric method and the tendon excursion method are widely employed for measuring the moment arm. The geometric method, which includes serial sectioning techniques such as X-ray and MRI, enables the direct measurement of muscle-tendon paths relative to joint centers[3,6].

Anatomical features, such as the acromion type and the subacromial and subcoracoid (coracohumeral) distances, have been associated with the etiology of shoulder disorders. For instance, Oh et al. emphasized the clinical importance of acromial spurs—particularly the heel-type—in the development of rotator cuff tears, suggesting that specific acromial morphologies increase mechanical impingement[7]. Similarly, Tan et al. used MRI analysis to demonstrate that variations in coracoid morphology and decreased coracohumeral distances are associated with subscapularis tendon pathology, indicating that anatomical narrowing may predispose patients to anterior impingement[8]. In surgical treatment, merely repairing the existing pathology without addressing the underlying anatomical factors contributing to the damage has often proven inadequate, frequently leading to the need for revision surgery. The literature includes studies that utilize measurements such as the critical shoulder angle, lateral acromial angle, and acromial index to predict these pathologies[9]. This study aimed to evaluate the correlation between the length of the supraspinatus tendon moment arm and shoulder pathologies, as well as the shoulder anatomical features previously examined in the existing literature. Furthermore, the roles of subacromial and coracohumeral distances in the development of shoulder disorders were also explored.

Material and Method

Ethical approval for the study was obtained from the Clinical Research Ethics Committee of Mersin University (approval number: 2023/729, dated 01.11.2023) prior to its initiation. Between January 2024 and May 2024, 83 shoulder MRI scans of patients who presented to our clinic and underwent MRI examination were reviewed using the digital archive system. Magnetic resonance imaging was performed using a 1.5 Tesla MR device (GE Signa, GE Healthcare Technologies, Chicago, Illinois). Images were acquired with the patients in the supine position using a standard shoulder coil. Axial PD FS, coronal T1 FSE , coronal PD FS, and sagittal PD FS sequences were used for all patients (TE 30 ms, TR 4500 ms, 4 mm slice thickness, 1 mm interslice gap, FOV 16 cm, 144x117 matrix). The images were examined to identify pathologies, including acromioclavicular joint degeneration, bone cysts around the rotator cuff footprint, and tendinopathies of the biceps, supraspinatus, infraspinatus, and subscapularis.

Subacromial and coracohumeral distance measurements, commonly investigated in the etiology of shoulder disorders, were conducted. The subacromial distance was measured using sagittal sequences, while axial sequences were employed for the measurement of the coracohumeral distance. **(**Figure 1**)**. Acromion morphology was classified according to the Bigliani system[10]. The measurement of supraspinatus moment arm length has been described through various methods in radiologic imaging literature; however, its association with shoulder disorders has not yet been established. In this study, a coronal PD FS was utilized to define the supraspinatus moment arm length. To account for anatomical variability among individuals, the moment arm length was expressed as a ratio (A/B), where "A" is the distance from the rotator cuff footprint to the joint center, and "B" is the radius of the humeral head. This approach minimized inter-individual anatomical variation and improved comparability across the study population. The ratio (A/B) of these two measurements was subsequently calculated **(**Figure 2**).** The relationship between the measurements obtained from MRI and the identified shoulder pathologies was investigated.

Statistical analysis of the obtained data was performed using SPSS (Statistical Package for the Social Sciences) version 22.0. For quantitative data, frequencies, percentages, means, and standard deviations were calculated. The chi-square test was used to assess differences in the frequency of categorical variables between groups. ANOVA and Kruskal-Wallis tests were applied to evaluate

differences in continuous variables such as age, subacromial distance, and coracohumeral distance among groups. For the analysis of differences between two groups, Mann-Whitney U and Student's t-tests were employed. Pearson correlation coefficient was calculated for correlation analyses. A p-value of less than 0.05 was considered statistically significant.

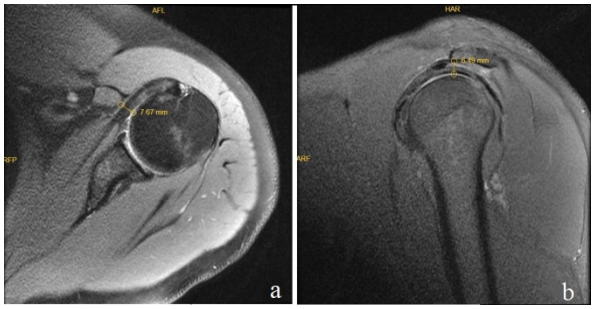

Figure 1:
a: *The technique for measuring the coracohumeral distance is employed in the axial PD FS section.* ***b****: The technique for measuring the subacromial distance is employed in the sagittal PD FS section.*

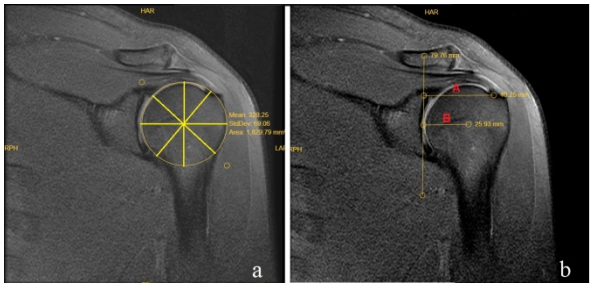

Figure 2:
a: *Finding the widest part of the humeral head in the coronal PD FS section and marking its center.* ***b****: The technique for measuring the moment arm length using the coronal PD FS section.*

Results

The study population comprised 46 females (55.4%) and 37 males (44.6%). Among the 83 shoulders examined, 47 (56.6%) were right shoulders, and 36 (43.4%) were left shoulders. The mean age was 57.5 ± 10.2 years (range: 45-91). Rotator cuff tears were present in 60 (72.3%) patients, with 11 (13.3%) having partial bursal tears, 22 (26.5%) partial articular tears, 15 (18.1%) small full-thickness tears, 7 (8.4%) medium full-thickness tears, 4 (4.8%) large full-thickness tears, and 1 (1.2%) massive full-thickness tear. Acromioclavicular degeneration was observed in 31 (37.3%) patients, and bone cysts around the rotator cuff footprint were found in 12 (14.5%) patients. Subscapularis tendinosis was identified in 38 (45.8%) patients, while subscapularis tears were seen in 7 (8.4%) patients.

The mean ratio of moment arm length (A/B) was calculated to be 1.53 ± 0.11, the mean subacromial distance was determined to be 8.56 ± 1.35 mm, and the mean coracohumeral distance was found to be 8.51 ± 1.51 mm. Subsequent to a thorough examination of the acromion morphology, the Bigliani classification system was employed to ascertain the acromion type. The findings revealed that 18 (21.7%) cases were classified as Type 1, 39 (47.0%) as Type 2, and 26 (31.3%) as Type 3 acromions.

The prevalence of acromioclavicular joint degeneration, bone cysts around the rotator cuff footprint, and rotator cuff tendon tears increased with age ($p < 0.05$). There was no association between age and rotator cuff tear size. No significant correlation was found between moment arm length and shoulder circumference pathologies that could impact the descriptive, diagnostic, and treatment processes. No association was observed between acromion morphology and rotator cuff tendinopathies. It was found that Type 2 and Type 3 acromions create a narrower subacromial space compared to Type 1 ($p < 0.05$). A positive correlation was identified between the subacromial space and the coracohumeral distance. It was observed that as the coracohumeral distance decreased, the frequency of tendinosis and tears in the subscapularis tendon increased ($p < 0.05$). Additionally, a reduction in the subacromial space was significantly associated with increased frequency of rotator cuff tears ($p < 0.05$). No significant effect of gender on the observed pathologies was found.

Discussion and Conclusion

The objective of this study is to investigate the biomechanical and anatomical determinants that contribute to the development of shoulder pathologies, with a specific emphasis on the interplay between moment arm length, subacromial distance, coracohumeral distance, and their association with various shoulder disorders. No statistically significant association was observed between supraspinatus tendon moment arm length and the presence of shoulder pathologies in a manner that would influence diagnostic interpretation, clinical characterization, or therapeutic planning. In contrast, a reduction in coracohumeral distance demonstrated a notable correlation with increased frequency of subscapularis tendinopathy and partial or full-thickness tears. Likewise, narrowing of the subacromial space was found to be positively associated with a higher prevalence of rotator cuff tendon tears.

The lack of a significant correlation between moment arm length and shoulder circumference pathologies

suggests that moment arm length may not be a critical determinant in the onset or progression of these pathologies. Previous studies investigating moment arm length have employed both cadaveric and imaging-based models, each with distinct advantages and limitations. For example, Ackland et al. and Pandy emphasized the anatomical variability and mechanical significance of moment arms in cadaveric simulations, while Zhang et al. demonstrated substantial interindividual variation in vivo using 3D MRI models, but found no significant correlation between moment arm length and pathological outcomes[5,6,11]. Similarly, Hughes et al. showed comparable moment arm lengths across techniques, underscoring methodological reliability[12]. In contrast, Leschinger et al.'s simulation highlighted the clinical importance of moment arm shortening after supraspinatus medialization[13]. Collectively, these mixed findings suggest that while moment arm length is biomechanically relevant, it may not independently predict shoulder pathology on imaging. Our study aligns with this view, as we also did not observe a significant association between the moment arm ratio and structural pathologies.

A study investigating the risk factors for partial rotator cuff tears using MRI identified several key factors, including age, subacromial distance, coracohumeral distance, and abnormal acromioclavicular signals. These findings are consistent with the results of our study, where similar risk factors were observed[14]. With advancing age, we observed an increase in the presence of degeneration in the acromioclavicular joint, the formation of bone cysts around the rotator cuff footprint, and the frequency of rotator cuff tendon tears. However, in our study, no relationship was found between acromion morphology and rotator cuff tendinopathies. Conversely, a comprehensive review on acromion morphology has identified a significant association between Type 3 acromion and rotator cuff tears[15].

Furthermore, we observed that a reduction in subacromial distance was associated with an increased frequency of rotator cuff tears; however, a cutoff value could not be determined. It has been reported that a reduced subacromial distance is associated with both rotator cuff tears and fatty degeneration[16]. Goutallier et al. have associated a subacromial distance of less than 6 mm with full-thickness rotator cuff tears[17]. A review of ultrasound studies has indicated that, in comparison to individuals who are healthy, those with rotator cuff injuries exhibit a reduced subacromial distance[18].

We found that the incidence of tendinosis and tears in the subscapularis tendon increased as the coracohumeral distance decreased. However, Tan et al. reported in their study using MRI and computed tomography (CT) that coracohumeral distance had no significant effect on subscapularis tendinopathies[8]. Çetinkaya et al. found no significant difference in the coracohumeral distance between shoulders with surgically treated subscapularis tears and the healthy contralateral shoulders of the same patients[19]. Mi et al. have identified that coracoid coverage and the presence of cysts around the rotator cuff footprint may serve as predictors for subscapularis tendon tears[20]. However, in our study, no association was found between the presence of cysts around the rotator cuff footprint and rotator cuff tendon tears.

This study has several limitations that should be acknowledged. First, intra- and inter-observer reliability for MRI-based measurements (moment arm length, subacromial, and coracohumeral distances) was not assessed. Although all measurements were performed by a single experienced musculoskeletal radiologist to ensure consistency, the lack of reproducibility analysis limits the assessment of measurement reliability. Additionally, the study was conducted at a single institution using a cross-sectional design, which may limit the generalizability and causality inference of the results. Longitudinal and multicenter studies are warranted to validate and expand upon these findings.

The observed association between reduced subacromial and coracohumeral distances and rotator cuff tendon tears may suggest a bidirectional relationship. It is uncertain whether anatomical narrowing predisposes to tendon tears, or whether tendon degeneration and retraction result in apparent narrowing of these spaces. Further comprehensive and longitudinal studies are necessary to address this question.

Acknowledgments

The authors would like to express their sincere gratitude to Dr. Gülçin Durukan Günaydın, Consultant Radiologist, for performing all radiological measurements in this study and for her invaluable contributions to the imaging analysis.

Researcher Contribution Statement:
Idea and design: İ.D., F.G.; Data collection and processing: İ.D., F.G., Ö.K., B.S.; Analysis and interpretation of data: İ.D., F.G., Ö.K., B.S.; Writing of significant parts of the article: İ.D., F.G., Ö.K., B.S.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Mersin Üniversitesi Rektörlüğü Klinik Araştırmalar Etik Kurulu
Approval Date: 06/11/2023
Decision No: 78017789/05.01.04/2525720

References

1. van der Heijden GJ. Shoulder disorders: a state-of-the-art review. Best Practice & Research Clinical Rheumatology. 1999;13(2):287-309.
2. Roy J-S, Braën C, Leblond J, et al. Diagnostic accuracy of ultrasonography, MRI and MR arthrography in the characterisation of rotator cuff disorders: a systematic review and meta-analysis. British journal of sports medicine. 2015;49(20):1316-1328.
3. Juul-Kristensen B, Bojsen-Møller F, Finsen L, et al. Muscle sizes and moment arms of rotator cuff muscles determined by magnetic resonance imaging. Cells Tissues Organs. 2000;167(2-3):214-222.
4. Maganaris CN. Imaging-based estimates of moment arm length in intact human muscle-tendons. European journal of applied physiology. 2004;91:130-139.
5. Ackland DC, Pak P, Richardson M, et al. Moment arms of the muscles crossing the anatomical shoulder. Journal of anatomy. 2008;213(4):383-390.
6. Pandy MG. 4 Moment A rm of a Muscle Force. Exercise and sport sciences reviews. 1999;27(1):79-118.
7. Oh JH, Kim JY, Lee HK, et al. Classification and clinical significance of acromial spur in rotator cuff tear: heel-type spur and rotator cuff tear. Clinical Orthopaedics and Related Research®. 2010;468(6):1542-1550.
8. Tan V, Moore Jr RS, Omarini L, et al. Magnetic resonance imaging analysis of coracoid morphology and its relation to rotator cuff tears. American journal of orthopedics (Belle Mead, NJ). 2002;31(6):329-333.
9. Heuberer PR, Plachel F, Willinger L, et al. Critical shoulder angle combined with age predict five shoulder pathologies: a retrospective analysis of 1000 cases. BMC musculoskeletal disorders. 2017;18:1-9.
10. Lu B. The morphology of the acromion and its relationship to rotator cuff tear. Orthop Trans. 1986;10:228.
11. Zhang Y, Herbert RD, Bilston LE, et al. Three-dimensional architecture and moment arms of human rotator cuff muscles in vivo: Interindividual, intermuscular, and intramuscular variations. Journal of Anatomy. 2024.
12. Hughes RE, Niebur G, Liu J, et al. Comparison of two methods for computing abduction moment arms of the rotator cuff. Journal of biomechanics. 1997;31(2):157-160.
13. Leschinger T, Birgel S, Hackl M, et al. A musculoskeletal shoulder simulation of moment arms and joint reaction forces after medialization of the supraspinatus footprint in rotator cuff repair. Computer Methods in Biomechanics and Biomedical Engineering. 2019;22(6):595-604.
14. Wu H, Zuo Z, Li Y, et al. Anatomic characteristics of shoulder based on MRI accurately predict incomplete rotator cuff injuries in patients: relevance for predictive, preventive, and personalized healthcare strategies. EPMA J 2023:553-570.
15. Yadav SK, Zhu WH. A systematic review: Of acromion types and its effect on degenerative rotator cuff tear. International Journal of Orthopaedics. 2017;3(1):453-458.
16. Saupe N, Pfirrmann CW, Schmid MR, et al. Association between rotator cuff abnormalities and reduced acromiohumeral distance. American Journal of Roentgenology. 2006;187(2):376-382.
17. Goutallier D, Le Guilloux P, Postel J-M, et al. Acromio humeral distance less than six millimeter: its meaning in full-thickness rotator cuff tear. Orthopaedics & Traumatology: Surgery & Research. 2011;97(3):246-251.
18. Seitz AL, Michener LA. Ultrasonographic measures of subacromial space in patients with rotator cuff disease: a systematic review. Journal of clinical ultrasound. 2011;39(3):146-154.
19. Çetinkaya M, Kaptan AY, Ulucaköy C, et al. Is it the subcoracoid impingement or the subacromial impingement that tears the subscapularis tendon? A comparison of the MRI findings of the operated and healthy shoulders of the patients. Turk J Med Sci. 2023;53(1):273-281.
20. Mi Y, Lin Y, Cheng B. Magnetic resonance imaging based coracoid process morphology and its associations with isolated subscapularis tendon tears in Chinese patients. Jt Dis Relat Surg. 2024;35(2):267-275.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 277-283, 2025
DOI: https://doi.org/10.32708/uutfd.1663996

ORIGINAL RESEARCH

Assessing Average Proteinuria and Blood Pressure as Predictors of Kidney Survival in Autosomal Dominant Polycystic Kidney Disease

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ABSTRACT

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common hereditary kidney disorder characterized by bilateral cyst formation and progressive kidney damage. Predicting prognosis at diagnosis and during follow-up can be challenging. Beyond genetics and total kidney volume, clinical features such as blood pressure profile and proteinuria may be valuable. We evaluated 116 ADPKD patients from medical records in terms of demographic and clinical parameters, as well as two new variables: average blood pressure (calculated as the mean across all visits) and average proteinuria (also calculated across all visits). Univariate and multivariate analyses of these parameters were performed to assess their association with renal replacement therapy and other renal outcome measures. The study included 116 ADPKD patients with a median age of 44.5 years and a follow-up period of 55 months. Hypertension was common (76.7%), with most patients receiving RAS blockade therapy. A lower eGFR at presentation was a key predictor of worse renal outcomes. Diastolic blood pressure and RAS blockade were significantly associated with disease progression. However, multivariate analysis did not confirm proteinuria, smoking, or extrarenal manifestations as independent predictors of creatinine doubling or eGFR halving time. Efforts to predict prognosis in ADPKD using clinical features beyond genetics remain a subject of debate. Future studies with larger cohorts are needed to validate the role of these parameters in predicting ADPKD progression.

Keywords: Polycystic Kidney Disease. Average blood pressure. Average proteinuria. Prognosis. Renal outcome.

Otozomal Dominant Polikistik Böbrek Hastalığında Böbrek Sağkalımı

ÖZET

Otozomal Dominant Polikistik Böbrek Hastalığı (ADPBH), bilateral kist oluşumu ve ilerleyici böbrek hasarı ile karakterize yaygın bir kalıtsal böbrek hastalığıdır. Tanı anında ve takip sürecinde prognozu öngörmek önem arz eder. Bu çalışmada genetik ve toplam böbrek hacmi gibi bilinen bulgular dışında klinikte rahatlıkla uygulanabilecek iki yeni parametre olan ortalama kan basıncı profili ve proteinürinin prognozu öngörmedeki yeri incelenmiştir. Tıbbi kayıtlar üzerinden 116 ADPKD hastasını demografik ve klinik parametreler açısından değerlendirdik ve ayrıca iki yeni değişken ekledik: ortalama kan basıncı (tüm ziyaretler boyunca hesaplanan ortalama) ve ortalama proteinüri (tüm ziyaretler boyunca hesaplanan ortalama). Böbrek replasman tedavisi ve diğer böbrek sonuç ölçütleri ile bu parametreler arasındaki ilişkiyi değerlendirmek için tek değişkenli ve çok değişkenli analizler gerçekleştirildi. Çalışmaya dahil edilen 116 ADPKD hastasının medyan yaşı 44,5 yıl olup, takip süresi 55 aydı. Hipertansiyon yaygındı (%76,7) ve hastaların çoğu RAS blokaj tedavisi almaktaydı. Başlangıçtaki düşük GFR, daha kötü böbrek sonuçlarının temel belirleyicisiydi. Diyastolik kan basıncı ve RAS blokaj tedavisi hastalığın ilerlemesiyle anlamlı şekilde ilişkiliydi. Ancak, çok değişkenli analiz proteinüri, sigara kullanımı veya ekstrarenal bulguların kreatininin ikiye katlanması veya GFR'nin yarılanma süresi için bağımsız belirleyiciler olduğunu doğrulamadı. Genetik dışındaki klinik özelliklerin ADPKD prognozunu öngörmede kullanılması hala tartışmalı bir konudur. Bu parametrelerin ADPKD ilerleyişini öngörmedeki rolünü doğrulamak için daha geniş hasta gruplarında ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Polikistik Böbrek Hastalığı. Ortalama kan basıncı. Ortalama proteinüri. Prognoz. Böbrek sağkalımı.

Date Received: 26.March.2025
Date Accepted: 21.July.2025

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common hereditary renal disorder, with a prevalence ranging from 1 in 1000 to 1 in 2,500 individuals[1,2]. ADPKD is characterized by bilateral renal cyst formation and progressive cyst enlargement, leading to chronic kidney damage. Approximately 45% to 70% of patients with ADPKD progress to end-stage renal disease (ESRD) by the age of 65 [3]. Several classical prognostic factors have been associated with faster progression to ESRD, including male sex, mutations in the *Polycystic Kidney Disease 1* (PKD1) gene, increased total kidney volume (greater than 600 cc), early-onset hypertension before the age of 35, diagnosis before 30 years of age, macroscopic hematuria, and recurrent urinary tract infections[4].

In addition to these well-known risk factors, other variables such as proteinuria, reduced fluid intake, elevated serum copeptin levels, high caffeine consumption, smoking, and increased dietary protein intake have also been linked to disease progression, albeit with less recognition. Hypertension is a common clinical feature of ADPKD, affecting approximately 60% of patients before significant renal impairment develops, and its early onset is strongly correlated with more rapid kidney function decline[5]. Conversely, patients without hypertension exhibit a more favorable prognosis[6].

Although proteinuria is less extensively studied in ADPKD, it has been associated with higher arterial blood pressure, increased total kidney volume, and lower glomerular filtration rate (eGFR)[7]. Recent research in glomerulonephritis has explored the concept of average proteinuria and variable proteinuria as potential prognostic metrics[8]. In this study, we aim to evaluate the role of average proteinuria and average blood pressure as novel prognostic parameters alongside

Material and Method

This retrospective study was conducted with approval from the Dokuz Eylül University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (Decision No: 2018/19-20). No funding was requested following ethical approval. Written permissions were obtained from the Nephrology Department and Archive Records Division of DEU Hospital (DEUH) to access patient data for clinical review in line with the study's objectives. Patients were invited to participate and provided informed consent for the use of their clinical, laboratory, and medical history data.

Data from 196 patients diagnosed with Autosomal Dominant Polycystic Kidney Disease (ADPKD) and followed up at the DEUH Nephrology Clinic were reviewed. Patients with follow-up periods shorter than one year or incomplete data (n = 80) were excluded. Demographic variables, including age, sex, history of hypertension (HT) and diabetes mellitus (DM), smoking status, and body mass index (BMI), were analyzed. Clinical variables evaluated included blood pressure measurements from the first and last visits, average blood pressure (calculated as the average across all visits), use of renin-angiotensin system (RAS) blockers, history of urinary tract infections, kidney stones, macroscopic hematuria, inguinal/umbilical hernias, cerebral aneurysms, liver cysts, and mitral valve prolapse. Average proteinuria was calculated by the mean of all the proteinuria values in each 6-month interval from the follow ups to the end of follow-up or an outcome event. Average blood pressure was calculated by the same method.

Estimated Glomerular filtration rate (eGFR) was estimated using the CKD-EPI formula at diagnosis, annually, and at the final follow-up. Proteinuria measurements were recorded at diagnosis and final visit, and average proteinuria (mg/day) was calculated for each patient. Proteinuria was quantified from 24-hour urine collections (mg/day). Average proteinuria was calculated by averaging proteinuria values across all visits, and its association with renal survival was analyzed. Blood pressure was measured with an Omron digital sphygmomanometer after 15 minutes of rest, using a single-arm reading at each visit. Hypertension was defined as a blood pressure of ≥140/90 mmHg on at least two measurements. Kidney survival outcomes included the last measured eGFR, time to doubling of serum creatinine, time to end-stage renal disease (ESRD), and the need for renal replacement therapy.

Statistical Analyses

Variable distributions were assessed by the Shapiro-Wilk normality test. According to the variable distribution, Student's *t*-test or Mann Whitney *U* test was applied for the comparison of groups regarding quantitative data. Categorical variables were compared by the χ^2 test, and fisher's exact test accordingly. Statistical analysis was performed using Statistical Package of Social Science (SPSS Inc., Chicago, IL), version 22.0 for Windows. Data were expressed as median (range) and a *p* value less than 0.05 was accepted as statistically significant. A sample size estimation was performed to determine the minimum number of patients required to detect a clinically meaningful difference in the time to renal replacement therapy (RRT) based on a Cox proportional hazards model. The analysis assumed a two-sided alpha of 0.05 and a target statistical power of 80%.In the univariate analysis, we evaluated both baseline and average (mean) values of blood pressure, proteinuria, and eGFR to identify potential predictors of kidney survival. However, to avoid the risk of multicollinearity and overfitting, only variables that were statistically significant in the univariate analysis were considered for inclusion in the multivariate Cox

regression model. Importantly, we did not include both baseline and average values of the same variable in the final multivariate model.

Results

116 ADPKD patients were included in to the study, median age was 44.5 (18-84) years. 57% of the patients were female and median follow up was 55 (12-136) months. Most of the patients had hypertension (76.7%) among these patients, 72.4% of them were receiving RAS blockade therapy. Diabetes mellitus (DM) was present in 8.6 % of patients. A history of smoking or current smoking was reported by 42.3% of the patients. Kidney stones were detected in 29.3% of patients and 17.24% of patients had a history of urinary tract infections. A history of macroscopic hematuria was present in 16% of patients. Regarding extrarenal involvement, the most common extrarenal manifestation was liver cysts, observed in 60.7% of them. Additionally, inguinal/umbilical hernias were noted in 19.5% of patients, colonic diverticula in 1.7%, mitral valve prolapse (MVP) in 14.3% of patients, and cerebral aneurysms were suspected in 14.5% of patients. The clinical characteristics and extrarenal organ involvement data of the patients are summarized in Table I.

Table I. Patient Characteristics and Outcome Variables

Variable	Frequency n=116
Age (years)	44.5 (18-84)
Gender (Female / Male %)	56.9 / 43.1
Diabetes Mellitus (Present / Absent %)	8.6 / 91.4
eGFR at presentation (ml/min/m2)	76 (11 – 145)
Systolic BP at presentation (mmHg)	130 (100 - 180)
Diastolic BP at presentation (mmHg)	80 (40 – 110)
Average Systolic BP (mmHg)	128.5 (99 – 163)
Average Diastolic BP (mmHg)	80 (54 – 110)
Hypertension at presentation (Present / Absent %)	76.7 / 26.3
Proteinuria at presentation (mg/day)	160.5 (30 – 1261)
Average Proteinuria (mg/day)	199 (36 – 2543)
Body Mass Index at presentation (kg/m2)	26.3 (18.4 – 38)
RAS Blockade (Present/Absent)	72.4 / 27.6
Smoking (Yes/No)	42.3 / 57.7
Hernia (Present/Absent)	19.5 / 80.5
Macroscopic Hematuria (Present/Absent)	16 / 84
Mvp (Present/Absent)	14.3 / 85.7
Hepatic Cyst (Present/Absent)	60.7 / 39.3
Cerebral Aneurysm (Present/Absent)	14.5 / 85.5
Follow Up Time (Months, median (min -max))	55 (12 – 136)
Renal Replacement Free Survival (Months, median, 95% CI)	80 (72.05 – 87.96)
Serum Creatinine Doubling Free Survival (Months, median 95%CI)	NR
eGFR Halving Free Survival (Months, median 95%CI)	NR

NR: Not Reached

The expected effect size was derived from a hazard ratio (HR) of 0.60, representing a substantial association between the clinical predictor (e.g., baseline eGFR or proteinuria) and the risk of requiring RRT. The corresponding log hazard ratio was approximately 0.51.

Using standard normal approximation methods for survival analysis, it was determined that a minimum of 100 patients would be sufficient to detect such an effect with a power of 95.1%. This sample size accounts for proportional hazards, balanced exposure distribution, and an anticipated number of outcome events appropriate for time-to-event analysis.

Median eGFR at presentation was 76 ml/min/m2 (11 – 145). Median systolic BP at presentation was 130 mmHg (100 - 180). Median proteinuria at presentation (mg/day) was 160.5 mg/day (30 – 1261). Median average systolic BP was 128.5 mmHg (99 – 163), Median average diastolic BP was 80 mmHg (54 – 110). Median average proteinuria was 199 mg/day (36 – 2543).

Renal replacement free survival was 80 months. Serum creatinine doubling free survival and eGFR halving free survival were not reached (figure 3-4). Among the 12 patients (10.3%) requiring RRT, 10 underwent hemodialysis, 1 received peritoneal dialysis, and 1 patient underwent kidney transplantation.

Variables having a potential to affect time to renal replacement therapy were evaluated. In the univariate analysis, a lower estimated glomerular filtration rate (eGFR) at presentation was significantly associated with worse outcomes (HR 0.91, CI 0.87–0.96, $p < 0.001$). Diastolic blood pressure at presentation ($p = 0.033$) and the presence of RAS blockade ($p = 0.016$) also showed significant associations. In the multivariate analysis, eGFR at presentation remained an independent predictor ($p = 0.003$). Diastolic blood pressure ($p = 0.014$) and RAS blockade ($p = 0.014$) were also significant, highlighting their roles in disease progression. Other variables, including age, gender, diabetes, proteinuria, hypertension, and extrarenal manifestations (e.g., hepatic cysts, cerebral aneurysms), did not show statistically significant associations in the multivariate model. Cox regression analysis of variables having a potential to affect time to renal replacement therapy were detailed in table II. Renal replacement free survival was 80 months (78-88) (Figure 1). Renal replacement free survival for patients on RAS blockade therapy were significantly lower comparing the patients who are not on ras blockers (figure 2).

Table II. Cox Regression Analysis of Variables Having a Potential To Affect Time To Renal Replacement Therapy

Variable	Univariate Analysis (HR (CI), p)	Multivariate Analysis p value[a,b]
Age at Diag (yrs)	0.96 (0.90-1.02), 0.183	-
Gender (M/F)	0.65 (0.19-2.27), 0.504	-
Diabetes (Present/Absent)	0.04 (0.01-544.98), 0.506	-
eGFR at presentation (ml/min/m2)	0.91 (0.87-0.96), **<0.001**	**0.003**[a]
Systolic BP at presentation (mmHg)	0.99 (0.96-1.03), 0.832	-
Diastolic BP at presentation (mmHg)	1.07 (1.01-1.13), **0.033**	0.145[a], **0.014**[b]
Average Systolic BP (mmHg)	0.99 (0.96-1.05), 0.967	-
Average Diastolic BP (mmHg)	0.99 (0.97-1.01), 0.472	-
Hypertension at presentation(Present/Absent)	1.68 (0.21-13.45), 0.624	-
Proteinuria at presentation (mg/day)	1.00 (0.99-1.00), 0.301	-
Average Proteinuria (mg/day)	1.00 (0.99-1.00), 0.775	-
Body Mass Index at presentation (kg/m2)	0.76 (0.51-1.13), 0.179	-
RAS Blockade (Present/Absent)	0.22 (0.06-0.75), **0.016**	0.124[a], **0.014**[b]
Smoking (Yes/No)	2.27 (0.23-22.06), 0.480	-
Hernia (Present/Absent)	0.44 (0.09-2.23), 0.317	-
Macroscopic Hematuria (Present/Absent)	2.29 (0.24-22.30), 0.475	-
Mvp (Present/Absent)	0.05 (0.01-12.94), 0.800	-
Hepatic Cyst (Present/Absent)	3.48 (0.68-17.75), **0.133**	0.253[a], **0.266**[b]
Cerebral Aneurysm (Present/Absent)	0.04 (0.01-25.69), 0.800	-

a. eGFR at presentation included in the equation
b. eGFR at presentation excluded in the equation

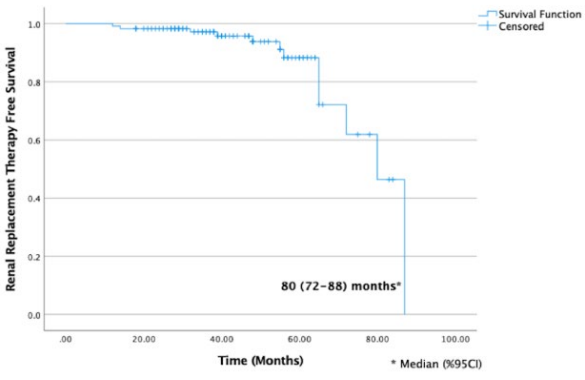

Figure 1.
Renal replacement therapy free survival

In the Cox regression analysis evaluating factors influencing creatinine doubling time, univariate analysis identified significant associations with age at diagnosis (HR 1.06, CI 1.02–1.11, p = 0.008), average proteinuria (HR 1.01, CI 1.01–1.02, p = 0.001), and eGFR at presentation (HR 0.94, CI 0.91–0.97, p < 0.001). Gender showed a borderline significance (p = 0.056).

However, multivariate analysis did not confirm these variables as independent predictors (p > 0.05). Specifically, average proteinuria (p = 0.095) and smoking (p = 0.216) showed trends without statistical significance. Variables such as hypertension, RAS blockade, and extrarenal manifestations (hepatic cysts, cerebral aneurysms) did not significantly influence creatinine doubling time in either analysis (Table III). Median creatinine doubling time and eGFR halving time were not reached (Fıgure 3-4).

Table III. Cox Regression Analysis of Variables Having a Potential To Affect Creatinine Doubling Time

Variable	Univariate Analysis (HR (CI), p)	Multivariate Analysis p value[a,b]
Age at Diag (yrs)	1.06 (1.02-1.11), **0.008**	0.505[a], 0.254[b]
Gender (M/F)	0.28 (0.07-1.04), **0.056**	0.966[a], 0.970[b]
Diabetes (Present/Absent)	1.75 (0.38-8.13), 0.476	-
eGFR at presentation (ml/min/m2)	0.94 (0.91-0.97), **<0.001**	0.418[a]
Systolic BP at presentation (mmHg)	1.01 (0.97-1.04), 0.846	-
Diastolic BP at presentation (mmHg)	0.99 (0.94-1.05), 0.846	
Mean Systolic BP (mmHg)	1.03 (0.99-1.08), 0.154	-
Mean Diastolic BP (mmHg)	0.99 (0.96-1.03), 0.843	-
Hypertension at presentation(Present/Absent)	25.36 (0.01-45.17), 0.397	-
Proteinuria at presentation (mg/day)	1.00 (0.99-1.01), 0.975	-
Average Proteinuria (mg/day)	1.01 (1.01-1.02), **0.001**	0.275[a], 0.095[b]
Body Mass Index at presentation (kg/m2)	0.95 (0.76-1.18), 0.642	-
RAS Blockade (Present/Absent)	0.63 (0.17-2.39), 0.501	
Smoking (Yes/No)	6.54 (0.73- 58.68), **0.094**	0.466[a], 0.216[b]
Hernia (Present/Absent)	1.07 (0.23-5.06), 0.929	-
Macroscopic Hematuria (Present/Absent)	2.01 (0.37-11.03), 0.422	-
Mvp (Present/Absent)	0.03 (0.01-847.12), 0.512	-
Hepatic Cyst (Present/Absent)	1.47 (0.31-7.01), 0.626	
Cerebral Aneurysm (Present/Absent)	0.04 (0.01-8815.70), 0.609	-

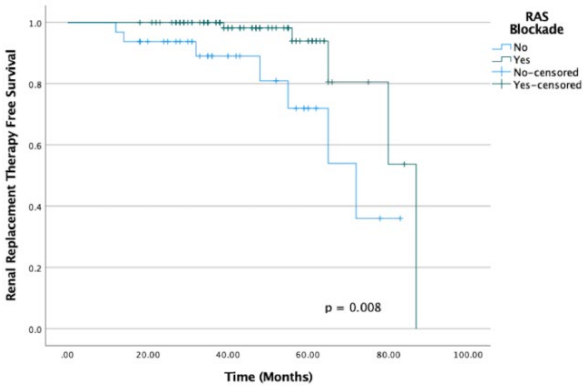

Figure 2.
Renal replacement therapy free survival and ras blockade.

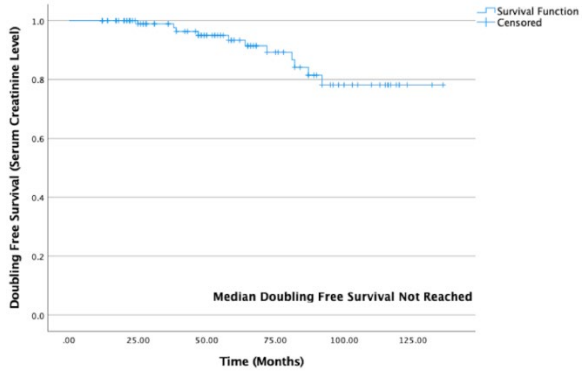

Figure 3.
Serum creatinine level doubling time free survival

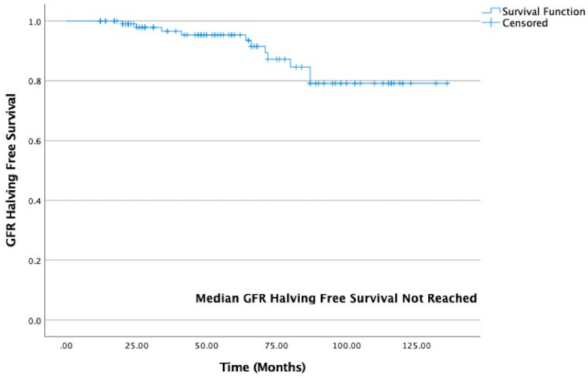

Figure 4.
eGFR halving time free survival

In the Cox regression analysis evaluating factors affecting eGFR halving time, univariate analysis identified significant associations with older age at diagnosis (HR 1.06, CI 1.02–1.11, p = 0.008), lower eGFR at presentation (HR 0.94, CI 0.91–0.97, p < 0.001), and average proteinuria (HR 1.01, CI 1.01–1.02, p = 0.002). Gender showed a borderline significance (p = 0.054).

However, in multivariate analysis, none of these factors remained statistically significant predictors of eGFR halving time (all p > 0.05). Other variables, including systolic and diastolic blood pressure, RAS blockade, smoking, and extrarenal manifestations (hepatic cysts, cerebral aneurysms), did not show significant associations with eGFR halving time in either analysis (Table IV).

Table IV. Cox Regression Analysis of Variables Having a Potential To Affect eGFR Halving Time

Variable	Univariate Analysis (HR (CI), p)	Multivariate Analysis p value[a,b]
Age at Diag (yrs)	1.06 (1.02-1.11), **0.008**	0.507[a], 0.242[b]
Gender (M/F)	0.27 (0.07-1.02), **0.054**	0.966[a], 0.955[b]
Diabetes (Present/Absent)	1.71 (0.37-7.94), 0.494	-
eGFR at presentation (ml/min/m2)	0.94 (0.91-0.97), **<0.001**	0.380[a]
Systolic BP at presentation (mmHg)	1.00 (0.97-1.04), 0.815	-
Diastolic BP at presentation (mmHg)	0.99 (0.94-1.05), 0.845	
Mean Systolic BP (mmHg)	1.03 (0.99-1.08), 0.144	-
Mean Diastolic BP (mmHg)	0.99 (0.96-1.04), 0.889	-
Hypertension at presentation(Present/ Absent)	25.86 (0.02-39.42), 0.384	-
Proteinuria at presentation (mg/day)	1.00 (0.99-1.01), 0.960	-
Average Proteinuria (mg/day)	1.01 (1.01-1.02), **0.002**	0.471[a], 0.095[b]
Body Mass Index at presentation (kg/m2)	0.95 (0.77-1.19), 0.657	-
RAS Blockade (Present/Absent)	0.63 (0.17-2.37), 0.490	
Smoking (Yes/No)	6.39 (0.71- 57.40), **0.098**	0.815[a], 0.323[b]
Hernia (Present/Absent)	1.09 (0.23-5.16), 0.910	-
Macroscopic Hematuria (Present/Absent)	2.12 (0.39-11.62), 0.387	-
Mvp (Present/Absent)	0.03 (0.01-770.32), 0.507	-
Hepatic Cyst (Present/Absent)	1.54 (0.32-7.32), 0.591	
Cerebral Aneurysm (Present/Absent)	0.04 (0.01-4485.75), 0.588	-

Discussion and Conclusion

ADPKD exhibits significant clinical variability, particularly in its progression to end-stage kidney disease. Patients and their families often seek to understand their risk of disease progression. Prognostic risk assessment can be achieved through two main approaches: genetic testing and total kidney volume evaluation[9]. However, in healthcare systems with limited resources, alternative clinical markers play a crucial role in evaluating disease progression. Assessing factors such as serum creatinine levels,

estimated glomerular filtration rate (eGFR) decline, blood pressure and proteinuria can provide critical insights when genetic testing or imaging techniques like MRI or CT scans are unavailable. Integrating these alternative assessments into routine care may help optimize patient management and resource allocation in settings where access to advanced diagnostics is restricted.

In our study we retrospectively evaluated clinical and laboratory findings with two additional parameters - average blood pressure and proteinuria- regarding to renal function outcomes. Patients who had lower eGFR at presentation more progressed to end stage renal disease. This finding confirms the association between early end stage renal disease progression and unpredictable nature of the disease. Diastolic blood pressure at presentation and the presence of RAS blockade also showed significant associations between end stage renal disease progression. These findings confirm the benefits of treatment with RAAS blocking medications and their effect of reducing the progression of renal disease in animal models of ADPKD[10,11].

On the oher hand in ADPKD glomerular filtration rate (eGFR) masks the underlying renal damage for several decades, due to hyperfiltration and hypertrophy of unaffected residual nephrons. Early detection of hypertension may help clinicians to detect such patients. Although average blood pressure was not significant in multivariate analysis, time varying blood pressure can serve as a potential clinical parameter for future studies.

Proteinuria is not a common finding in autosomal dominant polycystic kidney disease (ADPKD), but it is considered a prognostic factor. In a study conducted by Chapman A.B. and colleagues, the average proteinuria in 270 ADPKD patients under follow-up was found to be 259 mg/day. Only 18% of patients had proteinuria exceeding 300 mg/day. However, patients with proteinuria showed more severe signs of disease progression[7]. In this group, higher arterial blood pressure, larger kidney volumes, and lower creatinine clearance were observed.Similarly, in a study by Ecder T. and colleagues, which followed 323 ADPKD patients for an average of 100 months, it was reported that each 1 g/day increase in proteinuria increased the risk of rapid decline in kidney function by 2.35 times[12]. In our study, the baseline proteinuria level was found to be an average of 160.5 mg/day, while at the end of the follow-up period, the average proteinuria level had increased to 435 mg/day. Over an average follow-up of 55 months, a significant increase in proteinuria levels was observed.

The concept of average proteinuria has emerged in the search for an ideal proteinuria measurement method for monitoring glomerulonephritis. Instead of a single-point or periodic assessment, calculating the average of proteinuria measurements across all visits may provide a more accurate projection of disease progression. Our study also utilized the concept of average proteinuria. In this study we hypothesized that average blood pressure and avarege proteinuria can be an accessible and feasible approach to show renal outcomes and disease progression in ADPKD. Although average diastolic blood pressure and average proteinuria showed significant associations with creatinine doubling time and eGFRhalving time in the univariate analyses, this findings did not confirmed by multivariate analyses in our study. This findings can be related to fewer sample size rather than confounding or correlation effect.

The use of average proteinuria in ADPKD was not supported by our multivariate analyses. Nevertheless, the need for a clinical parameter beyond the PROPKD score remains an unmet need in clinical practice. Simple averaging may fail to reflect the dynamic nature of proteinuria and its cumulative impact on kidney function. In this context, average proteinuria might serve as a preliminary step toward identifying a more accurate method for quantifying proteinuria in ADPKD. Incorporating time-varying proteinuria measurements, including the intervals between measurements, could enhance the predictive utility of such models.

In conclusion, our study highlights the importance of alternative clinical markers in assessing disease progression in ADPKD, particularly in resource-limited settings. While lower baseline eGFR, diastolic blood pressure at presentation, and RAS blockade therapy were significantly associated with disease progression, our findings further support the protective role of RAAS inhibition in delaying end-stage renal disease. Additionally, although average blood pressure and proteinuria were hypothesized as potential prognostic indicators, their significance was not confirmed in multivariate analysis, likely due to sample size limitations. Future studies with larger cohorts are needed to validate the role of these parameters in predicting ADPKD progression and to refine risk stratification strategies for better patient management.

Researcher Contribution Statement:
Idea and design: Y.G.M, M.A.O, C.H, C.C; Data collection and processing: Y.G.M ; Analysis and interpretation of data: Y.G.M, O.G.S, C.C; Writing of significant parts of the article: Y.G.M, C.C
Support and Acknowledgement Statement:
This study received no financial support
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Dokuz Eylül Universitesi Girişimsel Olmayan Araştırmalar Etik Kurulu
Approval Date: 26.07.2018
Decision No: 2018/19-20

References

1. Lanktree MB, Haghighi A, Guiard E, et al. Prevalence Estimates of Polycystic Kidney and Liver Disease by Population Sequencing. *J Am Soc Nephrol*. Oct 2018;29(10):2593-2600. doi:10.1681/ASN.2018050493
2. Willey CJ, Blais JD, Hall AK, Krasa HB, Makin AJ, Czerwiec FS. Prevalence of autosomal dominant polycystic kidney disease in the European Union. *Nephrol Dial Transplant*. Aug 1 2017;32(8):1356-1363. doi:10.1093/ndt/gfw240
3. Soroka S, Alam A, Bevilacqua M, et al. Assessing Risk of Disease Progression and Pharmacological Management of Autosomal Dominant Polycystic Kidney Disease: A Canadian Expert Consensus. *Can J Kidney Health Dis*. 2017;4:2054358117695784. doi:10.1177/2054358117695784
4. Schrier RW, Brosnahan G, Cadnapaphornchai MA, et al. Predictors of autosomal dominant polycystic kidney disease progression. *J Am Soc Nephrol*. Nov 2014;25(11):2399-418. doi:10.1681/ASN.2013111184
5. Ecder T, Schrier RW. Cardiovascular abnormalities in autosomal-dominant polycystic kidney disease. *Nat Rev Nephrol*. Apr 2009;5(4):221-8. doi:10.1038/nrneph.2009.13
6. Sans-Atxer L, Torra R, Fernandez-Llama P. Hypertension in autosomal-dominant polycystic kidney disease (ADPKD). *Clin Kidney J*. Oct 2013;6(5):457-63. doi:10.1093/ckj/sft031
7. Chapman AB, Johnson AM, Gabow PA, Schrier RW. Overt proteinuria and microalbuminuria in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. Dec 1994;5(6):1349-54. doi:10.1681/ASN.V561349
8. Barbour SJ, Cattran DC, Espino-Hernandez G, Hladunewich MA, Reich HN. Identifying the ideal metric of proteinuria as a predictor of renal outcome in idiopathic glomerulonephritis. *Kidney Int*. Dec 2015;88(6):1392-1401. doi:10.1038/ki.2015.241
9. Cornec-Le Gall E, Alam A, Perrone RD. Autosomal dominant polycystic kidney disease. *Lancet*. Mar 2 2019;393(10174):919-935. doi:10.1016/S0140-6736(18)32782-X
10. Zafar I, Tao Y, Falk S, McFann K, Schrier RW, Edelstein CL. Effect of statin and angiotensin-converting enzyme inhibition on structural and hemodynamic alterations in autosomal dominant polycystic kidney disease model. *Am J Physiol Renal Physiol*. Sep 2007;293(3):F854-9. doi:10.1152/ajprenal.00059.2007
11. Kennefick TM, Al-Nimri MA, Oyama TT, et al. Hypertension and renal injury in experimental polycystic kidney disease. *Kidney Int*. Dec 1999;56(6):2181-90. doi:10.1046/j.1523-1755.1999.00783.x
12. Ozkok A, Akpinar TS, Tufan F, et al. Clinical characteristics and predictors of progression of chronic kidney disease in autosomal dominant polycystic kidney disease: a single center experience. *Clin Exp Nephrol*. Jun 2013;17(3):345-51. doi:10.1007/s10157-012-0706-3

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 285-290, 2025
DOI: https://doi.org/10.32708/uutfd.1701404

ORIGINAL RESEARCH

Evaluation of the Relationship Between Electrocardiographic Changes and the Calcium/Potassium Ratio in Patients with Acute and Chronic Renal Failure*

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ABSTRACT

The aim of this study was to investigate the relationship between serum calcium and potassium levels, as well as the calcium/potassium ratio, and electrocardiographic (ECG) changes in patients presenting to the emergency department with both renal failure and hyperkalemia. This cross-sectional study was conducted retrospectively by reviewing the medical records of patients diagnosed with renal failure and concomitant hyperkalemia (serum potassium>5.5 mEq/L) who presented to the emergency departments of a university hospital and a training and research hospital between November 1, 2022, and October 31, 2023. 55.4% of the patients were male, with a mean age of 68.31 ± 13.56 years. ECG abnormalities were detected in 69.5% of the cases. The most frequently observed ECG abnormalities were atrial fibrillation (22.0%) and peaked T waves (20.3%). Hemodialysis was performed in 52.5% of patients, 23.2% were admitted to the intensive care unit (ICU), and 15.8% died within 30 days of hospital presentation. Patients with T wave inversion had significantly lower potassium levels (6.42 ± 0.77 vs. 6.03 ± 0.71, p=0.018). In patients with any ECG abnormality, calcium levels (8.69 ± 0.87 vs. 8.36 ± 0.85, p=0.037) and calcium/potassium ratios (1.39 ± 0.19 vs. 1.32 ± 0.20, p=0.036) were significantly lower. The calcium/potassium ratio was also significantly lower in patients with peaked T waves (1.36 ± 0.20 vs. 1.27 ± 0.20, p=0.016). Among patients who died in-hospital or within 30 days, calcium/potassium ratios were significantly lower and potassium levels were significantly higher (p<0.05). In patients presenting to the emergency department with renal failure and concomitant hyperkalemia, the presence of ECG abnormalities was associated with lower calcium levels and a reduced calcium/potassium ratio. Moreover, 30-day mortality was found to be associated with higher potassium levels and a lower calcium/potassium ratio. These findings suggest that cardiac complications should be considered in clinical practice when hyperkalemia accompanies acute or chronic renal failure.

Keywords: Calcium. Potassium. ECG. Emergency service.

Akut ve Kronik Böbrek Yetmezliği olan Hastalarda Karşılaşılan EKG Değişikliklerinin Kalsiyum/ Potasyum Oranı ile İlişkisinin Değerlendirilmesi

ÖZET

Çalışmanın amacı acil serviste böbrek yetmezliğine ek olarak hiperkalemi tespit edilen hastalarda kalsiyum, potasyum düzeyinin ve kalsiyum/potasyum oranının EKG değişiklikleri ile ilişkisini araştırmaktır. Kesitsel tipte olan bu çalışma, 1 Kasım 2022 – 31 Ekim 2023 tarihleri arasında bir üniversite hastanesi ve eğitim araştırma hastanesi acil servislerinde böbrek yetmezliği tanısına ek olarak hiperkalemi (>5,5 mEq/L) saptanan olguların dosyalarının retrospektif olarak değerlendirilmesi ile gerçekleştirilmiştir. Olguların %55,4'ü erkekti ve yaş ortalaması 68,31 ± 13,56 yıldı. Olguların %69,5'inin EKG'sinde en az bir patolojik değişiklik saptandı. En sık saptanan patolojik değişiklikler atrial fibrilasyon (%22,0) ve sivri T dalgasıydı (%20,3). Olguların %52,5'ine hemodiyaliz uygulandığı, %23,2'si YBÜ'ye yatırıldığı ve %15,8'inin hastane başvurusu sonrasındaki 30 gün içinde exitus olduğu saptandı. T dalgası inversiyonu saptananlarda potasyum düzeyi istatistiksel olarak anlamlı düzeyde daha düşüktü (6,42±0,77 vs 6,03±0,71, p=0,018). EKG'de herhangi bir patoloji saptananlarda kalsiyum (8,69±0,87 vs 8,36±0,85, p=0,037) ve kalsiyum/potasyum oranı istatistiksel olarak anlamlı düzeyde daha düşük saptandı (1,39±0,19 vs 1,32±0,2, p=0,036). Sivri T dalgası saptananlarda kalsiyum/ potasyum oranı istatistiksel olarak anlamlı düzeyde daha düşüktü (1,36±0,2 vs 1,27±0,2, p=0,016). Hastanede veya 30 gün içinde ölen olguların kalsiyum/ potasyum düzeyi istatistiksel olarak anlamlı düzeyde daha düşük, potasyum düzeyi ise daha yüksekti (p<0,05). Sonuç olarak, acil servise böbrek yetmezliği tanısına ek olarak hiperkalemi ile başvuran olgularda EKG'de patoloji saptanma durumu daha düşük kalsiyum düzeyi ve kalsiyum/potasyum oranı ile ilişkilendirilmiştir. Ayrıca ilk 30 gün içinde mortalite, potasyum düzeyinin artışı ve kalsiyum/potasyum oranının azalışı ile ilişkili bulunmuştur. Rutin pratikte akut ya da kronik böbrek yetmezliğine ek olarak hiperkalemi saptanan olgularda kliniğe kardiyak sorunların eşlik edebileceği akılda tutulmalıdır

Anahtar Kelimeler: Kalsiyum. Potasyum. EKG. Acil servis.

Date Received: 17.May.2025
Date Accepted: 24.July.2025

* Presented as an oral presentation at the "XX. Ulusal Acil Tıp Kongresi & XI. Intercontinental Emergency Medicine Congress & XI. International Critical Care and Emergency Medicine Congress" (May 2024, Antalya)

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Renal failure is a significant clinical condition that leads to disturbances in the body's water, electrolyte, and acid-base balance. Both acute kidney injury (AKI) and chronic kidney disease (CKD) can alter serum levels of electrolytes such as potassium and calcium, potentially causing serious disruptions in the cardiac conduction system.

Hyperkalemia is a common clinical problem, often resulting from impaired urinary potassium excretion due to acute or chronic kidney disease and/or disorders or medications that inhibit the renin-angiotensin-aldosterone system (RAAS)[1]. It is typically defined as serum or plasma potassium levels exceeding the upper limit of normal, generally above 5.0–5.5 mEq/L. While mild hyperkalemia is often asymptomatic, higher levels can lead to life-threatening cardiac arrhythmias, muscle weakness, or paralysis[2]. Electrocardiographic manifestations of hyperkalemia may include a widened QRS complex, peaked T waves, prolonged QT interval, and absent P waves[3].

Hypokalemia, one of the most frequently encountered electrolyte disorders in clinical practice, can increase the risk of life-threatening arrhythmias. ECG features associated with hypokalemia (typically at potassium levels <2.7 mmol/L) include dynamic changes in T wave morphology, ST-segment depression, and U waves, particularly in mid-precordial leads (V2–V4). The PR interval may be prolonged with an increase in P wave amplitüde[4].

Another electrolyte disorder, hypercalcemia, is defined as a serum calcium concentration >10.5 mg/dL, with primary hyperparathyroidism and malignancies accounting for 90% of cases. Management focuses on treating the underlying cause. ECG changes associated with hypercalcemia may include a prolonged PR interval and shortened QT interval[5].

Hypocalcemia, most commonly due to hypoalbuminemia, is often associated with cirrhosis, nephrotic syndrome, malnutrition, burns, chronic illnesses, and sepsis. The hallmark ECG feature of hypocalcemia is a prolonged QT interval, resulting from ST segment prolongation[6,7].

Recent studies suggest that the serum calcium/potassium ratio may serve as a novel biomarker influencing cardiac electrical activity. This ratio reflects the balance between two critical electrolytes and may have clinical significance. However, its relationship with ECG findings has not been thoroughly explored, especially in patients with renal failure[8,9].

This study aimed to evaluate the association between ECG changes and serum calcium and potassium levels, as well as the calcium/potassium ratio, in patients diagnosed with acute or chronic renal failure accompanied by hyperkalemia.

Material and Method

This study was conducted with the approval of the Clinical Research Ethics Committee (November 23, 2022; decision number: 2022-18/43) by retrospectively reviewing the medical records of patients diagnosed with renal failure and hyperkalemia (serum potassium>5.5 mEq/L) who presented to the emergency departments of Bursa Uludağ University Faculty of Medicine Hospital (n=137) and Bursa High Specialization Training and Research Hospital (n=44) between November 1, 2022, and October 31, 2023.

Inclusion criteria were a confirmed diagnosis of acute or chronic renal failure, serum potassium levels above 5.5 mEq/L, and complete clinical and ECG data. Recorded parameters included age, sex, vital signs (blood pressure, oxygen saturation, heart rate), diagnosis of acute or chronic renal failure, laboratory results (arterial and venous blood gases), ECG findings, need for hemodialysis, clinical outcomes, and 30-day mortality.

All ECG's were evaluated retrospectively by two emergency physicians experienced in electrocardiography, each with more than five years of clinical experience and inter-observer agreement was not formally assessed. Inter-observer reliability was assessed using Cohen's kappa coefficient, which indicated substantial agreement ($\kappa = 0.78$).

Statistical Analysis

Data were analyzed using SPSS version 26.0. Categorical variables were summarized as counts and percentages, while continuous numerical variables were reported as means, standard deviations, and medians. Normal distribution of continuous data was assessed using the Shapiro-Wilk test and was found to be non-normal. Comparisons between two groups were performed using the Mann-Whitney U test, while

comparisons among three groups were conducted using the Kruskal-Wallis test. Bonferroni correction and pairwise comparisons were applied to identify significant group differences. A p-value of <0.05 was considered statistically significant. Box-and-whisker plots were used for data visualization. To assess the ability of the calcium/potassium (Ca/K) ratio to predict 30-day mortality, a receiver operating characteristic (ROC) curve analysis was conducted. The area under the curve (AUC) was calculated to determine the discriminative performance of the Ca/K ratio. The optimal cut-off value was determined using the Youden index, and corresponding sensitivity and specificity values were reported. Roc Analysis was performed using MedCalc® Statistical Software version 23.0.8 (MedCalc Software Ltd, Ostend, Belgium; 2024). Additionally, a multivariate logistic regression analysis was performed to identify independent predictors of 30-day mortality. Variables with p<0.10 in univariate comparisons (e.g., serum potassium, serum calcium, calcium/potassium ratio, need for hemodialysis, and ICU admission) were included in the model. The results were presented as odds ratios (OR) with 95% confidence intervals (CI), and a p-value<0.05 was considered statistically significant.

Results

Among the 177 patients included in the study, 55.4% were male and 44.6% were female. Of these, 44.6% were diagnosed with chronic renal failure, 52.0% with acute renal failure, and 3.4% with acute-on-chronic renal failure.

ECG abnormalities were detected in 69.5% of cases. The most common pathological findings were atrial fibrillation (22.0%) and peaked T waves (20.3%) (Table I) Hemodialysis was performed in 52.5% of the patients, 23.2% were admitted to the intensive care unit (ICU), and 15.8% died within 30 days of hospital admission.

Compared to patients without T wave inversion, those with this finding had significantly lower potassium levels (p=0.018).

Compared to discharged patients and those admitted for treatment, patients who died had significantly higher potassium levels (p=0.001). On day 30 following hospital admission, potassium levels were significantly higher in deceased patients compared to survivors (6.31 ± 0.71 vs. 6.85 ± 0.94, p = 0.006).

The calcium/potassium ratio was significantly lower in patients with peaked T waves compared to those without (1.36 ± 0.20 vs. 1.27 ± 0.20, p = 0.016). No statistically significant association was found between other ECG abnormalities and the calcium/potassium ratio (p > 0.05) (Table II)

Table I. Distribution of Electrocardiographic (ECG) Findings

Variables	Number (n)	Percent (%)
Pathological findings in ECG		
None	54	30.5
ECG findings present	123	69.5
Atrial fibrillation	39	22.0
Peaked T wave	36	20.3
Bundle branch block	18	10.2
T wave inversion	12	6.8
QRS prolongation	9	5.1
Prolonged PR interval	6	3.4
Loss of P wave	4	2.3
Asystole	4	2.3
Progressive QRSwidening	1	0.6
ST segment elevation	1	0.6
Ventricular tachycardia	1	0.6
Ventricular fibrillation	1	0.6

Table II. Calcium/Potassium Ratio Based on Specific ECG Changes

Variables	Calcium/Potassium Ratio (Mean±SD. Median)	*p*
Any ECG pathology		
None	1.39 ± 0.19 (1.39)	**0.036**
Present	1.32 ± 0.2 (1.33)	
Peaked T wave		
None	1.36 ± 0.2 (1.38)	**0.016**
Present	1.27 ± 0.2 (1.27)	
Bundle branch block		
None	1.34 ± 0.2 (1.37)	0.778
Present	1.33 ± 0.21 (1.3)	
Prolonged PR interval		
None	1.34 ± 0.2 (1.35)	0.670
Present	1.37 ± 0.21 (1.39)	
Loss of P wave		
None	1.34 ± 0.2 (1.36)	0.343
Present	1.25 ± 0.18 (1.26)	
QRS prolongation		
None	1.35 ± 0.2 (1.36)	0.167
Present	1.23 ± 0.23 (1.19)	
VF/VT/Asystole		
None	1.34 ± 0.2 (1.36)	0.370
Present	1.27 ± 0.18 (1.28)	
T wave inversion		
None	1.34 ± 0.2 (1.35)	0.276
Present	1.39 ± 0.21 (1.41)	
Atrial fibrillation		
None	1.34 ± 0.21 (1.36)	0.733
Present	1.33 ± 0.17 (1.34)	

VF: Ventricular fibrillation. VT: Ventricular tachycardia

Compared to discharged and hospitalized patients, those who died had significantly lower calcium/potassium ratios ($p<0.001$). Similarly, on day 30, deceased patients had significantly lower calcium/potassium ratios compared to survivors (1.36 ± 0.19 vs. 1.23 ± 0.21, p = 0.002) (Table III).

Table III. Calcium/Potassium Ratio by Type of Renal Failure and Clinical Outcomes

Variables	Calcium/Potassium Ratio (Mean±SD. Median)	p
Type of renal failure		
Chronic	1.33 ± 0.18 (1.34)	0.171
Acute	1.34 ± 0.21 (1.38)	
Acute on chronic renal failure	1.52 ± 0.23 (1.43)	
Clinical outcome		
Treatment rejection	1.33 ± 0.18 (1.27)	**<0.001**
Discharged	1.42 ± 0.19 (1.39)[a]	
Clinical admission (hospitalized)	1.37 ± 0.19 (1.39)[a]	
ICU admission	1.28 ± 0.2 (1.31)	
Eksitus	1.18 ± 0.17 (1.16)[b]	
30 day mortality		
None	1.36 ± 0.19 (1.38)	**0.002**
Present	1.23 ± 0.21 (1.2)	

ICU: Intensive care unit

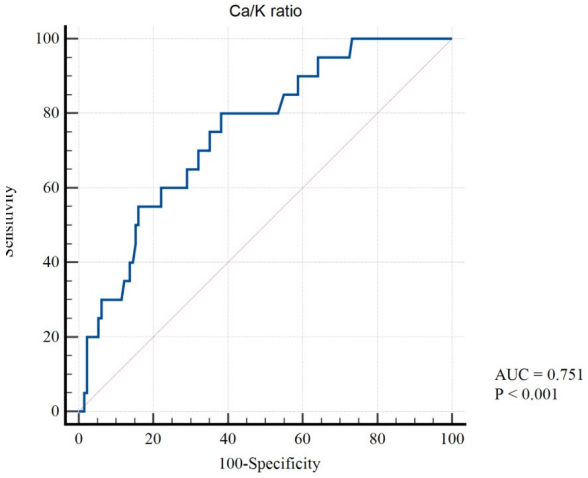
Multivariate logistic regression analysis revealed that a lower calcium/potassium ratio (OR: 0.37, 95% CI: 0.15–0.91, p=0.031) and higher serum potassium levels (OR: 2.28, 95% CI: 1.14–4.53, p=0.021) were independently associated with 30-day mortality (Table IV). Other variables (e.g., serum calcium, ICU admission, hemodialysis) did not remain statistically significant in the final model.

Table IV. Multivariate logistic regression analysis results

Variable	OR	95% CI	p
Calcium/Potassium ratio	0.37	0.15-0.91	0.031
Serum Potassium (mEq/L)	2.28	1.14-4.53	0.021
Serum Calcium (mg/dL)	0.89	0.53-1.48	0.653
Hemodialysis	1.16	0.45-2.95	0.756
ICU Admission	1.78	0.67-4.72	0.246

ICU: Intensive care unit

To evaluate the predictive value of the calcium/potassium (Ca/K) ratio for 30-day mortality, a receiver operating characteristic (ROC) curve analysis was performed. The area under the curve (AUC) was calculated as 0.751, indicating modest discriminative ability. The optimal cut-off value for the Ca/K ratio was determined to be 1.317, with a sensitivity of 80.0% and specificity of 61.83% (Figure 1).



(AUC: Area Under the Curve)

Figure 1.
ROC curve showing the predictive performance of the calcium/potassium (Ca/K) ratio for 30-day mortality in patients with renal failure and hyperkalemia. The AUC was 0.751

Discussion and Conclusion

To our knowledge, this is one of the few studies to explore the calcium/potassium ratio as a potential predictor of cardiac complications and short-term mortality in hyperkalemic renal failure patients. The observed associations may be explained by the synergistic impact of calcium and potassium on cardiac myocyte membrane stability and depolarization thresholds.

In our cohort, 52.5% of patients underwent hemodialysis, 23.2% were admitted to the ICU, and 15.8% died within 30 days. In a similar study by Davis et al[10]., involving 6,222 hyperkalemic patients, 24.3% underwent hemodialysis, 27.2% were admitted to the ICU, and the emergency department mortality rate was 15.4%. Peacock et al.[11] reported that 20.1% of 41 patients underwent hemodialysis, 79% were hospitalized, 19% required ICU care, and the 15-day mortality rate was 1.5% . These findings align in part with the literature, though differences in sample size and study settings may account for variations.

In our study, ECG abnormalities were detected in 69.5% of patients, with atrial fibrillation (22%) and peaked T waves (20%) being the most common. Potassium levels were significantly lower in patients with T wave inversions. In a study by Mulia et al[12]. involving 191 patients, ECG abnormalities were found

in 92.1%, with the most frequent being prolonged QTc interval (36.6%), fragmented QRS complex (29.8%), poor R wave progression (24.6%), peaked T waves (22.0%), and left ventricular hypertrophy (16.7%). Rafee et al. reported similar findings in their study of 67 hyperkalemic patients[13].

Studies by Thongprayoon et al. and Singer et al. also demonstrated associations between potassium levels and hospitalization or mortality[14,15]. Goyal et al. found that in patients with acute myocardial infarction, maintaining potassium levels between 3.5–4.5 mEq/L was associated with lower mortality[16].

In our study, patients with ECG abnormalities had significantly lower calcium levels compared to those without. Hou et al. showed that maintaining serum calcium levels between 8.2–10.4 mg/dL reduced the incidence of cardiac arrest in stroke patients[17]. Yarmohammadi et al. reported that low serum calcium levels were independently associated with increased risk of sudden cardiac arrest in the general population[18].

Furthermore, in our study, patients with ECG abnormalities—particularly those with peaked T waves—had significantly lower calcium/potassium ratios. Diercks and Webster also highlighted that ECG changes in hyperkalemia are strongly associated with electrolyte imbalances, and may inversely correlate with calcium levels[3,19].

Our multivariate analysis showed that both lower calcium/potassium ratio and higher serum potassium were independently associated with increased 30-day mortality, supporting their potential role as clinical markers. The ROC analysis showed that the Ca/K ratio had a modest discriminatory ability for predicting 30-day mortality (AUC = 0.751), suggesting its potential utility when combined with other clinical and laboratory parameters.

In this study, ECG abnormalities were observed in approximately 70% of patients presenting to the emergency department with renal failure and hyperkalemia. Half of these patients underwent hemodialysis, and the 30-day mortality rate was notably high (15.8%). ECG abnormalities were significantly associated with lower serum calcium levels and calcium/potassium ratios. Additionally, increased potassium levels and reduced calcium/potassium ratios were significantly linked to 30-day mortality. These findings suggest that clinicians should consider the potential for cardiac complications in patients with renal failure and hyperkalemia. Prompt recognition, comprehensive evaluation, and timely intervention may reduce morbidity and mortality. Further prospective, multicenter studies are warranted to better understand the cardiac implications of potassium and calcium imbalances in this patient population.

Limitations

The cross-sectional design of this study limits causal inferences. The absence of a normokalemic control group prevents assessment of the specificity and predictive value of ECG findings in hyperkalemia. The relatively small sample size, limited to emergency department admissions, and evaluation by a small number of ECG interpreters further restrict generalizability. Serum calcium values were not corrected for albumin levels. Given the prevalence of hypoalbuminemia in patients with renal failure, uncorrected calcium measurements may not fully reflect true ionized calcium status. This may have impacted the interpretation of the calcium/potassium ratio. Additionally, the retrospective nature of the study did not allow comparison of current ECGs with pre-illness ECGs, raising the possibility that some findings may have preceded hyperkalemia. Future prospective, multicenter studies are needed to validate these findings and assess ECG changes in hyperkalemic versus normokalemic patients while accounting for other clinical and biochemical variables.

Researcher Contribution Statement:
Idea and design: O.K, M.Y.,V.A.D, Q.M.A.; Data collection and processing: Q.M.A, E.K.,Analysis and interpretation of data: E.K, M.Y., Q.M.A.; Writing of significant parts of the article: V.A.D, O.K, G.A, M.Y., Q.M.A.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Uludağ Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu
Approval Date: 23.11.2022
Decision No: 2022-18/43

References

1. Clase CM, Carrero JJ, Ellison DH, et al. Potassium homeostasis and management of dyskalemia in kidney diseases: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int. 2020;97(1):42-61.
2. Lindner G, Burdmann EA, Clase CM, et al. Acute hyperkalemia in the emergency department: a summary from a Kidney Disease: Improving Global Outcomes conference. Eur J Emerg Med. 2020;27(5):329-337.
3. Diercks DB, Shumaik GM, Harrigan RA, et al. Electrocardiographic manifestations: electrolyte abnormalities. J Emerg Med. 2004;27(2):153-160.
4. Wang X, Han D, Li G. Electrocardiographic manifestations in severe hypokalemia. J Int Med Res. 2020;48(1):300060518811058.
5. Merali Z, Srivaratharajah K, Panju M, et al. Hypercalcemia. In: McMaster Textbook of Internal Medicine. Kraków, Poland: Medycyna Praktyczna. Accessed August 2, 2024. https://empendium.com/mcmtextbook/chapter/B31.II.19.1.6.2. Accessed August 2, 2024

6. Slovis C, Jenkins R. ABC of clinical electrocardiography: conditions not primarily affecting the heart. BMJ. 2002;324(7349):1320-1323.
7. Panju M, Srivaratharajah K, Merali Z, et al. Hypocalcemia. In: McMaster Textbook of Internal Medicine. Kraków, Poland: Medycyna Praktyczna. Accessed August 2, 2024. https://empendium.com/mcmtextbook/chapter/B31.II.19.1.6.1. Accessed August 2, 2024
8. Noordam R, Young WJ, Salman R, et al. Effects of calcium, magnesium, and potassium concentrations on ventricular repolarization in unselected individuals. J Am Coll Cardiol. 2019;73(24):3118-3131.
9. Bukhari HA, Sánchez C, Ruiz JE, et al. Monitoring of serum potassium and calcium levels in end-stage renal disease patients by ECG depolarization morphology analysis. Sensors (Basel). 2022;22(8):2951.
10. Davis J, Israni R, Betts KA, et al. Real-world management of hyperkalemia in the emergency department: an electronic medical record analysis. Adv Ther. 2022;39(2):1033-1044.
11. Peacock WF, Rafique Z, Clark CL, et al. Real world evidence for treatment of hyperkalemia in the emergency department (REVEAL-ED): a multicenter, prospective, observational study. J Emerg Med. 2018;55(6):741-750.
12. Mulia EPB, Nugraha RA, A'yun MQ, et al. Electrocardiographic abnormalities among late-stage non-dialysis chronic kidney disease patients. J Basic Clin Physiol Pharmacol. 2020;32(3):155-162.
13. Rafee LA, Alawneh KZ, Ababneh MJ, et al. Clinical and electrocardiogram presentations of patients with high serum potassium concentrations within emergency settings: a prospective study. Int J Emerg Med. 2022;15(1):23.
14. Thongprayoon C, Cheungpasitporn W, Thirunavukkarasu S, et al. Serum potassium levels at hospital discharge and one-year mortality among hospitalized patients. Medicina (Kaunas). 2020;56(5):236.
15. Singer AJ, Thode HC Jr, Peacock WF. A retrospective study of emergency department potassium disturbances: severity, treatment, and outcomes. Clin Exp Emerg Med. 2017;4(2):73-79.
16. Goyal A, Spertus JA, Gosch K, et al. Serum potassium levels and mortality in acute myocardial infarction. JAMA. 2012;307(2):157-164.
17. Hou J, Huang Z, Zeng W, et al. Serum calcium is associated with sudden cardiac arrest in stroke patients from ICU: a multicenter retrospective study based on the eICU collaborative research database. Sci Rep. 2024;14(1):1700.
18. Yarmohammadi H, Uy-Evanado A, Reinier K, et al. Serum calcium and risk of sudden cardiac arrest in the general population. Mayo Clin Proc. 2017;92(10):1479-1485.
19. Webster A, Brady W, Morris F. Recognising signs of danger: ECG changes resulting from an abnormal serum potassium concentration. Emerg Med J. 2002;19(1):74-77.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 291-294, 2025
DOI: https://doi.org/10.32708/uutfd.1723791

ORIGINAL RESEARCH

Investigation of Drug Resistant *Mycobacterium tuberculosis* Strains with Molecular Methods

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ABSTRACT

The aim of this study was to investigate mutations in the ***rpo*B** and ***emb*B** genes using molecular methods in *Mycobacterium tuberculosis* isolates phenotypically resistant to rifampicin and/or ethambutol. Drug susceptibility testing of 110 isolates collected in Turkey between 2005 and 2014 was performed using the modified agar proportion method. Molecular analysis was conducted on 28 selected isolates, including 7 rifampicin-resistant strains. Mutations were detected within the Rifampicin Resistance-Determining Region (RRDR) between codons 507–533 of the ***rpo*B** gene. The most common mutation was **Ser531Leu (TCG→TTG)**, found in 10 strains; one strain had a **His526Asn (CAC→AAC)** mutation. These mutations were considered important molecular markers of rifampicin resistance. Rapid molecular detection of these mutations is important for early and effective tuberculosis treatment planning

Keyword: *Mycobacterium tuberculosis*. Drug resistance. Molecular analysis.

Moleküler Yöntemlerle İlaç Dirençli *Mycobacterium tuberculosis* Suşlarının Araştırılması

ÖZET

Bu çalışmanın amacı, fenotipik olarak rifampisin ve/veya etambutol dirençli *Mycobacterium tuberculosis* izolatlarında, ***rpo*B** ve ***emb*B** genlerindeki mutasyonları moleküler yöntemlerle araştırmaktır. 2005–2014 yılları arasında Türkiye'den toplanan 110 izolatın ilaç duyarlılıkları modifiye agar proporsiyon yöntemiyle test edilmiştir. Seçilen 28 suşta moleküler analiz yapılmış, 7'si rifampisin dirençli bulunmuştur. ***rpo*B** geninde, Rifampisin Direnç Belirleyici Bölge (RRDR) olan 507–533. kodonlar arasında mutasyonlar tespit edilmiştir. En yaygın mutasyon, 10 suşta görülen **Ser531Leu (TCG→TTG)** mutasyonu, bir suşta ise **His526Asn (CAC→AAC)** mutasyonudur. Bu mutasyonlar, rifampisin direncinin moleküler göstergeleri olarak önemli bulunmuştur. Moleküler yöntemlerle bu mutasyonların hızlı tespiti, tüberküloz tedavisinin erken planlanması için önemlidir.

Anahtar Kelimeler: *Mycobacterium tuberculosis*. Ilaç direnci. Moleküler analiz.

Tuberculosis is an important public health problem all over the world despite modern diagnostic, treatment, and control methods.[1] The gold standard for diagnosis is still *Mycobacterium tuberculosis* culture, which is essential for identification and drug susceptibility testing.[2]

Date Received: 23.June.2025
Date Accepted: 29.July.2025

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Rifampicin (RIF) and ethambutol (ETB), both of which have antimycobacterial effects, are major anti-tuberculosis drugs used in the treatment of the disease. RIF affects bacterial protein synthesis, while ETB inhibits arabinogalactan synthesis through inhibition of the arabinosyl transferase enzyme.[3]

Multidrug resistance in *Mycobacterium tuberculosis* represents a global health burden.[4] Resistance to RIF in the *M. tuberculosis* complex is caused by mutations in the 81-base pair (bp) region of the **rpoB** gene, which encodes the β-subunit of RNA polymerase.[4–7] The frequency of codon mutations in the *rpoB* gene among RIF-resistant *M. tuberculosis* isolates varies by geographic region. Sequence analysis of the *rpoB* gene in 37 isolates from Italy revealed mutations in codons 531 (59.4%), 526 (35.1%), and 516 (8.1%).[7] Data from 86 isolates in China showed mutations at codons 531 (41.0%), 526 (40.0%), 516 (4.0%), 513 (2.0%), and 533 (2.0%).[8] Two reports from Taiwan in the past decade also analyzed the prevalence of *rpoB*

mutations.[8–10] Further surveillance is clearly needed to monitor the evolution of mutations and their associated resistance.

This study aimed to investigate the presence of mutations in the **embB** and **rpoB** gene regions, as well as mutation patterns, through DNA sequence analysis of isolates that exhibited phenotypic resistance to RIF and/or ETB in drug susceptibility tests.

Material and Method

M. tuberclosis isolates

In our study, a hundred ten *M. tuberculosis* samples, which detected at least one phenotypic anti-tuberculosis drug resistance, were collected the Tuberculosis Laboratory of the Medical Microbiology Department of the Uludağ University Health Practice and Research Center between 2005 and 2014. Seven of these strains were only resistance to RIF, 15 were ETB, 6 were both. Sterile clinical specimens were centrifuged and then centrifuged at the bottom of the centrifuge tube. Non-sterile specimens were inoculated to Löwenstein-Jensen and MGIT media after homogenization-decontamination and incubated at 37 ° C in an incubator for 6 weeks. Strains were separated from MOTT using the PNB test and the MGIT TBc test.

DNA isolation and Polymerase chain reactions (PCR)

DNA was extracted from the *M. tuberculosis* using a Tris-EDTA. DNA from the samples was amplified with selected primers designed with Ensembl (Table I).

Table I. Primers for the amplification of *rpo*B and *emb*B.

Gene	Primers	Base-pair
*rpo*B-F	CTTGCACGAGGGTCAGACCA	543
*rpo*B-R	ATCTCGTCGCTAACCACGCC	
*emb*B-F	CTGACCGACGCCGTGGTGATAT	490
*emb*B-R	TGAATGCGGCGGTAACGACG	

Amplifications with Taq polymerase were performed in 25-µl reaction mixtures containing 0.15 mM of deoxyribonucleoside triphosphate (dNTP, Promega-U1515, USA), 10 pmol of each primer, 5 U/ml of Taq DNA polymerase (Promega-M8305, USA), and 150 ng of genomic DNA. Polymerase chain reactions (PCR) were performed with a 2-min initial denaturation at 94°C, followed by 30 cycles of 1 min at 94°C, 30 s at 55°C, 1 min at 72°C, and a 10-min final extension at 72°C. The PCR products were separated on a 2% agarose gel and stained with ethidium bromide.

Sequencing analysis

The PCR products were purified according to the manufacturer's instructions by OMEGA. The RIF-resistance-determining hot-spot region of *rpo*B and *emb*B samples was sequenced by Dye Terminator Cycle Sequencing (DTCS) and analyzed using a CEQ-8000 Automated DNA Sequencing System (Beckman Coulter, Inc., Fullerton, CA, USA). The results of the sequencing analysis were compared with wild-type samples and the wild-type sequences of these genes. The relationships between the defined alterations and the risk of resistance were verified using the Ensembl Genome Browser (http://www.ensembl.org).

This study was conducted with the approval of the Clinical Research Ethics Committee of Uludağ University Faculty of Medicine (decision dated 09.06.2015, no. 2015-12/17) and was supported by the Uludağ University Scientific Research Projects Unit (project no. KUAP(T)-2015/43).

Results

In our study, *M. tuberculosis* complex strains that detected at least one phenotypic anti-tuberculosis drug resistance were investigated in the Tuberculosis Laboratory of the Medical Microbiology Department of Medical Utilization Research Center of Uludağ University between 2005 and 2014. These tuberculosis complex strains examined belong to 105 patients. The samples were mostly sent from the Department of Chest Diseases (60%) followed by the Department of Infectious Diseases and Pediatric Infectious Diseases. A total of 110 strains were tested; 75 strains (68.2%) had single-strand resistance, 23 (21%) had two strains, and 12 strains (11%) had very little resistance. Molecular analysis included a total of 28 strains, 7 with RIF resistance, 15 with ETB resistance, and 6 with both RIF and ETB resistance, and investigated the presence of mutations in the *rpo*B and *emb*B gene regions. In our study, a mutation in the *rpo*B gene was detected in 11 (84.6%) of 13 strains phenotypically resistant to RIF. Two of them (16.4%) were phenotypically resistant to RIF, but no mutations were observed in the examined gene locus. Mutations detected were found between codons 507 and 533, which are defined as the RRDR region. Mutation is detected in 11 strains of 10 strains, with the Ser531Leu (TCG-TTG) mutation found in 531. (Figure-1). In one strain, the His526Asn (CAC-AAC) mutation was detected (Figure-2).

In 21 strains resistant to ETB phenotypically, mutation in *the embB* gene was detected in 14 (66.6%). (Figure 3). Seventy-three percent (33.3%) were phenotypically resistant to ETB, but no mutation was observed in the examined gene locus. Mutations were detected in 14 strains, 3 in binary mutation and 11 in single mutation.

The most frequently identified mutation was the Ser297Ala (TCG-GCG) mutation in the 297th codon and was detected in 8 strains. In four strains, the Met306Val (ATG-GTG) mutation was detected at the 306th codon.

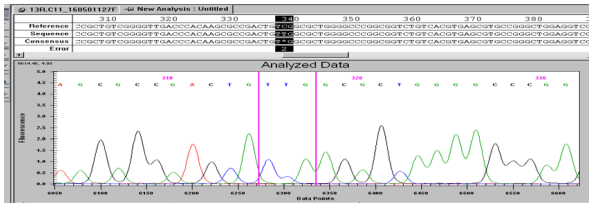

Figure 1.
Codon 531 mutation (Mutation is shown in black).

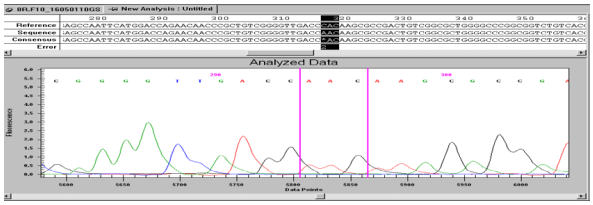

Figure 2:
Codon 526 mutation (Mutation is shown in black)

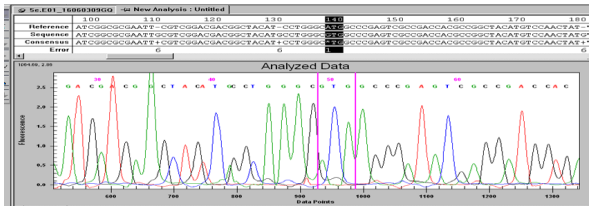

Figure 3:
Codon 306 mutation. The location of the mutation is shown in black.

Discussion and Conclusion

Although tuberculosis has a very long history, it remains an infectious disease that continues to evolve. Despite precise knowledge of its cause and the availability of effective medications, high morbidity rates still contribute to significant mortality.[11,12] Early diagnosis, effective and regular treatment of tuberculosis patients, and appropriate follow-up of treatment are the foundations of the tuberculosis control program.[13] One of the most important roles of the microbiology laboratory in tuberculosis control is the rapid and reliable detection of drug resistance. Culture-based phenotypic susceptibility testing for *Mycobacterium tuberculosis* is time-consuming and labor-intensive, which has led to the widespread use of molecular methods.

Rifampicin (RIF) is one of the most effective drugs in tuberculosis treatment and forms the basis of combination therapy alongside isoniazid. Numerous studies have shown that RIF resistance-associated mutations most frequently occur in codons 531, 526, and 516.[14–19]

Ethambutol (ETB) is a narrow-spectrum, first-line antimycobacterial agent used in tuberculosis treatment. It plays an important role in combination therapy and enhances the efficacy of RIF, aminoglycosides, and quinolones.[20] The most frequent mutations in the *embB* gene of ETB-resistant strains are missense mutations at codon 306.[21] However, Mokrousov et al. identified the embB 306 mutation in both ETB-sensitive and ETB-resistant strains.[22]

In our study, a total of 21 strains phenotypically resistant to ETB were examined, and mutations in the *embB* gene were found in 14 of them (66.6%). No mutations were detected in the examined gene region in 7 strains (33.3%) despite phenotypic resistance. Among the 14 mutated strains, 11 had single mutations and 3 had double mutations. The most frequently observed mutation was Ser297Ala (TCG → GCG) at codon 297, found in 8 strains. Four strains had the Met306Val (ATG → GTG) mutation at codon 306. Additional mutations were detected at codons 405 (Glu405Asp: GAG → GAC), 406 (Glu408Asp: GGC → GAC), and 378 (Glu378Ala: GAG → GCG). Our findings of mutations at codons 378, 405, and 406 are consistent with the results of Campbell et al.[23]

Mutations at different *rpoB* codons are associated with varying levels of RIF resistance. Mutations at codons 531 and 526 are typically linked to high-level resistance (MIC > 64 µg/mL) and cross-resistance to all rifamycins, whereas mutations at codon 516 are associated with moderate-level resistance (MIC = 32 µg/mL) and susceptibility to rifabutin.[24–27] However, in India, some isolates with mutations at codons 516 or 533 were shown to have high-level resistance (MIC > 128 µg/mL).[13] Although MIC testing was not performed in our study, multidrug resistance was observed in all strains with mutated alleles.[11,23]

In previous studies, strains with the same *rpoB* genotype from different geographic regions showed similar drug resistance patterns.[14,23] In contrast, the 14 isolates with allele 2 in our study demonstrated three different resistance profiles. These differences may result from the selective pressure of therapeutic regimens over time in various geographic regions.[12]

As a result, the presence of genes and mutations associated with RIF and ETB resistance in *M. tuberculosis* strains isolated from Bursa and surrounding regions was investigated for the first time. We believe that these findings will contribute to the literature not only for the Marmara Region but also for our country, even though the study was conducted with regional strains.

Molecular methods are important for the rapid diagnosis of *M. tuberculosis* and the identification of resistance. DNA sequence analysis of drug resistance-related genes is recommended as a reference method for establishing proper treatment protocols. However, the absence of mutations in frequently studied gene regions of phenotypically resistant strains complicates diagnosis and suggests the need to study additional gene regions. Conversely, detecting mutations in strains that are phenotypically susceptible may be the only way to reveal existing genetic alterations. Still, such findings are only meaningful if the corresponding proteins encoded by the mutated genes are also present and functional. Therefore, resistance to antituberculous drugs may open new research areas in proteomics.

Researcher Contribution Statement:
Idea and design: M.P.; Data collection and processing: S.A.; Analysis and interpretation of data: C.Ö.; Writing of significant parts of the article: M.P., C.Ö., S.S.
Support and Acknowledgement Statement:
This study was supported by the Uludağ University Scientific Research Projects Unit (project no. KUAP(T)-2015/43).
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Clinical Research Ethics Committee of Uludağ University Faculty of Medicine
Approval Date: 09.06.2015
Decision No: 2015-12/17

References

1. Gagneux S. Ecology and evolution of *Mycobacterium tuberculosis*. Nat Rev Microbiol. 2018;16(4):202–213. doi:10.1038/nrmicro.2018.8
2. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, et al. Tuberculosis. Nat Rev Dis Primers. 2016;2:16076. doi:10.1038/nrdp.2016.76
3. World Health Organization. Global Tuberculosis Report 2023. 2023. [Accessed: June 23, 2024]
4. Dong B, He Z, Li Y, Xu X, Wang C, Zeng J. Improved conventional and new approaches in the diagnosis of tuberculosis. Front Microbiol. 2022;13:924410.
5. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med. 2010;363(11):1005–1015. doi:10.1056/NEJMoa0907847
6. Ohno H, Koga H, Kohno S, Tashiro K, Hara K. Relationship between rifampin MICs for and rpoB mutations of *Mycobacterium tuberculosis* strains isolated in Japan. Antimicrob Agents Chemother. 1996;40(5):1053–1056.
7. Heep M, Rieger U, Beck D, Lehn N. Mutations in the beginning of the rpoB gene can induce resistance to rifamycins in both *Helicobacter pylori* and *Mycobacterium tuberculosis*. Antimicrob Agents Chemother. 2000;44(4):1075–1077.
8. Taniguchi H, Aramaki H, Nikaido Y, Mizuguchi Y, Nakamura M, Koga T, et al. Rifampicin resistance and mutation of the rpoB gene in *Mycobacterium tuberculosis*. FEMS Microbiol Lett. 1996;144(1):103–108.
9. Lee PH, Chan PC, Peng YT, Chu PW, Wu MH, Jou R, et al. Impact of universal drug susceptibility testing and effective management of multidrug-resistant tuberculosis in Taiwan. PLoS One. 2019;14(4):e0214792.
10. Yu MC, Chiang CY, Lee JJ, Chien ST, Lin CJ, Lee SW, et al. Treatment outcomes of multidrug-resistant tuberculosis in Taiwan: tackling loss to follow-up. Clin Infect Dis. 2018;67(2):202–210. doi:10.1093/cid/ciy066
11. Chan PC, Huang SH, Yu MC, Lee SW, Huang YW, Chien ST, et al. Effectiveness of a government-organized and hospital-initiated treatment for multidrug-resistant tuberculosis patients—a retrospective cohort study. PLoS One. 2013;8(2):e57719. doi:10.1371/journal.pone.0057719
12. Georghiou SB, Gomathi NS, Rajendran P, Nagalakshmi V, Prabakaran L, Prem Kumar MM, et al. Accuracy of the Truenat MTB-RIF Dx assay for detection of rifampicin resistance-associated mutations. Tuberculosis (Edinb). 2021;127:102064.
13. Singh UB, Singh M, Sharma S, Mahajan N, Bala K, Srivastav A, et al. Expedited diagnosis of pediatric tuberculosis using Truenat MTB-Rif Dx and GeneXpert MTB/RIF. Sci Rep. 2023;13:6976.
14. Meaza A, Tesfaye E, Mohamed Z, Zerihun B, Seid G, Eshetu K, et al. Diagnostic accuracy of Truenat Tuberculosis and Rifampicin-Resistance assays in Addis Ababa, Ethiopia. PLoS One. 2021;16(5):e0261084.
15. Omer ZB, Mekonnen Y, Worku A, Zewde A, Medhin G, Mohammed T, et al. Evaluation of the GenoType MTBDRplus assay for detection of rifampicin-and isoniazid-resistant *Mycobacterium tuberculosis* isolates in central Ethiopia. Int J Mycobacteriol. 2016;5(4):475–481.
16. Siddiqui S, Brooks MB, Malik AA, Fuad J, Nazish A, Bano S, et al. Evaluation of GenoType MTBDR plus for the detection of drug-resistant *Mycobacterium tuberculosis* on isolates from Karachi, Pakistan. PLoS One. 2019;14(8):e0221485.
17. Meaza A, Kebede A, Yaregal Z, Dagne Z, Moga S, Yenew B, et al. Evaluation of genotype MTBDRplus VER 2.0 line probe assay for the detection of MDR-TB in smear positive and negative sputum samples. BMC Infect Dis. 2017;17(1):280.
18. Abanda NN, Djieugoué JY, Lim E, Pefura-Yone EW, Mbacham WF, Vernet G, et al. Diagnostic accuracy and usefulness of the Genotype MTBDRplus assay in diagnosing multidrug-resistant tuberculosis in Cameroon: A cross-sectional study. BMC Infect Dis. 2017;17(1):379 doi: 10.1186/s12879-017-2489-3.
19. Jian J, Yang X, Yang J, Chen L. Evaluation of the GenoType MTBDRplus and MTBDRsl for the detection of drug-resistant *Mycobacterium tuberculosis* on isolates from Beijing, China. Infect Drug Resist. 2018;11:1627–1634.
20. Singh BK, Sharma R, Kodan P, Soneja M, Jorwal P, Nischal N, et al. Diagnostic evaluation of non-interpretable results associated with rpoB gene in Genotype MTBDRplus Ver 2.0. Tuberc Respir Dis (Seoul). 2020;83(4):289–294.
21. Maurya AK, Singh AK, Kant S, Umrao J, Kumar M, Kushwaha RAS, et al. Use of GenoType® MTBDRplus assay to assess drug resistance and mutation patterns of multidrug-resistant tuberculosis isolates in northern India. Indian J Med Microbiol. 2013;31(3):230–236.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 295-303, 2025
DOI: https://doi.org/10.32708/uutfd.1707053

ORIGINAL RESEARCH

Drug Repurposing Targeting Mitochondrial Dysfunction in Parkinson's Disease: FBXO7-Focused Approach Through Network Analysis and *In Silico* Molecular Docking

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ABSTRACT

FBXO7 is a promising but underexplored therapeutic target in Parkinson's disease (PD), having role in mitophagy, proteasomal degradation, and synaptic function. This study aims to investigate the therapeutic potential of targeting mitochondrial dysfunction in PD through an FBXO7-centered drug repurposing approach. A protein-protein interaction (PPI) network was constructed using the STRING database, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to identify key pathways associated with FBXO7. Additionally, *in silico* molecular docking was conducted using the AutoDock Vina algorithm in SwissDock to evaluate the binding affinities of selected clinically approved drugs to FBXO7 and identify promising candidates for potential repurposing in PD treatment. Docking analysis identified several compounds with high binding affinity to FBXO7, including fluorometholone (-6.367 kcal/mol), bendroflumethiazide (-6.354 kcal/mol), lasofoxifene (-6.173 kcal/mol), penicillin V (-6.102 kcal/mol), hydromorphone (-6.067 kcal/mol), and cefamandole (-6.036 kcal/mol). These drugs are involved in biological pathways related to mitochondrial function, neuroinflammation, and cellular stress responses, highlighting their potential as disease-modifying agents in PD. However, limitations such as the potential for exacerbating disease progression or systemic side effects may restrict their direct repurposing. This study highlights several clinically approved drugs with high binding affinities to FBXO7, suggesting their potential for targeting mitochondrial dysfunction in PD. While some compounds may present challenges for or direct use, their molecular interactions offer valuable insights for developing novel mitochondrial-targeted therapies. Further experimental validation and structural optimization are required to enhance their therapeutic potential and minimize side effects, paving the way for novel therapeutic strategies in PD.

Keywords: FBXO7. Drug repurposing. Mitochondrial dysfunction. Neurodegeneration. Parkinson's disease.

Parkinson Hastalığında Mitokondriyal Disfonksiyonu Hedefleyen İlaç Yeniden Konumlandırma: Network Analizi ve *In Silico* Moleküler Docking Aracılı FBXO7 Odaklı Bir Yaklaşım

ÖZET

FBXO7, mitofaji, proteazomal degradasyon ve sinaptik işlevlerde rol oynayan, Parkinson hastalığı (PH)'nda umut vadeden ancak yeterince araştırılmamış bir terapötik hedeftir. Parkinson hastalığında PH'de mitokondriyal disfonksiyonu hedef alan, FBXO7 merkezli bir ilaç yeniden konumlandırma yaklaşımının terapötik potansiyelini araştırmayı amaçlamaktadır. Bu amaçla, STRING veritabanı kullanılarak bir protein-protein etkileşim (PPI) ağı oluşturulmuş; ardından FBXO7 ile ilişkili temel biyolojik yolları belirlemek için Gen Ontolojisi (GO) ve KEGG zenginleştirme analizleri gerçekleştirilmiştir. Ayrıca, seçilen klinik olarak onaylı ilaçların FBXO7'ye bağlanma afinitelerini değerlendirmek ve PH tedavisi için yeniden konumlandırılmaya uygun adayları belirlemek üzere SwissDock üzerinden AutoDock Vina algoritması kullanılarak *in silico* moleküler docking çalışmaları yapılmıştır. Docking analizi sonucunda FBXO7'ye yüksek bağlanma afinitesi gösteren çeşitli bileşikler tanımlanmıştır: florometolon (–6,367 kcal/mol), bendroflumetiyazid (–6,354 kcal/mol), lasofoksifen (–6,173 kcal/mol), penisilin V (–6,102 kcal/mol), hidromorfon (–6,067 kcal/mol) ve sefamandol (–6,036 kcal/mol). Bu ilaçlar, mitokondri işlevi, nöroinflamasyon ve hücresel stres yanıtlarıyla ilişkili biyolojik yollarla bağlantılı olup, PH'de hastalık modifiye edici ajanlar olma potansiyeline sahiptir. Ancak, hastalık progresyonunu kötüleştirme veya sistemik yan etkiler gibi sınırlılıklar doğrudan yeniden kullanımını kısıtlayabilir. Bu çalışma, FBXO7 ile yüksek bağlanma afinitesi gösteren klinik olarak onaylı çeşitli ilaçları ortaya koyarak, bunların PH'de mitokondriyal disfonksiyonu hedeflemek için potansiyel taşıdığını göstermektedir. Bazı bileşiklerin doğrudan kullanımıyla ilgili zorluklar bulunsa da, elde edilen moleküler etkileşim verileri mitokondri odaklı yeni tedavi stratejileri geliştirmek için değerli bilgiler sunmaktadır. Terapötik potansiyelin artırılması ve yan etkilerin azaltılması için ileri düzey deneysel doğrulama ve yapısal optimizasyon gereklidir; bu da PPH için yeni tedavi yaklaşımlarının önünü açabilir.

Anahtar Kelimeler: FBXO7. İlaç yeniden konumlandırma. Mitokondriyal disfonksiyon. Nörodejenerasyon. Parkinson hastalığı.

Date Received: 27.May.2025
Date Accepted: 30.July.2025

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the presence of Lewy bodies that leads to motor and non-motor symptoms[1]. Although the etiology of PD is multifactorial, mitochondrial dysfunction has emerged as a central pathological mechanism. Mitochondria are critical regulators of cellular energy production, redox balance, and apoptosis. Any dysfunction in these organelles is linked to increased neuronal susceptibility in PD[2]. Since mitochondria are highly multifunctional organelles, their proper functioning is critical for the health and longevity of neurons[3]. Therefore, targeting mitochondrial dysfunction is increasingly recognized as a promising strategy for therapeutic intervention in PD.

Genetic studies on monogenic forms of PD have highlighted the significant role of mitochondrial dysfunction in the disease pathogenesis[4]. Although only about 10% of PD cases are linked to specific genetic mutations, investigations into familial PD (PARK) genes have significantly enhanced our knowledge of the causes and mechanisms of PD. Familial PD cases often involve mutations in genes such as Parkin (PRKN), glucocerebrosidase (GBA1), leucine-rich repeat kinase 2 (LRRK2), vacuolar protein sorting-associated protein 35 (VPS35), PTEN-induced putative kinase 1 (PINK1), and F-box only protein 7 (FBXO7), many of which are directly associated with mitochondrial dysfunction and compromised cellular integrity [3,4]. FBXO7 has gained attention in recent years due to its significant role in maintaining mitochondrial homeostasis. It is a part of the Skp-Cullin-F-box (SCF) E3 ubiquitin ligase, playing a crucial role in mitophagy, a process disrupted in PD[5]. Mutations of PARK15, which encodes FBXO7 protein, cause early-onset autosomal recessive PD, highlighting its importance in neurodegenerative disease mechanisms[6]. In addition to its role in mitophagy, FBXO7 is involved in mitophagy, proteasome assembly, synaptic function, and motor control, processes that are less emphasized in other PARK genes[7,8]. It also causes a unique clinical syndrome combining parkinsonian and pyramidal signs, suggesting a distinct pathogenic pathway[8]. These features make FBXO7 an important target for drug repurposing efforts. Despite its critical involvement in pathways related to mitochondrial quality control and protein degradation, FBXO7 remains underexplored as a therapeutic target. To our knowledge, this study represents the first structure-based repurposing approaches focused specifically on FBXO7, highlighting its novelty and potential impact.

Drug repurposing is an appealing strategy for speeding up the development of new therapies, particularly in complex diseases like PD, where traditional drug discovery demands much time and money[9]. This approach allows for identifying new targets for drugs that are already approved for the treatment of a disease. In this context, *in silico* methodologies, including molecular docking and network analysis, are effective tools for facilitating the identification of drug-target interactions quickly and cost-effectively[10].

This study aims to investigate the therapeutic potential of addressing mitochondrial dysfunction in PD through an FBXO7-focused approach. We performed a comprehensive network analysis to identify key protein-protein interactions involving FBXO7 and its related pathways. Furthermore, using *in silico* molecular docking studies, we assessed the binding affinities of selected drugs to FBXO7 to identify promising candidates for repurposing. The integration of network pharmacology and molecular modelling comprehensive perspective on the potential mechanisms through which repurposed drugs could modulate FBXO7-related pathways, offering insights into their suitability for PD therapy.

Material and Method

Drugbank Database Search and Selection of Mitochondria-Related Drugs

This study focused on identifying drugs that influence mitochondrial function and have the potential to be repurposed for PD based on the FBXO7 protein, which is a significant contributor to both mitochondrial dysfunction and PD pathology.

An initial query was performed in the DrugBank database (https://www.drugbank.com) (accessed on 09 September 2024), specifically targeting drugs associated with FBXO7. Following the search, the initial list of drug candidates was generated. The criteria for selection were:

- Drugs are known to interact with or modulate FBXO7 or related mitochondrial pathways.
- Only drugs approved for clinical use to prioritize safety and feasibility for repurposing.

Python programming scripts were utilized to further process the data. The list of drugs obtained from DrugBank was subjected to a literature review to identify their known effects on mitochondrial function.

Automated Literature Screening Using Python

To ensure comprehensive screening, automated searches were performed on PubMed using the following two queries for each drug:

- *Mitochondria-related research:* The search term "(Drug Name[Title/Abstract]) AND (mitochondria

[Title/Abstract])" was used to identify research focused on the drug's impact on mitochondrial function, including energy production, oxidative stress regulation, and apoptosis.

- *Parkinson-related research*: The search term "(Drug Name [Title/Abstract]) AND (Parkinson [Title/Abstract])" was used to filter out drugs that had been previously studied in the context of Parkinson's disease.

Python's requests library was used to automate the interaction with PubMed's Application Programming Interface (API), and pandas were utilized for data management and processing. The API enabled automated retrieval of article metadata for each compound, while pandas facilitated the filtering, organization, and comparison of the resulting datasets.

Filtering and Final Drug Selection

Following the retrieving the PubMed data, the subsequent step was to filter the drugs based on the following criteria:

- *Inclusion Criteria:* Drugs with at least one study documenting mitochondrial function effects but no studies linking them to Parkinson's disease were selected for further analysis.
- *Exclusion Criteria:* Drugs that had documented neurotoxic effects, exacerbated PD symptoms, or negatively impacted mitochondrial function in neurodegenerative conditions were excluded through a manual literature review.

This filtering process resulted in a final list of 53 drugs, all of which were previously studied for their effects on mitochondrial processes but had no known research linking them to PD. These drugs were selected as candidates for further in silico analysis and potential repurposing.

Network Pharmacology Study

Protein-Protein Interaction Network

To investigate the protein-protein interactions (PPIs) involving FBXO7 and its associated proteins, we utilized the STRING database (version 11.5; https://string-db.org) (accessed on 17 October 2024) to construct the PPI network. STRING aggregates known and predicted PPIs from multiple sources, including direct (physical) and indirect (functional) associations. For this study, we set the minimum required interaction score to high confidence (0.7). Disconnected nodes were hidden to focus on the core network. The PPI network was visualized and further analyzed using Cytoscape software (version 3.9.1). Nodes represent proteins, while edges indicate the interactions between them. No additional filtering for hub genes was applied. This analysis aimed to clarify the role of FBXO7 in pathways related to PD, especially through its interactions with proteins involved in mitochondrial function and cellular stress responses.

GO and KEGG Pathway Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the STRING database (https://string-db.org/) (accessed on 17 October 2024). The interaction network for FBXO7 and its associated proteins was constructed using a high confidence interaction score of 0.7. GO enrichment analysis was conducted to assess the biological processes, cellular components, and molecular functions relevant to PD pathology. Additionally, KEGG pathway enrichment analysis was used to explore key pathways associated with the interactome of FBXO7. The top enriched terms were visualized as bubble charts, generated using the STRING platform, where the size and color of the bubbles represent the significance and gene count for each enriched term, respectively. Only terms with a False Discovery Rate (FDR) < 0.05 were considered statistically significant.

In Silico Studies

Molecular Docking

The *Autodock Vina* algorithm provided by SwissDock was used for evaluating the binding affinities of ligands targeting the FBXO7 protein. This platform is widely utilized for predicting potential binding conformations and energies between target proteins and ligands, allowing for the identification of optimal binding sites. The crystal structure of the target protein, FBXO7, was obtained from the Protein Data Bank (PDB). The binding region of the protein was identified, unnecessary water molecules were removed, and only amino acids crucial for the binding site were retained. The prepared protein structure was uploaded to the SwissDock platform. The canonical SMILES of the selected compounds were obtained from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 9 October 2024) and uploaded to the SwissDock platform. These ligands were included in the docking analysis to investigate their potential interactions with FBXO7. The *Autodock Vina* algorithm provided by SwissDock utilizes a specific energy function to estimate the binding energies between the ligand and the protein. Default parameters were used for docking, considering a rigid target protein and flexible ligand conformations. The minimum binding score was evaluated based on the reference ligand BC1464 (-5.870), a small molecule that has been experimentally shown to bind directly to the FBXO7[11].

Data Analysis

Docking scores obtained from the SwissDock platform were analyzed. These scores represent the binding energies, with lower (more negative) energy values indicating stronger binding. The lowest energy score for each ligand was compared to the reference molecules, BC1464. The binding affinity of the drugs was determined by comparing its score to that of the reference molecules. The ligand with the most negative binding energy was considered to have the strongest potential interaction with FBXO7.

Visualization of Results

Output files and three-dimensional binding positions provided by SwissDock were visualized using ChimeraX v1.9 for detailed structural analysis. The binding affinities of the selected drugs to FBXO7 were assessed using results from docking studies, which allowed for an evaluation of their potential interactions. By using BC1464 as a reference ligand known for its strong binding affinity, other candidate molecules were compared to it in terms of their binding strength to FBXO7. This comparative approach, visualized using ChimeraX, provided a reliable method for determining the relative binding affinities of each drug to the target protein. Additionally, it offered insights into the potential therapeutic efficacy of the drugs based on these binding interactions.

Statistical Analysis

The docking scores of selected compounds were qualitatively compared with the reference ligand (BC1464) to identify potential candidates for FBXO7 inhibition. No statistical tests were applied to compare docking scores quantitatively. Statistical significance in enrichment analyses was determined using an FDR threshold of <0.05, corrected by the Benjamini-Hochberg method to account for multiple comparisons. The significance of PPI networks was assessed using STRING's PPI enrichment p-value, ensuring that observed interactions were not due to random chance.

Results

Protein-Protein Interaction Network Analysis

The STRING analysis demonstrated a well-structured protein-protein interaction (PPI) network, comprising 21 nodes and 93 edges, resulting in an average node degree of 8.86 (Figure 1). This network showed significant PPI enrichment with a p-value of <1.0e-16, which suggests that these proteins interact more than would be expected by random chance, further supporting their functional relationships. The clustering coefficient of 0.892 also supports that the proteins in this network are highly connected, proposing a common functional role, particularly in the context of PD. FBXO7 interactors PRKN, PINK1, and PARK7 are known players in the regulation of mitochondria, further implicating FBXO7 in key PD-related mechanisms.

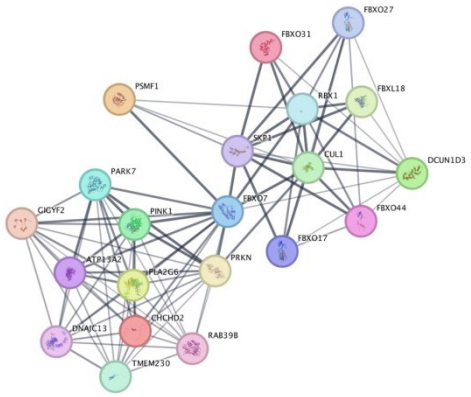

Figure 1.
Protein-protein interaction network of FBXO7 and its functional partners. This network illustrates the interaction between FBXO7 and its associated proteins. Nodes represent individual proteins, while edges indicate the predicted or known interactions based on experimental data, databases, gene neighbourhood, gene co-occurrence, and text mining. The node color distinguishes different protein groups, and the node size is proportional to the degree of interaction. The network shows a densely interconnected cluster, highlighting the key proteins involved in ubiquitination and mitochondrial regulation, which are critical processes in Parkinson's disease pathology.

GO and KEGG Enrichment Analyses

GO and KEGG Enrichment analyses covered KEGG pathways and GO terms, including biological process, molecular function, and cellular component. Bubble charts were used to visualize the top 10 enriched terms in each category (Figure 2). GO biological process enrichment analysis identified key pathways related to ubiquitin-proteasome system dysfunction, mitochondrial homeostasis, and oxidative stress response, all of which are central to PD pathology (Fig 2a). The most significantly enriched process was SCF-dependent proteasomal ubiquitin-dependent protein catabolic process (FDR = 7.78e-12), followed by Proteasome-mediated ubiquitin-dependent protein catabolic process (FDR = 2.21e-09) and Ubiquitin-dependent protein catabolic process (FDR = 5.54e-10). These findings reinforce the role of FBXO7 in protein degradation, highlighting its contribution to proteostasis imbalance in PD. Additionally, pathways directly related to mitochondrial function were enriched, including Regulation of autophagy of

mitochondria (FDR = 5.22e-07) and Positive regulation of mitochondrion organization (FDR = 1.37e-05). Negative regulation of oxidative stress-induced neuron death (FDR = 8.85e-06) was also significantly enriched, emphasizing the neuroprotective mechanisms modulated by FBXO7.

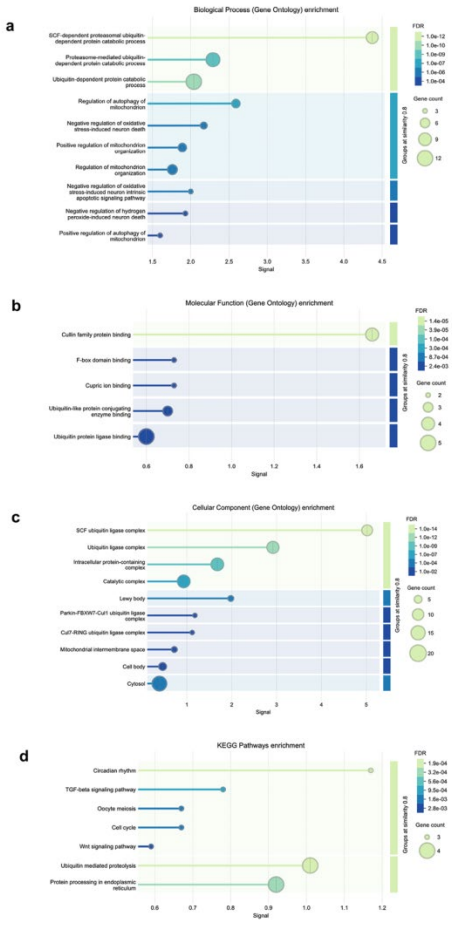

Figure 2.
GO and KEGG Pathway Enrichment Analysis of FBXO7-associated Proteins. The bubble chart displays the enriched Gene Ontology (GO) terms and KEGG pathways related to FBXO7 and its interacting proteins. The size of each bubble represents the gene count, while the color gradient indicates the False Discovery Rate (FDR), reflecting the significance of enrichment.

GO molecular function enrichment analysis further confirmed the involvement of FBXO7 in ubiquitin-related interactions (Fig 2b). The most significant terms were Ubiquitin protein ligase binding (FDR = 2.40e-03) and Ubiquitin-like protein conjugating enzyme binding (FDR = 8.70e-04). The identification of Cullin family protein binding (FDR = 1.40e-05) and F-box domain binding (FDR = 3.60e-05) suggests that FBXO7 participates in Cullin-RING ubiquitin ligase (CRL) complexes, which are critical for protein turnover and mitochondrial quality control.

GO cellular component analysis highlighted key subcellular structures involved in protein degradation and mitochondrial function (Fig 2c). The most significantly enriched terms included SCF ubiquitin ligase complex (FDR = 1.00e-14) and Ubiquitin ligase complex (FDR = 1.00e-12), confirming the importance of FBXO7 in ubiquitin-mediated proteolysis. Notably, Lewy bodies (FDR = 1.00e-06), a hallmark of Parkinson's disease pathology, were also significantly enriched. Additionally, the Parkin-PINK1 ubiquitin ligase complex (FDR = 1.00e-06) was among the top-ranked terms, reinforcing the well-established role of these proteins in mitochondrial autophagy (mitophagy) and neuroprotection.

KEGG pathway enrichment analysis identified Ubiquitin-mediated proteolysis (FDR = 3.20e-04) and Protein processing in the endoplasmic reticulum (FDR = 2.80e-03) as the most enriched pathways, aligning with the role of FBXO7 in maintaining protein homeostasis. Additionally, TGF-beta signaling pathway (FDR = 3.20e-04) and Wnt signaling pathway (FDR = 9.80e-04) were significantly enriched, suggesting potential regulatory mechanisms that could influence neurodegeneration and neuronal survival (Fig 2d).

Results of Docking Analysis

Table I provides a summary of the docking scores, ranking the tested compounds based on their binding strength relative to BC1464. Among the tested compounds, fluorometholone (-6.367), bendroflumethiazide (-6.354 kcal/mol), lasofoxifene (-6.173 kcal/mol), penicillin V (-6.102 kcal/mol), cefamandole (-6.036 kcal/mol) and hydromorphone (-6.067) exhibited the strongest binding affinities, surpassing the reference ligand.

Table I. Molecular Docking Scores and Binding Affinities of Selected Compounds for FBXO7

Drug	Docking Score (kcal/mol)	Binding Affinity Category
Fluorometholone	-6.367	High
Bendroflumethiazide	-6.354	High
Lasofoxifene	-6.173	High
Penicillin V	-6.102	High
Cefamandole	-6.036	High
Hydromorphone	-6.067	High
BC1464 (Reference)	-5.870	Reference
Bimatoprost	-5.651	Moderate
Cilastatin	-5.542	Moderate
Doconexent	-5.354	Moderate
Elvitegravir	-4.97	Low
Amlexanox	-4.89	Low
Gadobutrol	-3.808	Low
Lumefantrine	-2.832	Low

A subset of compounds exhibited docking scores close to the reference ligand but did not surpass it. Bimatoprost (-5.651 kcal/mol), cilastatin (-5.542 kcal/mol), and doconexent (-5.354 kcal/mol) fell into this category, indicating moderate binding affinity. These compounds showed potential for FBXO7 interaction, albeit with weaker binding strength compared to the high-affinity group.

Several compounds demonstrated weaker binding affinities relative to the reference ligand. Amlexanox (-4.89 kcal/mol) and elvitegravir (-4.97 kcal/mol) exhibited moderate-to-low binding potential. Lumefantrine (-2.832 kcal/mol) and gadobutrol (-3.808 kcal/mol) showed the least favorable docking scores, suggesting minimal interaction with FBXO7.

The docking analysis revealed that the top-binding compounds exhibited stable interactions with critical residues within the FBXO7 binding pocket. The structural alignment of these drugs within the active site of FBXO7, as visualized in Figure 3, supports their potential role in modulating mitochondrial dysfunction in PD.

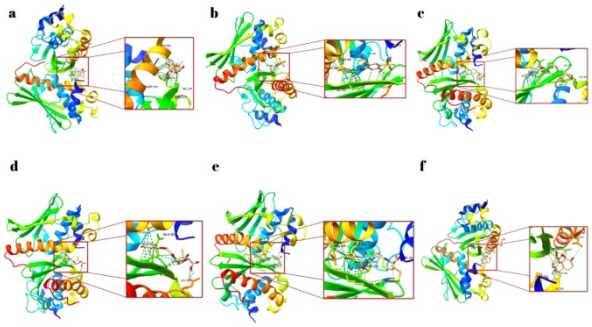

Figure 3.

Best docking poses for the top-binding drugs with FBXO7. (a) Fluorometholone interacts with key residues VAL274, LEU307, ASP310, ARG276 and ARG272, (b) bendroflumethiazide interacts with key residues GLN302, ARG306, VAL274, LYS275, LEU277, LEU255 and MET207, (c) lasofoxifene interacts with key residues GLN302, ARG276, ASN196, VAL274, and LEU280, (d) penicillin V interacts with key residues LYS275, VAL274, LEU280, GLU209 and GLN278, (e) cefamandole interacts with key residues ARG276, ARG306, SER182, GLN278, GLN302, LEU208, and GLU209. (f) hydromorphone interacts with key residues LYS275, VAL274, GLN302, LEU301, and GLN311. Hydrogen bonds are shown in blue and van der Waals interactions are shown in green.

Discussion and Conclusion

PD is a complex neurodegenerative disorder characterized by dopaminergic neuronal loss, mitochondrial dysfunction, oxidative stress, and disruption of proteostasis[12]. Despite extensive research, disease-modifying treatments remain unavailable, necessitating the exploration of novel therapeutic targets. In this study, we identified FBXO7 as a central player in PD-associated mitochondrial dysfunction and conducted *in silico* molecular docking to assess the potential of clinically approved drugs for repurposing. Our findings indicate that several high-affinity binders to FBXO7 could serve as candidates for modulating key PD-related pathways, particularly those involved in mitophagy, ubiquitin-proteasome degradation, and oxidative stress regulation.

While most cases of PD occur sporadically, approximately 10% have a hereditary origin. In recent years, multiple mutations linked to an increased risk of PD have been discovered, providing a deeper understanding of the disease's pathogenic mechanisms[13]. Genetic mutations in PARK15, the gene encoding FBXO7, have been linked to early-onset autosomal recessive PD, supporting its involvement in neurodegeneration[14,15]. Consistent with previous findings, our network analysis confirmed FBXO7's strong interaction with well-known PD-related proteins, including PRKN (Parkin), PINK1, and PARK7, which are key regulators of mitophagy [16]. Disruptions in these pathways contribute to the accumulation of damaged mitochondria, increased oxidative stress, and neuronal loss, reinforcing the significance of FBXO7 as a potential therapeutic target. GO and KEGG enrichment analyses further corroborated the role of ubiquitin-mediated proteolysis and mitochondrial quality control pathways in PD. The enrichment of SCF-dependent proteasomal ubiquitin-dependent protein catabolic process and regulation of autophagy of mitochondria suggests that targeting proteasomal degradation and mitochondrial homeostasis could be key to developing neuroprotective strategies. Notably, the enrichment of Lewy body-related processes aligns with the histopathological hallmark of PD, indicating a possible link between FBXO7 and abnormal protein aggregation.

The *in silico* screening process identified several drugs with high binding affinity to FBXO7, positioning them as potential candidates for disease-modifying interventions in PD. Among these, fluorometholone, bendroflumethiazide, lasofoxifene, penicillin V, hydromorphone, and cefamandole demonstrated stronger interactions with FBXO7 than the reference ligand. Given the role of FBXO7 in mitophagy regulation, protein degradation, and oxidative stress response, compounds that effectively bind to this target may have therapeutic implications in slowing or modifying disease progression in PD.

Fluorometholone is a synthetic glucocorticoid with well-established anti-inflammatory effects[17]. Glucocorticoids have been implicated in modulating mitochondrial biogenesis, reducing oxidative stress, and regulating the Nuclear Factor kappa B signaling pathway, which is a key driver of neuroinflammation in PD[18]. Given the chronic neuroinflammatory component of PD, glucocorticoids have been hypothesized to offer therapeutic benefits[19]. However, the long-term use of systemic glucocorticoids in PD remains controversial, as these drugs may both alleviate neuroinflammation and exacerbate neurodegeneration by affecting mitochondrial function and synaptic plasticity[20,21]. Fluorometholone has a high anti-inflammatory potency but is exclusively available as a topical formulation, primarily in the form of eye drops or ointments[17]. It is not used systemically due to its design and efficacy as a topical treatment, which provides significant local anti-inflammatory effects with minimal systemic absorption[22]. Given its strong docking affinity for FBXO7, fluorometholone could theoretically play a role in mitigating mitochondrial dysfunction in PD. However, the lack of systemic formulations and blood-brain barrier (BBB) permeability significantly limits its applicability in treating neurodegenerative diseases. Although fluorometholone itself is not an ideal candidate for systemic PD therapy, its structure and pharmacodynamics provide insights for developing safer glucocorticoid-based treatments with neuroprotective potential.

Bendroflumethiazide is a thiazide diuretic primarily used for hypertension and fluid retention management. However, recent studies suggest that thiazides may exert neuroprotective effects through their ability to modulate oxidative stress[23]. Although bendroflumethiazide's current clinical use is limited to its diuretic effects, and there is no direct evidence supporting its efficacy in neurodegenerative diseases, given its high binding affinity to FBXO7, future studies should investigate whether bendroflumethiazide can modulate mitochondrial ion homeostasis and reduce oxidative stress in PD models.

Lasofoxifene is a third-generation selective estrogen receptor modulator (SERM) used for osteoporosis prevention and estrogen-related disorders[24]. Current research has highlighted estrogen's neuroprotective actions, particularly in mitochondrial regulation, oxidative stress reduction, and inflammatory modulation, all of which are relevant to PD pathophysiology[25,26]. Estrogen signaling supports mitochondrial biogenesis and improves mitophagy efficiency, which is in line with FBXO7's role in modulating mitochondrial clearance[27]. In addition, epidemiological evidence indicates that women are less likely to develop PD prior to menopause, likely due to the protective effects of estrogen on dopaminergic neurons [28]. Lasofoxifene's high affinity for FBXO7 suggests a possible synergistic role in mitochondrial repair and proteostasis, that needs further investigation. However, SERMs have limited BBB penetration[29], and their long-term systemic use may pose cardiovascular risks and thromboembolic complications[30]. Therefore, nanoparticle-based formulations of lasofoxifene or structural modifications may be necessary to optimize its neuroprotective potential in PD.

Beta-lactam antibiotics such as penicillin V and cefamandole also demonstrated strong interactions with FBXO7. Beta-lactam antibiotics have been shown to reduce neuroinflammation by modulating microglial activation, which aligns with FBXO7's role in cellular stress responses and mitophagy regulation[31]. For example, ceftriaxone has been shown to upregulate glutamate transporters, reducing glutamate excitotoxicity, a key contributor to PD neurodegeneration[32]. Although there is no evidence from these abstracts to support its neuroprotective properties, cefamandole, a cephalosporin antibiotic, showed high binding affinity to FBXO7, suggesting a possible role in mitochondrial quality control and proteostasis regulation. However, some β-lactams, including penicillin V, have the propensity to cause neurologic problems in a subpopulation of geriatric patients[33]. Moreover, in a study using a penicillin-induced epilepsy model in rats, penicillin was found to significantly increase the levels of proinflammatory cytokines, indicating a potential to promote neuroinflammation[34]. Given these limitations, direct repurposing of Penicillin V for PD appears unfeasible. However, its strong binding to FBXO7 suggests that beta-lactam scaffolds could serve as a basis for novel drug development.

Hydromorphone is a potent opioid receptor agonist primarily used for pain management[35]. Opioid receptor activation has been reported to protect against PD-related mitochondrial dysfunction by enhancing mitophagy[12]. Although the most prominent tag of these opioid receptors is their modulation of pain signaling, the analgesic effects are primarily through activation of mu opioid receptors[36]. Interestingly, delta opioid receptors, while less significant in pain management than mu opioid receptors with a lower risk of abuse[36], exhibit a distinct potential in neuroprotection and inflammatory regulation[37,38]. These studies suggest that there is insufficient evidence to support hydromorphone as neuroprotective, with some studies indicating potential neurotoxic effects in renal impairment[39]. Given its high binding affinity to FBXO7, Hydromorphone may influence mitochondrial autophagy and oxidative stress pathways, but its systemic opioid effects raise concerns regarding addiction, tolerance, and potential neurotoxicity[40-42]. Thus, while hydromorphone's

interaction with FBXO7 is compelling, its clinical utility as a disease-modifying agent in PD remains uncertain. Future studies must target opioid derivatives that have less liability for addiction and better mitochondrial targeting action.

Although the primary focus of this study was on high-affinity binders, moderate-affinity compounds such as bimatoprost, cilastatin, and doconexent may still hold biological relevance. These drugs may interact with FBXO7 in an allosteric manner or influence secondary pathways involved in mitochondrial function and protein degradation. While their therapeutic implications are less direct than high-affinity binders, their moderate interaction strength suggests a potential role in combinatorial therapeutic approaches. In contrast, low-affinity compounds such as amlexanox and elvitegravir demonstrated minimal interaction with FBXO7, suggesting limited therapeutic potential in PD.

While this study provides compelling evidence for FBXO7 as a druggable target in PD, several limitations must be acknowledged. As an *in silico* analysis, this study predicts binding interactions but does not account for pharmacokinetics, bioavailability, or *in vivo* efficacy. Experimental verification using molecular dynamics simulations, enzymatic assays, and cell-based assays is required to confirm the stability and biological relevance of these interactions. Additionally, BBB permeability remains a major challenge for most compounds revealed herein, and therefore, structural optimization or novel delivery strategies will be required to maximize central nervous system bioavailability. Off-target effects and toxicity must also be thoroughly evaluated in preclinical models before advancing these candidates to clinical translation.

The present study indicates FBXO7 as a promising therapeutic target for modulating mitochondrial dysfunction in PD and identifies a number of clinically approved drugs with strong binding affinity. The findings pave the way for exploring repurposed drugs as an approach to treat PD with considerations of optimal pharmacokinetics, enhancing central nervous system penetration, and validating neuroprotective effects in experimental models. Future studies should prioritize the most promising candidates, such as Bendroflumethiazide, lasofoxifene and cefamandole, for in-depth preclinical evaluation, with the ultimate goal of developing effective disease-modifying therapies for PD.

Researcher Contribution Statement:
Idea and design: D.N.S., Data collection and processing: D.N.S., Analysis and interpretation of data: D.N.S.; Writing of significant parts of the article: D.N.S.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.

References

1. Koeglsperger T, Rumpf S-L, Schließer P, et al. Neuropathology of incidental Lewy body & prodromal Parkinson's disease. *Molecular Neurodegeneration*. 2023/05/12 2023;18(1):32. doi:10.1186/s13024-023-00622-7
2. Büeler H. Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease. *Exp Neurol*. Aug 2009;218(2):235-46. doi:10.1016/j.expneurol.2009.03.006
3. Henrich MT, Oertel WH, Surmeier DJ, Geibl FF. Mitochondrial dysfunction in Parkinson's disease - a key disease hallmark with therapeutic potential. *Mol Neurodegener*. Nov 11 2023;18(1):83. doi:10.1186/s13024-023-00676-7
4. Choong C-J, Mochizuki H. Involvement of Mitochondria in Parkinson's Disease. *International Journal of Molecular Sciences*. 2023;24(23):17027.
5. Kraus F, Goodall EA, Smith IR, et al. PARK15/FBXO7 is dispensable for PINK1/Parkin mitophagy in iNeurons and HeLa cell systems. *EMBO Rep*. Aug 3 2023;24(8):e56399. doi:10.15252/embr.202256399
6. Fonzo AD, Dekker M, Montagna P, et al. FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. *Neurology*. 2009;72(3):240-245.
7. Vingill S, Brockelt D, Lancelin C, et al. Loss of FBXO7 (PARK15) results in reduced proteasome activity and models a parkinsonism-like phenotype in mice. *Embo j*. Sep 15 2016;35(18):2008-25. doi:10.15252/embj.201593585
8. Joseph S, Schulz JB, Stegmüller J. Mechanistic contributions of FBXO7 to Parkinson disease. *J Neurochem*. Jan 2018;144(2):118-127. doi:10.1111/jnc.14253
9. Han M, Jung S, Lee D. Drug repurposing for Parkinson's disease by biological pathway based edge-weighted network proximity analysis. *Scientific Reports*. 2024/09/11 2024;14(1):21258. doi:10.1038/s41598-024-71922-1
10. MacMahon M, Hwang W, Yim S, et al. An in silico drug repurposing pipeline to identify drugs with the potential to inhibit SARS-CoV-2 replication. *Informatics in Medicine Unlocked*. 2023/01/01/ 2023;43:101387. doi:https://doi.org/10.1016/j.imu.2023.101387
11. Liu Y, Lear TB, Verma M, et al. Chemical inhibition of FBXO7 reduces inflammation and confers neuroprotection by stabilizing the mitochondrial kinase PINK1. *JCI Insight*. Jun 4 2020;5(11)doi:10.1172/jci.insight.131834
12. Xu Y, Zhi F, Mao J, et al. δ-opioid receptor activation protects against Parkinson's disease-related mitochondrial dysfunction by enhancing PINK1/Parkin-dependent mitophagy. *Aging (Albany NY)*. Nov 10 2020;12(24):25035-25059. doi:10.18632/aging.103970
13. Navarro E, Esteras N. A new mutation in the Parkinson's-related FBXO7 gene impairs mitochondrial and proteasomal function. *Febs j*. Jun 2024;291(12):2562-2564. doi:10.1111/febs.17155
14. Shojaee S, Sina F, Banihosseini SS, et al. Genome-wide linkage analysis of a Parkinsonian-pyramidal syndrome pedigree by 500 K SNP arrays. *Am J Hum Genet*. Jun 2008;82(6):1375-84. doi:10.1016/j.ajhg.2008.05.005
15. Di Fonzo A, Dekker MC, Montagna P, et al. FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. *Neurology*. Jan 20 2009;72(3):240-5. doi:10.1212/01.wnl.0000338144.10967.2b
16. Imberechts D, Vandenberghe W. Defects in PINK-PRKN-PARK7/DJ-1-dependent mitophagy and autosomal recessive Parkinson disease. *Autophagy*. Jun 2023;19(6):1872-1873. doi:10.1080/15548627.2022.2139129

17. Minigh J. Fluorometholone. In: Enna SJ, Bylund DB, eds. *xPharm: The Comprehensive Pharmacology Reference*. Elsevier; 2007:1-4.
18. Claros S, Gil A, Martinelli M, et al. Impact of Glucocorticoid on a Cellular Model of Parkinson's Disease: Oxidative Stress and Mitochondrial Function. *Brain Sci*. Aug 22 2021;11(8)doi:10.3390/brainsci11081106
19. Tentillier N, Etzerodt A, Olesen MN, et al. Anti-Inflammatory Modulation of Microglia via CD163-Targeted Glucocorticoids Protects Dopaminergic Neurons in the 6-OHDA Parkinson's Disease Model. *J Neurosci*. Sep 7 2016;36(36):9375-90. doi:10.1523/jneurosci.1636-16.2016
20. Vyas S, Rodrigues AJ, Silva JM, et al. Chronic Stress and Glucocorticoids: From Neuronal Plasticity to Neurodegeneration. *Neural Plasticity*. 2016/01/01 2016;2016(1):6391686. doi:https://doi.org/10.1155/2016/6391686
21. Choi GE, Han HJ. Glucocorticoid impairs mitochondrial quality control in neurons. *Neurobiol Dis*. May 2021;152:105301. doi:10.1016/j.nbd.2021.105301
22. Schlagel CA. COMPARATIVE EFFICACY OF TOPICAL ANTI-INFLAMMATORY CORTICOSTEROIDS. *J Pharm Sci*. Mar 1965;54:335-54. doi:10.1002/jps.2600540302
23. DeLoach T, Beall J. Diuretics: A possible keystone in upholding cognitive health. *Ment Health Clin*. Jan 2018;8(1):33-40. doi:10.9740/mhc.2018.01.033
24. Gennari L. Lasofoxifene: a new type of selective estrogen receptor modulator for the treatment of osteoporosis. *Drugs Today (Barc)*. Jun 2006;42(6):355-67. doi:10.1358/dot.2006.42.6.973583
25. Simpkins JW, Yi KD, Yang SH, Dykens JA. Mitochondrial mechanisms of estrogen neuroprotection. *Biochim Biophys Acta*. Oct 2010;1800(10):1113-20. doi:10.1016/j.bbagen.2009.11.013
26. Villa A, Vegeto E, Poletti A, Maggi A. Estrogens, Neuroinflammation, and Neurodegeneration. *Endocr Rev*. Aug 2016;37(4):372-402. doi:10.1210/er.2016-1007
27. Mei R, Lou P, You G, Jiang T, Yu X, Guo L. 17β-Estradiol Induces Mitophagy Upregulation to Protect Chondrocytes via the SIRT1-Mediated AMPK/mTOR Signaling Pathway. *Front Endocrinol (Lausanne)*. 2020;11:615250. doi:10.3389/fendo.2020.615250
28. Gillies GE, Pienaar IS, Vohra S, Qamhawi Z. Sex differences in Parkinson's disease. *Front Neuroendocrinol*. Aug 2014;35(3):370-84. doi:10.1016/j.yfrne.2014.02.002
29. Arevalo MA, Santos-Galindo M, Lagunas N, Azcoitia I, Garcia-Segura LM. Selective estrogen receptor modulators as brain therapeutic agents. *Journal of Molecular Endocrinology*. 01 Feb. 2011 2011;46(1):R1-R9. doi:10.1677/JME-10-0122
30. Cosman F, Baz-Hecht M, Cushman M, et al. Short-term effects of estrogen, tamoxifen and raloxifene on hemostasis: a randomized-controlled study and review of the literature. *Thrombosis Research*. 2005/01/01/ 2005;116(1):1-13. doi:https://doi.org/10.1016/j.thromres.2004.09.014
31. Yimer EM, Hishe HZ, Tuem KB. Repurposing of the β-Lactam Antibiotic, Ceftriaxone for Neurological Disorders: A Review. *Front Neurosci*. 2019;13:236. doi:10.3389/fnins.2019.00236
32. Rothstein JD, Patel S, Regan MR, et al. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature*. Jan 6 2005;433(7021):73-7. doi:10.1038/nature03180
33. Stock ML, Fiedler KJ, Acharya S, et al. Antibiotics acting as neuroprotectants via mechanisms independent of their anti-infective activities. *Neuropharmacology*. 2013/10/01/ 2013;73:174-182. doi:https://doi.org/10.1016/j.neuropharm.2013.04.059
34. Karabulut S, Filiz AK. The effect of penicillin-induced epileptiform activity on proinflammatory cytokines levels in the rat brain. *Cumhuriyet Science Journal*. March 2021;42(1):1-6. doi:10.17776/csj.775122
35. Murray A, Hagen NA. Hydromorphone. *Journal of Pain and Symptom Management*. 2005;29(5):57-66. doi:10.1016/j.jpainsymman.2005.01.007
36. Vicente-Sanchez A, Segura L, Pradhan AA. The delta opioid receptor tool box. *Neuroscience*. 2016/12/03/ 2016;338:145-159. doi:https://doi.org/10.1016/j.neuroscience.2016.06.028
37. Zhang J, Gibney GT, Zhao P, Xia Y. Neuroprotective role of delta-opioid receptors in cortical neurons. *Am J Physiol Cell Physiol*. Jun 2002;282(6):C1225-34. doi:10.1152/ajpcell.00226.2001
38. Huang JZ, Ren Y, Xu Y, et al. The delta-opioid receptor and Parkinson's disease. *CNS Neurosci Ther*. Dec 2018;24(12):1089-1099. doi:10.1111/cns.13045
39. Lee KA, Ganta N, Horton JR, Chai E. Evidence for Neurotoxicity Due to Morphine or Hydromorphone Use in Renal Impairment: A Systematic Review. *J Palliat Med*. Nov 2016;19(11):1179-1187. doi:10.1089/jpm.2016.0101
40. Guenther S, Mickle TC, Barrett AC, et al. Pharmacokinetics and Abuse Potential of Asalhydromorphone, a Novel Prodrug of Hydromorphone, After Intranasal Administration in Recreational Drug Users. *Pain Med*. Mar 1 2020;21(3):511-520. doi:10.1093/pm/pnz066
41. Martin EJ, Vaughan CL, Atayee R, Hirst JM, O'Donnell K, Edmonds KP. Hydromorphone-induced chorea as an atypical presentation of opioid neurotoxicity: A case report and review of the literature. *Palliat Med*. Oct 2018;32(9):1529-1532. doi:10.1177/0269216318786861
42. Fainsinger R, Schoeller T, Boiskin M, Bruera E. Palliative care round: cognitive failure and coma after renal failure in a patient receiving captopril and hydromorphone. *Journal of palliative care*. 1993;9(1):53-55.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 305-309, 2025
DOI: https://doi.org/10.32708/uutfd.1653680

ORIGINAL RESEARCH

Evaluating the Performance of Large Language Models in Generating Impressions for Radiology Reports

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ABSTRACT

The aim of the study was to evaluate and compare the performance of three popular large language models (LLMs) in generating impressions for radiology reports in Turkish. ChatGPT, Gemini, and Copilot were used to generate impressions for 50 anonymized radiology reports using a "few-shot" prompt. The impressions were scored by three radiologists using a Likert scale, based on whether they included all relevant information from the report, provided an appropriate summary of the report, contained no misleading information, and could be added to the report without modification. Friedman's test was used to evaluate whether there was a difference between the scores of the LLMs. The 50 reports included 32 magnetic resonance examinations, 11 computed tomography examinations, 5 ultrasound examinations, and 2 fluoroscopy examinations. Of these, 15 were neuroradiology studies, 14 were musculoskeletal studies, 13 were abdominal studies, and 8 were thoracic radiology studies. The median scores for the models' outputs were 4 and 5. This finding indicates that the radiologists generally found the models successful in generating impressions. Furthermore, no statistically significant difference was found among the models in terms of their performance in containing all information, providing an appropriate summary, avoiding misleading information, and being suitable for inclusion in the report without modification (p = 0.607, 0.327, 0.629, 0.089, respectively). In conclusion, ChatGPT, Gemini, and Copilot were found to be successful in generating impressions for radiology reports in Turkish, and no significant difference in performance was detected among the models.

Keywords: Radiology. Artificial intelligence. Large language models.

Büyük Dil Modellerinin Radyoloji Raporları İçin Sonuç Bölümü Oluşturmadaki Performanslarının Değerlendirilmesi

ÖZET

Çalışmamızın amacı popüler üç büyük dil modelinin (BDM) Türkçe radyoloji raporları için sonuç bölümü oluşturma konusundaki performansını değerlendirip mukayese etmekti. Anonimize edilmiş 50 radyoloji raporu için, "few-shot" bir komut ile, ChatGPT, Gemini ve Copilot dil modellerine sonuç bölümü oluşturuldu. Sonuçlar; rapordaki tüm bilgileri içerme, raporu uygun bir şekilde özetleme, yanıltıcı bilgi içermeme ve değiştirilmeden rapora eklenebilme açısından üç radyolog tarafından bir Likert skalası kullanılarak skorlandı. Friedman testi ile BDM'lerin skorları arasında fark olup olmadığı değerlendirildi. Çalışmaya dahil edilen 50 raporun 32'si manyetik rezonans, 11'i bilgisayarlı tomografi, 5'i ultrason ve 2'si floroskopi tetkikleriydi. Bu tetkiklerden 15'i nöroradyoloji, 14'ü kas-iskelet, 13'ü abdomen ve 8'i toraks radyolojisi çalışmalarıydı. Üç radyoloğun yaptığı skorlamalarda modellerin aldığı skorların medyan değerleri 4 ve 5 idi. Bu bulgu modellerin sonuç oluşturmada radyologlar tarafından genel olarak başarılı bulunduğunu göstermekteydi. Ayrıca modeller arasında bütün bilgileri içerme, raporu uygun bir şekilde özetleme, yanıltıcı bilgi içermeme ve değiştirilmeden rapora eklenebilme performansı açısından istatistiksel bir farklılık saptanmadı (*p* değerleri sırasıyla 0,607; 0,327; 0,629; 0,089). Sonuç olarak ChatGPT, Gemini ve Copilot Türkçe radyoloji raporları için sonuç bölümü oluşturmada başarılı bulunmuş ve modellerin performansı arasında anlamlı bir farklılık saptanmamıştır.

Anahtar Kelimeler: Radyoloji. Yapay zeka. Büyük dil modelleri.

Large language models (LMMs) are advanced artificial intelligence applications built using deep neural networks and trained using very large amounts of text to generate human-like responses. ChatGPT, one of the most popular of these models, is developed by OpenAI and designed as a chatbot that can interact with users through deep learning and natural language processing algorithms. Gemini, developed by Google, is another artificial intelligence model that can engage in text-based interactions with humans thanks to similar natural language processing capabilities. Copilot, developed by Microsoft, is another popular artificial intelligence model built using natural language processing and deep learning algorithms.

Date Received: 10.March.2025
Date Accepted: 31.July.2025

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It has been suggested that ChatGPT can be useful for radiologists in reporting by assisting in creating clinical information or impression sections, summarizing reports for patients, and increasing patient interaction[1]. In a study evaluating the performance of ChatGPT, Google Bard (now Gemini), and Microsoft Bing (now Copilot) in simplifying radiology reports, the models were shown to be able to perform this task accurately[2]. In another study evaluating ChatGPT's performance in generating impression sections for radiology reports, the impressions produced by the model scored lower than those generated by radiologists[3]. A language model specifically developed to generate radiology report impressions has successfully produced professional and linguistically appropriate impressions for a wide range of radiological examinations[4].

In studies evaluating the performance of popular LLMs in generating impressions for radiology reports, the models have generally been used with "zero-shot" prompts. "Zero-shot" refers to a model's ability to perform a new task without having been explicitly trained on examples of that specific task. On the other hand, "few-shot" learning in LLMs refers to the ability of the model to perform a task after being provided with a few examples (or "shots"). The aim of our study is to use three popular LLMs (ChatGPT, Gemini, and Copilot) to generate impressions for radiology reports in Turkish using a few-shot prompt, evaluate the appropriateness of these impressions, and compare the performance of the LLMs in this task.

Material and Method

After obtaining approval for the study from the ethics committee of our university (Decision number: 2024-19/1), 50 radiology reports from our institution's picture archiving and communication system (PACS) that were created in 2024 were selected and anonymized. The impressions of these reports were then removed, leaving only the clinical information section and the body of the report. Then, a detailed prompt was prepared for the language models (ChatGPT o1, Gemini 1.5 Pro, Copilot) to create an impression for the reports. When creating the prompt, instead of a "zero-shot" prompt such as "simplify this report"[5], a more detailed prompt was written to obtain an impression more similar to what radiologists create during their daily practice. Additionally, two examples were given to make it easier for the models to learn to do the desired task. Below is the English translation of the prompt used in the study (Original Turkish prompt can be found as a supplementary file):

"You are a radiologist. Create an impression for the radiology report provided. When doing this, follow these guidelines: 1) Create the impression in a way that appeals to health professionals. 2) Do not include normal structures and findings in the impression. 3) Include only pathologic findings in the impression. 4) Specify the diagnosis or possible diagnoses by interpreting the findings. 5) Make the impression as concise as possible.

I have given an example below.

Clinical Information: Knee pain

Findings: The amount of fluid within the knee joint is increased. There is a horizontal tear in the posterior horn of the medial meniscus. No tear in the lateral meniscus. Anterior and posterior cruciate ligaments are intact. Medial and lateral collateral ligaments are intact. Quadriceps and patellar tendons are normal. Cartilage defects involving less than 50% thickness are observed in the medial femorotibial joint. Subchondral bone marrow edema-like signal intensity is noted on posterior medial tibial plateau. No bone or soft tissue masses.

Impression: 1. Effusion 2. Horizontal tear of the posterior horn of the medial meniscus 3. Cartilage defects involving less than 50% thickness in the medial femorotibial joint 4. Subchondral bone marrow edema-like signal intensity on posterior medial tibial plateau.

Another example:

Clinical Information: Liver mass characterization

Findings: A mass lesion of approximately 5.5 cm in diameter is observed on segment 8 of the liver. There is a T2 hyperintense branching structure in the center of the lesion (consistent with vascular scar). The mass is slightly hypointense on T1-weighted images and iso-hyperintense on T2-weighted images compared to the liver parenchyma. On dynamic examination, the lesion enhances in the arterial phase and does not show washout. In the hepatobiliary phase, the enhancement is heterogeneous but persistent. No lesion in other parts of the liver. Gallbladder and bile ducts are normal. Spleen, pancreas, both adrenal glands, and kidneys are normal. No intra-abdominal free fluid, localized fluid collections, or lymphadenopathy are observed. The bladder and bony structures in the imaging field are normal.

Impression: Focal nodular hyperplasia in segment 8 of the liver.

Now generate an impression to this report in accordance with the above instructions and examples:"

To assess the quality and appropriateness of the impressions generated by the models, three radiologists with 27, 10, and 6 years of experience were asked to score the following four statements about the impressions: 1) The impression generated by the model contains all the necessary information specified in the report. 2) The impression generated by

the model summarizes the report appropriately. 3) The impression generated by the model does not contain false or misleading information. 4) I can add the impression generated by the model to my report without editing it. A 5-point Likert scale was used for scoring (1 = Strongly disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Strongly agree). A total of 12 scores were obtained for each impression (4 statements x 3 radiologists). Scores are reported as median and interquartile ranges. Friedman's test was used to assess differences among the LLM scores. Data analysis was performed using SPSS software (IBM SPSS Statistics for Windows, Version 27.0; Armonk, NY: IBM Corp.). A p-value < 0.05 was deemed significant.

Results

Of the 50 reports retrieved from PACS and anonymized, 32 were magnetic resonance imaging (MRI), 11 were computed tomography (CT), 5 were ultrasound and 2 were fluoroscopy studies. Of these, 15 were neuroradiology, 14 were musculoskeletal, 13 were abdominal and 8 were thoracic radiology examinations. The median scores provided by the three radiologists regarding whether the impressions generated by the models included all necessary information, summarized the reports appropriately, contained no misleading information, and could be added without modification were 4 and 5 (Table). This finding indicates that the models were generally considered successful by the radiologists in generating impressions (Figures 1a–d). In addition, no statistical difference was found between the models in terms of including all information, summarizing the report appropriately, not containing misleading information and being able to be added to the report without editing (Table).

Table. Likert scores of the LLMs (Data are given as median and interquartile range [25th and 75th percentile])

	ChatGPT	Gemini	Copilot	*p**
The impression generated by the model contains all the necessary information specified in the report.	5 (5–5)	5 (5–5)	5 (5–5)	0.607
The impression generated by the model summarizes the report appropriately.	5 (5–5)	5 (4–5)	5 (4–5)	0.327
The impression generated by the model does not contain false or misleading information.	5 (5–5)	5 (5–5)	5 (5–5)	0.629
I can add the impression generated by the model to my report without editing it.	5 (4–5)	5 (4–5)	4 (4–5)	0.089

*Friedman test

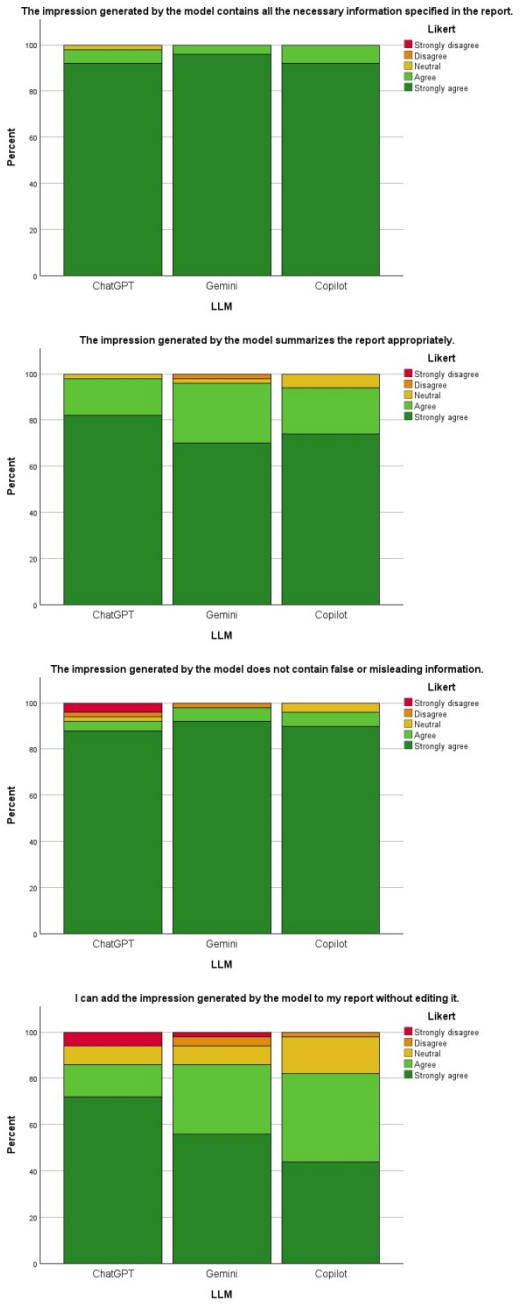

Figure 1.
Stacked bar charts of the scores of LLMs for (a) including all information, (b) summarizing the report appropriately, (c) not containing misleading information, and (d) being able to be added to the report without editing.

Discussion and Conclusion

Our study evaluating the performance of three popular LLMs in generating impressions for radiology reports in Turkish shows that the models can successfully perform this task and that there is no significant difference between the performance of the models.

Studies on the use of LLMs to generate impressions for radiology reports have focused more on evaluating the success of the models in simplifying radiology reports and generating patient-friendly impressions. For example, in Doshi et al.'s study evaluating the performance of ChatGPT-3.5, ChatGPT-4, Bard (now Gemini) and Bing (now Copilot) in generating simplified impressions for radiology reports[5] the authors used the following prompts: "Simplify this radiology report", "I am a patient. Simplify this radiology report", and "Simplify this radiology report at the 7th grade level". Can et al. used GPT-4, GPT-3.5 Turbo, Claude-3-Opus, Gemini Ultra as well as open-source models such as Mistral-7b and Mistral-8×7b to evaluate the performance of the models in simplifying interventional radiology reports in a way that patients can easily understand[6]. There are not many studies assessing the performance of the models in creating impressions that radiologists can include in their reports. In the study by Sun et al., the authors asked GPT-4 to *"*Generate a new short one-line impression from the findings section using medical vocabulary*"* for 50 chest x-rays[3]. However, in this study, the radiologist-generated impressions were shown to be better than the model-generated ones in terms of coherence, comprehensiveness, factual consistency and medical harmfulness. We believe that the better results we found in our study are related to the more detailed prompt we used and the fact that we used a few-shot prompt instead of a zero-shot one as used by Sun et al.

Zhang et al. have developed a new language model designed for creating impressions for radiology reports, using 20 gigabytes of medical and general purpose text for pre-training, and the impressions generated by the model were found to be in close agreement with the impressions of radiologists (median, 5 [IQR, 5–5] vs 5 [IQR, 5–5])[4]. This study shows that language models can be made more effective for specific tasks through fine-tuning.

Although the impressions generated by the models were generally appropriate, they were not entirely free of errors. For example, when presented with a chest CT showing unilateral absence of the pulmonary artery, ChatGPT suggested a potential diagnosis of scimitar syndrome. Likewise, for a pituitary MRI revealing a Rathke cleft cyst, the model's impression leaned toward a microadenoma. These cases illustrate that, in their current form, the models still require supervision and cannot be relied upon for independent use.

Our primary aim in this study was to evaluate how effectively the LLMs generate accurate, concise summaries suitable for daily practice, rather than assessing their diagnostic accuracy. Indeed, there are many studies in the literature evaluating the success of LLMs in diagnosing using text-based data. In a study evaluating the performance of ChatGPT on diagnosing cases published in the "Diagnosis Please" section of the journal Radiology, the accuracy of the model was 54%[7]. In another study evaluating the performance of ChatGPT in correctly diagnosing 100 "Case of the Week" examples published in the American Journal of Neuroradiology, the diagnostic accuracy of the model was found to be 50%[8]. In our study, none of the models could generate an impression section with the correct diagnosis for a brain MRI report with findings consistent with Aicardi syndrome. In an ankle MRI report of a tenosynovial giant cell tumor in the tibialis posterior tendon sheath, two of the models (ChatGPT and Gemini) were able to generate an impression with the correct diagnosis.

Limitations of our study include its retrospective nature and being a single-center study. Different reporting habits of different centers may affect the evaluation of the performance of the models. In addition, although we tried to create a detailed prompt, it may be possible to see the actual performance of the models by better prompting. In addition, since the models do not have access to the patients' electronic files, they lack some important laboratory or clinical information, which may also affect their performance. The impressions of radiology reports are written mostly for the referring clinician. The fact that we did not evaluate what clinicians think about the impressions generated by the models can be considered another limitation of this study.

Our study found that three popular LLMs generated generally appropriate impressions for radiology reports, with no significant differences in their performance. However, larger multi-center studies involving clinicians may provide a more comprehensive assessment of the effectiveness of LLMs in this task.

Researcher Contribution Statement:
Idea and design: H.E.K., D.S.; Data collection and processing: H.E.K., D.S., Z.Y.; Analysis and interpretation of data: H.E.K., D.S., Z.Y., G.G.; Writing of significant parts of the article: H.E.K., D.S.
Support and Acknowledgement Statement:
N/A
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Uludağ University Faculty of Medicine Clinical Research Ethics Committee
Approval Date: 20.11.2024
Decision No: 2024-19/1

References

1. Elkassem AA, Smith AD. Potential Use Cases for ChatGPT in Radiology Reporting. AJR Am J Roentgenol 2023;221(3):373–6.
2. Amin KS, Davis MA, Doshi R, Haims AH, Khosla P, Forman HP. Accuracy of ChatGPT, Google Bard, and Microsoft Bing for Simplifying Radiology Reports. Radiology 2023;309(2).

3. Sun Z, Ong H, Kennedy P, Tang L, Chen S, Elias J, et al. Evaluating GPT4 on Impressions Generation in Radiology Reports. Radiology 2023;307(5).
4. Zhang L, Liu M, Wang L, Zhang Y, Xu X, Pan Z, et al. Constructing a Large Language Model to Generate Impressions from Findings in Radiology Reports. Radiology 2024;312(3).
5. Doshi R, Amin KS, Khosla P, Bajaj S, Chheang S, Forman HP. Quantitative Evaluation of Large Language Models to Streamline Radiology Report Impressions: A Multimodal Retrospective Analysis. Radiology 2024;310(3).
6. Can E, Uller W, Vogt K, Doppler MC, Busch F, Bayerl N, et al. Large Language Models for Simplified Interventional Radiology Reports: A Comparative Analysis. Acad Radiol. 2024 Sep 30:S1076-6332(24)00690-1. doi: 10.1016/j.acra.2024.09.041. Epub ahead of print. PMID: 39353826.
7. Ueda D, Mitsuyama Y, Takita H, Horiuchi D, Walston SL, Tatekawa H, et al. ChatGPT's Diagnostic Performance from Patient History and Imaging Findings on the Diagnosis Please Quizzes. Radiology 2023;308(1).
8. Horiuchi D, Tatekawa H, Shimono T, Walston SL, Takita H, Matsushita S, et al. Accuracy of ChatGPT generated diagnosis from patient's medical history and imaging findings in neuroradiology cases. Neuroradiology 2024;66(1):73–9.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 311-318, 2025
DOI: https://doi.org/10.32708/uutfd.1655566

ORIGINAL RESEARCH

Prevalence of Infectious Diseases and the Assessment of Antibiotic Use in the Anesthesia Intensive Care Unit*

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ABSTRACT

Infections remain a major cause of morbidity and mortality in intensive care units. In this retrospective study, conducted to determine the prevalence of infections and resistance patterns in intensive care patients and to highlight the prognostic value of biomarkers and disease severity scores, 195 patients with suspected or confirmed infections admitted to the Anesthesia ICU of XXXXX Hospital between March 2020 and March 2021 were evaluated. Demographic data, infection foci, microbiological findings, biomarkers (WBC, CRP, PCT), and disease severity scores (APACHE II, SAPS II, SOFA) were analyzed. The infection prevalence was 60.6%, and ICU mortality was 49.7%. The median age was 67 years (IQR: 56–77), and 61% were male. Age, sex, and BMI were not associated with mortality. Non-survivors had significantly higher severity scores (APACHE II: 28.6 vs. 18.1; SAPS II: 63.2 vs. 41.2; SOFA: 10.8 vs. 6.4; all $p<0.001$). Common comorbidities included cardiovascular disease (69.2%), diabetes (21.0%), and chronic respiratory disease (19.5%). Respiratory (36.0%), intra-abdominal (18.0%), and bloodstream infections (17.4%) were most common. Tracheal aspirates were the most frequent culture-positive samples (35.3%). *K. pneumoniae*, *A. baumannii*, and *E. coli* were the predominant pathogens. Antimicrobial resistance was found in 56.0% of culture-positive cases, without a significant mortality association ($p=0.118$). Empirical antimicrobial therapy was initiated in 87.7% of patients. By the 72nd hour of treatment, significant reductions were observed in PCT (1.4→0.9 ng/mL, $p<0.001$) and WBC ($15.3\rightarrow12.4\times10^3/\mu L$, $p<0.001$) levels, while the change in CRP was not statistically significant ($p=0.181$). In the mortality group, initial CRP (111.0 vs. 78.5 mg/L, $p=0.032$) and PCT (2.4 vs. 1.0 ng/mL, $p=0.034$) levels were higher, whereas WBC did not differ significantly ($p=0.787$). Our findings suggest that severity scores and biomarker changes have prognostic value in infected critically ill patients, and integrating host response with microbiological data may aid clinical management.

Keywords: Intensive care infections. Antimicrobial resistance. Biomarkers. Empirical antibiotics. Mortality.

Anestezi Yoğun Bakım Ünitesinde Enfeksiyöz Hastalıkların Prevalansı ve Antibiyotik Kullanımının Değerlendirilmesi

ÖZET

Enfeksiyonlar, yoğun bakım ünitelerinde morbidite ve mortalitenin başlıca nedenlerindendir. Yoğun bakım hastalarında enfeksiyonların yaygınlığını ve direnç paternlerini ortaya çıkararak, biyobelirteçler ile hastalık şiddeti skorlarının prognozdaki önemini vurgulamayı amaçladığımız bu retrospektif çalışmada; Mart 2020–Mart 2021 tarihleri arasında XXXXX Hastanesi Anestezi YBÜ'nde şüpheli veya doğrulanmış enfeksiyon tanısı ile izlenen 195 hasta değerlendirildi. Demografik veriler, enfeksiyon odakları, mikrobiyolojik bulgular, biyobelirteçler (WBC, CRP, PCT) ve hastalık şiddeti skorları (APACHE II, SAPS II, SOFA) analiz edildi. Enfeksiyon prevalansı %60,6; mortalite oranı %49,7 olarak bulundu. Medyan yaş 67,0 yıl (IQR: 56,0-77,0) olup hastaların %61,0'ı erkekti. Yaş, cinsiyet ve vücut kitle indeksi mortalite ile ilişkili bulunmadı. Mortalite grubunda APACHE II (28,6 vs. 18,1), SAPS II (63,2 vs. 41,2) ve SOFA (10,8 vs. 6,4) skorları anlamlı olarak daha yüksekti (tüm skorlar için $p<0,001$). Kardiyovasküler hastalıklar (%69,2), diyabet (%21,0) ve kronik solunum yolu hastalıkları (%19,5) en yaygın komorbiditelerdi. En yaygın enfeksiyon odakları solunum sistemi (%36,0), intraabdominal (%18,0) ve kan dolaşım sistemi (%17,4) olarak belirlendi. Toplam 133 kültür pozitif örnek arasında en sık üreme, (n=47, %35,3) trakeal aspirat kültürlerinde saptandı. İzole edilen patojenler arasında en sık *K. pneumoniae*, *A. baumannii* ve *E. coli* görüldü. Kültür pozitif vakaların %56,0'sında antimikrobiyal direnç mevcut olup, mortaliteyle istatistiksel olarak ilişkilendirilmedi ($p=0,118$). Hastaların %87,7'sine ampirik antimikrobiyal tedavi başlandı. Antimikrobiyal tedavinin 72. saatinde PCT (1,4→0.9 ng/mL, $p<0,001$) ve WBC ($15,3\rightarrow12,4\times10^3/\mu L$, $p<0,001$) düzeylerinde anlamlı düşüş izlenirken, CRP değişimi anlamlı değildi ($p=0,181$). Mortalite grubunda başlangıç CRP (111,0 vs. 78,5 mg/L, $p=0,032$) ve PCT (2,4 vs. 1,0 ng/mL $p=0,034$) düzeyleri daha yüksekken, WBC de anlamlı fark saptanmadı ($p=0,787$). Bulgularımız, enfeksiyonu olan kritik hastalarda hastalık şiddeti skorları ve biyobelirteç değişimlerinin prognostik değerini ortaya koymakta; konak yanıtı ile mikrobiyolojik verilerin birlikte değerlendirilmesi, hasta yönetimini kolaylaştırabilir.

Anahtar Kelimeler: Yoğun bakım enfeksiyonları. Antimikrobiyal direnç. Biyobelirteçler. Ampirik antibiyotikler. Mortalite.

Date Received: 11.March.2025
Date Accepted: 5.August.2025

* Presented as a poster presentation at the "Turkish Society of Anesthesiology and Reanimation 56th National Congress" (November, 2022, Antalya)"

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Intensive care units (ICUs) are among the hospital settings where infectious diseases are most prevalent and associated with high mortality. ICU patients are at increased risk for infections due to comorbidities, prolonged hospitalization, and invasive procedures. The EPIC III study demonstrated that 54% of ICU patients had suspected or confirmed infections, with a mortality rate of 30%[1].

Understanding ICU infection epidemiology, risk factors, and resistance patterns is critical for reducing mortality and improving infection control. Depending on patient populations and local practices, infection rates and resistance profiles vary widely between centers. The increasing prevalence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) organisms further complicates management and highlights the need for ongoing surveillance and robust local data[2].

Early diagnosis and timely, appropriate empirical antimicrobial therapy are essential for improving outcomes in critically ill patients, as delays or inappropriate regimens can significantly increase morbidity, mortality, and healthcare costs[3]. Biomarkers such as C-reactive protein (CRP), procalcitonin (PCT), and white blood cell (WBC) count are increasingly used to support diagnosis, prognostication, and guidance of therapy[4]. In addition, the severity of illness is routinely assessed in ICU practice using scoring systems such as Acute Physiology and Chronic Health Evaluation II (APACHE II), Simplified Acute Physiology Score II (SAPS II), and Sequential (or Sepsis-related) Organ Failure Assessment (SOFA), which play an important role in predicting patient prognosis[1]. Although microbiological cultures are considered the gold standard, they frequently fail to confirm infection; therefore, empirical treatment remains the cornerstone of initial ICU management. However, inappropriate empirical therapy is common due to underlying bacterial resistance, leading to suboptimal outcomes and increased healthcare costs[5,6].

In this study, we aimed to describe the prevalence of infections among critically ill patients, identify the microbiological characteristics and resistance patterns observed, and examine how infection-related biomarkers and severity scores relate to infection status. The results are intended to contribute to the growing body of data on ICU infections by providing observations from a single center.

Material and Method

Ethics

This study was approved by the Izmir Kâtip Çelebi University Non-Interventional Clinical Studies Institutional Review Board (Decision No: 0351, August 26, 2021). All procedures adhered to ethical guidelines and complied with the principles of the Declaration of Helsinki. We anonymized patient data and handled it in compliance with confidentiality principles to ensure patient privacy.

Study Population and Inclusion Criteria

We conducted this retrospective study between March 1, 2020, and March 1, 2021, in the 23-bed ICU of İzmir, Turkey. Adult patients (≥18 years) hospitalized for at least 72 hours were screened for inclusion.

The prevalence of infectious diseases was determined by calculating the proportion of patients diagnosed with a suspected or confirmed infection among all screened patients, regardless of microbiological culture positivity. Only patients with a suspected or confirmed infectious disease were included in the final analysis.

Patient data were obtained from the hospital information management system, archived medical records, and electronic patient files. For patients with multiple ICU admissions, only the first episode was analyzed. Exclusion criteria were incomplete medical records, ICU stay of less than 72 hours, confirmed COVID-19 diagnosis, or pregnancy.

Data Collection and Analysis

We reviewed a total of 700 patient records. The study flow chart is presented in Figure 1. The final analysis included 195 patients diagnosed with a suspected or confirmed infectious disease.

We collected demographic data, including age, sex, and body mass index (BMI), clinical characteristics, major comorbidities (e.g., cardiovascular disease, diabetes, malignancy), and infection-predisposing factors present at ICU admission (such as indwelling devices, recent surgery, prolonged hospitalization, or

immunosuppression), as well as data on suspected or confirmed infection sites, microbiological cultures, antibiograms, and biomarker levels (WBC, CRP, and PCT). Biomarker measurements were obtained at ICU admission and approximately 72 hours after initiation of antimicrobial therapy, per the standard clinical protocol. In the retrospective analysis, when laboratory results were not available exactly at 72 hours, the value closest to this time point was used for statistical analysis. BMI categories were defined according to the World Health Organization (WHO) criteria as follows: underweight (<18.5 kg/m²), normal weight (18.5–24.9 kg/m²), pre-obesity (25.0–29.9 kg/m²), obesity class I (30.0–34.9 kg/m²), obesity class II (35.0–39.9 kg/m²) and obesity class III (≥40 kg/m²) [7]. APACHE II, SAPS II, and SOFA scores at ICU admission were also documented.

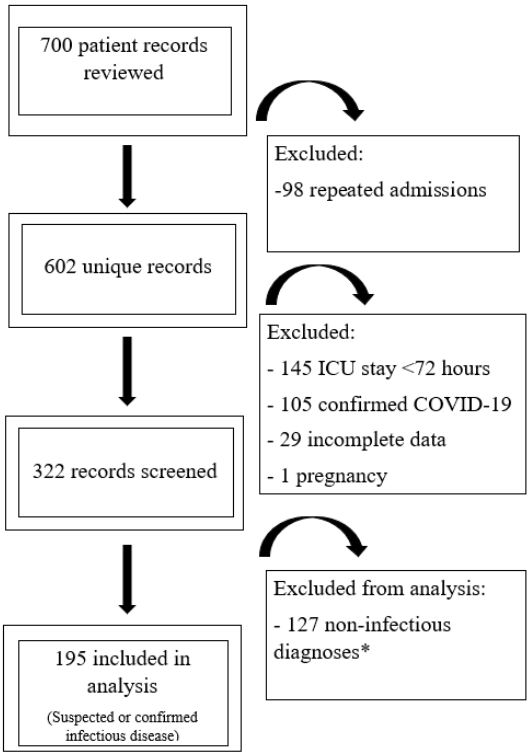

Figure 1.
*Flow Diagram of the Patient Selection Process (*Included in the denominator for prevalence calculation)*

Infection foci were determined based on clinical judgment supported by physical examination and laboratory parameters. In certain infection types, such as intra-abdominal infections and deep-seated soft tissue infections, microbiological confirmation was frequently not feasible due to clinical context or sampling limitations. Diagnoses were made based on clinical and perioperative findings in these cases.

As part of the routine ICU diagnostic protocol, microbiological samples, including blood, urine, and respiratory tract specimens, were obtained from all patients at ICU admission, wherever clinically and technically feasible. Based on clinical judgment, additional cultures were collected from suspected infection sites, such as surgical fields, wounds, or indwelling catheters. Some patients provided multiple samples, and a subset of cultures yielded more than one pathogen.

Antimicrobial treatments were classified as empirical or targeted based on microbiological evidence. Since individual patients could receive multiple antibiotics during their ICU stay, antimicrobial usage patterns were analyzed according to the number of antibiotic initiations, not the number of patients.

ICU mortality was defined as death during ICU stay. Patients discharged or transferred were considered survivors at ICU disposition.

Statistical Analysis

We performed statistical analyses using IBM SPSS version 21.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR), and categorical variables as frequencies and percentages. For comparisons, the independent samples t-test or Mann–Whitney U test was used for continuous variables, depending on distribution. Categorical variables were compared using the Pearson's chi-square (χ^2) test. Changes in biomarker levels were assessed using the paired samples t-test or the Wilcoxon signed-rank test. The association between AMR and ICU mortality was evaluated using the chi-square test. A p-value of <0.05 was considered statistically significant. All tests were two-tailed.

Results

Patient Characteristics and Association with Mortality

After applying the exclusion criteria, the prevalence of suspected or confirmed infectious diseases among eligible ICU patients was 60.6% (195/322). The median age was 67.0 years (IQR: 56.0–77.0). Among the study population, 60% were older than 65 years. Of the cohort, 61.0% were male. The most prevalent BMI category was pre-obesity, with 40.5% of patients, followed by obesity class I at 28.2%.

The ICU mortality rate in this cohort was 49.7% (97/195). When examining the association between demographic characteristics and ICU mortality, there was no statistically significant relationship between mortality and age (median age, 72.0 (61.0–81.0) vs. 64.5 (54.3–80.0) years; p = 0.085), sex (p = 0.847), or BMI category (p = 0.703). These are summarized in Table I.

Table I. Association of Demographic and Clinical Characteristics with ICU Mortality

Variable	Non-Survivors (n=97)	Survivors (n=98)	Total (n=195)	p-value
Age (year), median (IQR)	72.0 (61.0–81.0)	64.5 (54.3–80.0)	67.0 (56–77)	0.085
Sex, n (%)				0.847
Female	39 (40.2)	37 (37.8)	76 (39.0)	
Male	58 (59.8)	61 (62.2)	119 (61.0)	
BMI category, n (%)				0.703
Normal (18.5–24.9)	22 (22.7)	18 (18.4)	40 (20.5)	
Pre-obesity (25–29.9)	41 (42.3)	38 (38.8)	79 (40.5)	
Obesity I (30–34.9)	26 (26.8)	29 (29.6)	55 (28.2)	
Obesity II (35–39.9)	8 (8.2)	11 (11.2)	19 (9.7)	
Obesity III (≥40)	0 (0.0)	2 (2.0)	2 (1.0)	
Severity Scores, mean ± SD				
APACHE II	28.6 ± 8.6	18.1 ± 7.4	23.3 ± 9.6	<0.001
SAPS II	63.2 ± 16.3	41.2 ± 16.3	52.2 ± 19.1	<0.001
SOFA	10.8 ± 3.7	6.4 ± 2.5	8.6 ± 3.9	<0.001

Abbreviations: *BMI = Body Mass Index; IQR = Interquartile Range; SD = Standard Deviation; APACHE II = Acute Physiology and Chronic Health Evaluation II; SAPS II = Simplified Acute Physiology Score II; SOFA = Sequential Organ Failure Assessment*

The mean APACHE II, SAPS II, and SOFA scores for the entire cohort were 23.3 ± 9.6, 52.2 ± 19.1, and 8.6 ± 3.9, respectively. All three scores were significantly higher in non-survivors compared to survivors. Details are provided in Table I.

Cardiovascular diseases (69.2%) and diabetes mellitus (21.0%) were the leading comorbidities (Table II). Frequently observed infection-related risk factors included prolonged hospitalization (14.4%), urinary catheterization (9.4%), mechanical ventilation (8.3%), recent surgery (7.6%), and malignancy (8.6%) (Table III).

Table II. Distribution of Comorbidities in the Study Population

Comorbidity	n	(%)
Cardiovascular disease	135	69.2
Chronic respiratory disease	38	19.5
Chronic kidney disease	33	16.9
Diabetes mellitus	41	21.0
Malignancy	26	13.3
Chronic liver disease	11	5.6
Neurological disease	22	11.3
Immunosuppression	14	7.2

Some patients had more than one comorbidity; thus, totals exceed the number of patients. Comorbidities with a prevalence below 5% are not shown.

Table III. Infection-Predisposing Factors Present at ICU Admission

Category	n	%
Indwelling devices		
Mechanical ventilation (intubation, tracheostomy, NIV)	55	8.3
Central venous catheter	36	5.5
Urinary catheter	62	9.4
Nasogastric tube	46	7.0
Other foreign bodies (drains, nephrostomy, etc.)	46	7.0
Nutrition/Treatment		
Total parenteral nutrition	10	1.5
Dialysis	12	1.8
Surgery/Procedures		
Recent surgery (<30 days)	50	7.6
Prolonged Hospitalization	95	14.4
Immunosuppression		
Immunosuppressive drug use	11	1.7
Neutropenia	6	0.9
Hematological disease	7	1.1
Malnutrition	20	3.0
Malignancy	57	8.6
Chemotherapy/radiotherapy	22	3.3
Transplantation (bone marrow/organ)	2	0.3
Autoimmune/rheumatologic disease	6	0.9
Comorbidities		
Diabetes mellitus	54	8.4
Dementia/head trauma	20	3.0
Loss of skin integrity (burn, pressure ulcer, open wound)	32	4.8
Other	22	3.3

Abbreviations*: NIV, noninvasive mechanical ventilation.*

Multiple factors may coexist in a single patient. "Other" includes IV drug abuse, obesity, chronic alcoholism, and a history of aspiration. Data refer to risk factors identified on ICU admission.

Infection Distribution and Culture Results

To evaluate the overall infection burden at ICU admission, we analyzed clinically diagnosed infection foci, regardless of culture positivity. A total of 328 infection sites were identified in 195 patients, as multiple foci could be present in a single individual. The most common site of infection was the respiratory tract (n = 118, 36.0%), followed by intra-abdominal infections (n = 59, 18.0%) and bloodstream infections (n = 57, 17.4%) **(**Table IV**).**

Table IV. Distribution of Top Five Most Common Clinically Diagnosed Infection Foci at ICU Admission (n=328)

Infection Focus	n	%
Respiratory tract infections	118	36.0
Intra-abdominal infections	59	18.0
Bloodstream infections	57	17.4
Urinary tract infections	40	12.2
Skin and soft tissue	33	10.1

Less common infections, including endocarditis, mediastinitis, and CNS infections, were observed in individual cases and are not shown here.

Distribution of Culture-Positive Samples, Resistance Patterns, and Mortality

A total of 133 culture-positive samples were obtained from 100 patients admitted to the ICU. The most frequently sampled sites with positive results were tracheal aspirate (n = 47, 35.3%), urine (n = 37, 27.8%), and bloodstream or catheter tip specimens (n = 33, 24.8%) (Table V).

Table V. Distribution of Culture-Positive Samples by Sample Type (n = 133)

Culture Type	n	%
Tracheal Aspirate	47	35.3
Urine	37	27.8
Bloodstream (Blood + Catheter Tip)	33	24.8
Other (Wound, Surgical, Sputum, etc.)	16	12.0

The distribution of pathogen groups varied by culture site. In tracheal aspirate cultures (n=47), Gram-negative bacteria were the most frequently isolated group (n=31), followed by Gram-positive bacteria (n=8), fungal pathogens (n=7), and one atypical isolate. Among urine isolates (n=40), Gram-negative organisms predominated (n=24), while fungal (n=11) and Gram-positive pathogens (n=5) were less common. In blood and catheter tip cultures (n=36), Gram-positive bacteria were the leading group (n=21), followed by Gram-negative bacteria (n=9) and fungal isolates (n=6) (Figure 2). In some samples, more than one pathogen was identified, resulting in a higher total number of isolates than the number of culture samples.

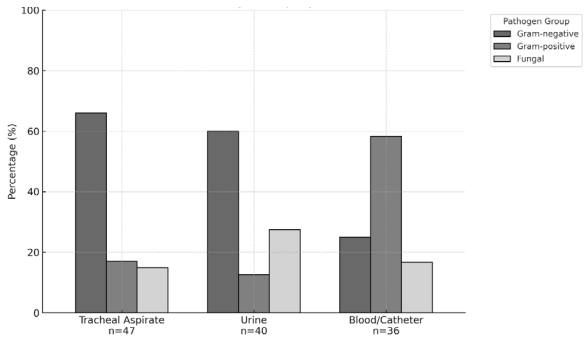

Figure 2.
Distribution of Pathogen Groups by Culture Site

Of the 100 patients with culture-positive results, antimicrobial resistance was found in 56 patients (56.0%). Of these, 39 (69.6%) died during their ICU stay. Among patients with susceptible pathogens (n = 44), 21 (47.7%) died. Although mortality was higher in the resistant group, the difference was not statistically significant (p = 0.118).

Our study revealed that 55.0% (11/20) of *Klebsiella pneumoniae* isolates produced Extended-Spectrum Beta-Lactamase (ESBL), while 41.2% (7/17) of *Escherichia coli* isolates did the same. Carbapenem resistance was noted in 20.0% (4/20) of *K. pneumoniae* and 11.8% (2/17) of *E. coli* isolates. Remarkably, 81.3% (13/16) of *A. baumannii* isolates exhibited carbapenem resistance (CRAB). Additionally, ampicillin resistance was found in 77.8% (7/9) of *Enterococcus faecium* isolates, and MRSA (Methicillin-Resistant *Staphylococcus aureus*) was detected in 25.0% (2/8) of *Staphylococcus aureus* isolates.

Antibiotic Utilization Patterns

In 87.7% (n=171) of patients, empirical therapy was administered, while only 12.3% (n=24) received culture-guided treatment. A total of 329 antibiotic initiations were recorded, as some patients received more than one agent during their ICU stay. The most commonly used agent was meropenem (n = 100, 30.4%), followed by piperacillin-tazobactam (n = 60, 18.2%), teicoplanin (n = 32, 9.7%), tigecycline (n = 29, 8.8%), and ceftriaxone (n = 27, 8.2%).

Biomarker Trends and Association with Mortality

At 72 hours, PCT and WBC levels showed a significant decrease from baseline ($p<0.001$ for both), while CRP levels declined without reaching statistical significance (p = 0.181) (Table VI).

Table VI. Comparison of Biomarker Levels at ICU Admission and at 72 Hours After Initiation of Antimicrobial Therapy

Biomarker	Admission Values (n = 195) *Median (IQR)*	72nd Hour Values (n = 195) *Median (IQR)*	p-value
WBC (×10³/μL)	15.3 (10.1–20.6)	12.4 (8.5–16.4)	**<0.001**
CRP (mg/L)	100.2 (42.7–180.4)	80.0 (34.0–171.5)	0.181
PCT (ng/mL)	1.4 (0.4–9.3)	0.9 (0.3–4.8)	**<0.001**

In the non-survivor group, 72-hour PCT and CRP values remained significantly higher than in survivors (p = 0.032 and p = 0.034), with no significant difference observed in WBC counts (p = 0.787) (Table VII).

Table VII. Mortality Impact of Initial WBC, CRP, and PCT Values

Biomarker	Non-Survivors (n = 97) *Median (IQR)*	Survivors (n = 98) *Median (IQR)*	p-value
WBC (×10³/μL)	16.0 (9.5–21.9)	14.8 (10.4–19.2)	0.787
CRP (mg/L)	111.0 (50.0–198.2)	78.5 (24.2–147.5)	**0.032**
PCT (ng/mL)	2.4 (0.5–11.1)	1.0 (0.4–7.0)	**0.034**

Discussion and Conclusion

The primary objective of this study was to investigate the epidemiological features, infection profiles, antimicrobial resistance patterns, and prognostic markers among critically ill patients with suspected or confirmed infections in the ICU. Our findings demonstrate that respiratory tract infections constituted the predominant infection focus. Multidrug-resistant pathogens were frequently isolated, and biomarker trends and severity scores provided valuable prognostic insights.

Patient Demographics and Infection Risk

The mean age of our ICU cohort was 67 years, with 60% of patients aged 65 or older. While advanced age has consistently been identified as a risk factor for ICU-acquired infections and worse outcomes in the literature[8,9], our study did not observe a statistically significant association between age and ICU mortality. However, a non-significant trend toward increased mortality in older patients was evident, suggesting that age remains a clinically important consideration, particularly in larger populations[8].

Neither sex nor BMI category demonstrated a significant association with ICU mortality, consistent with prior studies that have reported variable or weak relationships between these demographic factors and ICU outcomes in heterogeneous populations[10-12]. Some meta-analyses even highlight an "obesity paradox" in critical illness, where higher BMI does not necessarily predict poorer outcomes[12]. Significant comorbidities and infection-predisposing factors at ICU admission were summarized to provide a comprehensive view of our population, as these are well-established contributors to infection risk and ICU morbidity[1,13].

Severity scores in our cohort reflected a high burden of critical illness, with mean APACHE II, SOFA, and SAPS II values indicating moderate to severe disease. Among eligible ICU patients, the prevalence of suspected or confirmed infection was 60.6%, and the ICU mortality rate was 49.7%. Both infection prevalence and mortality were slightly higher than in large multicenter studies such as EPIC III, EPIC II, and the SOAP study[1,14,15], likely reflecting a more severely ill patient population in our cohort.

Overall, while our cohort's outcomes reflect a substantial disease burden, the observed mortality rate is within the range expected for populations with comparable severity of illness. Interpretation of these results should consider the heterogeneity of the patient population and local ICU practices.

Infection Distribution and Microbiological Findings

Respiratory tract infections (RTIs) were our cohort's most common infection focus, accounting for 36% of all clinically diagnosed infection sites. This finding is consistent with previous research highlighting the significant burden of respiratory infections, including ventilator-associated pneumonia, among ICU patients[13].

Tracheal aspirate samples were the most frequently positive among patients with positive cultures, representing 35.3% of all culture-positive specimens. Our cohort's most frequently isolated pathogens were *Klebsiella pneumoniae, Acinetobacter baumannii, and Escherichia coli,* particularly in respiratory tract samples. These Gram-negative organisms predominated in clinical and microbiological findings and were followed by Gram-positive bacteria and fungal pathogens in lower proportions.

This distribution reflects the local epidemiology of our ICU and aligns with the literature reporting the frequent isolation of MDR Gram-negative bacteria in intensive care settings[13]. However, given our study's single-center and retrospective nature, our results should be interpreted within the context of our institution's patient population.

Respiratory tract cultures, especially tracheal aspirates, yielded the highest rates of pathogen isolation, further emphasizing the importance of ongoing surveillance and infection control measures to reduce the burden of respiratory infections in the ICU.

Microbiology and Antimicrobial Resistance: Clinical Implications

The empirical antimicrobial therapy initiation rate in our study was notably high (87.7%). This finding can be attributed to several factors, including delays in infection diagnosis, frequent negative culture results, and the necessity for rapid treatment initiation in critically ill patients[1,5]. The literature similarly reports the widespread use of empirical therapy in intensive care settings and emphasizes that this approach contributes to the increasing risk of antimicrobial resistance[5,6]. Therefore, improving the proportion of culture-guided therapy in the ICU requires implementing rapid diagnostic methods and robust antimicrobial stewardship strategies.

Regarding microbiological findings, high ESBL and carbapenem resistance rates were observed among *Klebsiella pneumoniae* and *Escherichia coli* isolates, while *Acinetobacter baumannii* demonstrated a particularly high prevalence of carbapenem resistance. Similarly, significant rates of ampicillin resistance were noted in *Enterococcus faecium*, and MRSA was detected among *Staphylococcus aureus* isolates. These results are consistent with recent European and global surveillance reports, underscoring the ongoing

challenge of multidrug resistance in intensive care settings[16,17]. However, given the limited number of isolates in our cohort, these findings should be interpreted in the context of our institution's patient population.

Interestingly, antimicrobial resistance was not statistically associated with increased mortality in our cohort. This observation aligns with previous reports suggesting that, although resistant organisms complicate clinical management and may prolong hospitalization, patient outcomes are often more closely related to underlying disease severity, comorbidities, and organ dysfunction rather than resistance status alone[18,19]. Nevertheless, our study found higher mortality rates among patients with resistant infections than those with susceptible pathogens. However, this difference did not reach statistical significance, likely due to the limited sample size. The high ESBL and carbapenem resistance rates among Gram-negative isolates in our cohort highlight the ongoing clinical and economic burden of multidrug-resistant organisms in the ICU, as these pathogens can increase broad-spectrum antibiotic use and escalate healthcare costs[20]. Taken together, these findings emphasize the need for continuous surveillance, robust infection control measures, and regular updates of antimicrobial treatment strategies to prevent the emergence and spread of resistant organisms in critical care settings[20]. Further studies with larger cohorts are warranted to validate these results.

Prognostic Value of Biomarkers

Biomarkers such as PCT and CRP are important in evaluating infection severity, guiding antimicrobial therapy, and predicting outcomes[4,22]. In our study, higher PCT and CRP baseline levels were significantly associated with mortality, and persistent elevation of PCT at 72 hours was predictive of worse outcomes. These findings support serial biomarker measurements, particularly PCT, as a valuable tool in the prognostication and management of critically ill patients. However, the utility of CRP kinetics was less robust, likely reflecting its sensitivity to non-infectious inflammatory conditions. WBC count did not provide substantial prognostic information, which aligns with previous studies[23]. Integrating biomarker trends with comprehensive clinical evaluation may enhance risk stratification and inform treatment decisions in the ICU[24,25].

Limitations

This study has several limitations that must be considered. The retrospective, single-center design restricts the generalizability of the results to other ICUs with differing patient profiles and antimicrobial resistance patterns. The absence of long-term follow-up precludes assessment of sustained clinical impact. A lack of multivariable adjustment for potential confounders, such as comorbidities and other critical illness factors, also limits causal inference. The lack of radiological data may also affect diagnostic accuracy, particularly in ventilated patients, where imaging can be nonspecific or misleading. The unavailability of advanced molecular diagnostics may have led to missed pathogen identification, especially in culture-negative cases. While biomarkers and severity scores provide valuable prognostic data, their specificity in distinguishing infectious from non-infectious inflammation is uncertain, particularly among septic patients with multiple comorbidities. Collectively, these limitations may influence both clinical decision-making and the broader applicability of our findings.

Future Directions

Further prospective, multicenter studies with larger sample sizes and multivariable analyses are needed to clarify the complex relationships among infection, host factors, antimicrobial resistance, and ICU outcomes.

Researcher Contribution Statement:
Idea and design: G.K.K., M.A., N.K.; Data collection and processing: G.K.K., M.A., A.Ş., M.Ç, E.Ç., S.G.; Analysis and interpretation of data: G.K.K., M.A., A.Ş., M.Ç, E.Ç., S.G.;; Writing of significant parts of the article: G.K.K., M.A., A.Ş., M.Ç, E.Ç., S.G.;
Support and Acknowledgement Statement:
No financial support was provided for this study.
We are grateful to Dr. Nagihan Karahan for her invaluable guidance during the development of this study. Her wisdom and dedication will be remembered.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Izmir Kâtip Çelebi University Non-Interventional Clinical Studies Institutional Review Board
Approval Date: 26.08.2021
Decision No: 0351

References

1. Vincent JL, Sakr Y, Singer M, et al. Prevalence and outcomes of infection among patients in intensive care units in 2017. *JAMA*. 2020;323(15):1478-1487. doi:10.1001/jama.2020.2717
2. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant, and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18(3):268-281. doi:10.1111/j.1469-0691.2011.03570.x
3. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med*. 2006;34(6):1589-1596. doi:10.1097/01.CCM.0000217961.75225.E9

4. Magrini L, Gagliano G, Travaglino F, et al. Comparison between white blood cell count, procalcitonin, and C-reactive protein as diagnostic and prognostic biomarkers of infection or sepsis in patients presenting to the emergency department. *Clin Chem Lab Med*. 2014;52(10):1465-1472. doi:10.1515/cclm-2014-0210
5. Versporten A, Zarb P, Caniaux I, et al. Antimicrobial consumption and resistance in adult hospital inpatients in 53 countries: results of an internet-based global point prevalence survey. *Lancet Glob Health*. 2018;6(6):e619-e629. doi:10.1016/S2214-109X(18)30186-4
6. Centers for Disease Control and Prevention (CDC). Antibiotic resistance threats in the United States, 2019. Atlanta, GA: US Department of Health and Human Services; 2019. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf
7. World Health Organization (WHO). *Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation.* WHO Technical Report Series No. 894. Geneva: World Health Organization; 2000. https://iris.who.int/handle/10665/42330
8. Martin GS, Mannino DM, Moss M. The effect of age on the development and outcome of adult sepsis. *Crit Care Med*. 2006;34(1):15-21. doi:10.1097/01.CCM.0000194535.82812.BA
9. Flaatten H. The impact of age in intensive care. *Acta Anaesthesiol Scand*. 2014;58(1):3-4. doi:10.1111/aas.12221
10. Papathanassoglou E, Middleton N, Benbenishty J, et al. Systematic review of gender- dependent outcomes in sepsis. *Nurs Crit Care*. 2017;22(5):284-292. doi:10.1111/nicc.12280
11. Pepper DJ, Demirkale CY, Sun J, et al. Does Obesity Protect Against Death in Sepsis? A Retrospective Cohort Study of 55,038 Adult Patients. *Crit Care Med*. 2019;47(5):643-650. doi:10.1097/CCM.0000000000003692
12. Nie W, Zhang Y, Jee SH, Jung KJ, Li B, Xiu Q. Obesity survival paradox in pneumonia: a meta-analysis. *BMC Med*. 2014;12:61. doi:10.1186/1741-7015-12-61
13. Gagneja D, Goel N, Aggarwal R, Chaudhary U. Changing trend of antimicrobial resistance among gram-negative bacilli isolated from lower respiratory tract of ICU patients: a 5-year study. *Indian J Crit Care Med*. 2011;15(3):164-167. doi:10.4103/0972-5229.84900
14. Vincent JL, Rello J, Marshall J, et al. International study of the prevalence and outcomes of infection in intensive care units. *JAMA*. 2009;302(21):2323-2329. doi:10.1001/jama.2009.1754
15. Vincent JL, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med*. 2006;34(2):344-353.doi:10.1097/01.CCM.0000194725.48928.3A
16. European Centre for Disease Prevention and Control (ECDC). *Surveillance of antimicrobial resistance in Europe 2023–2022 data*. Stockholm: ECDC; 2023. https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2023-2022-data
17. World Health Organization (WHO). *Global antimicrobial resistance and use surveillance system (GLASS) report: 2022.* Geneva: World Health Organization; 2022. https://www.who.int/publications/i/item/9789240062702
18. Maskarinec SA, Park LP, Ruffin F, et al. Positive follow-up blood cultures identify high mortality risk among patients with Gram-negative bacteraemia. *Clin Microbiol Infect*. 2020;26(7):904-910. doi:10.1016/j.cmi.2020.01.025
19. Hariyanto H, Yahya CQ, Cucunawangsih C, Pertiwi CLP. Antimicrobial resistance and mortality. *Afr J Infect Dis.* 2022;16(2):13–20. doi:10.21010/Ajid.v16i2.2
20. Phua J, Ngerng W, See K, et al. Characteristics and outcomes of culture-negative versus culture-positive severe sepsis. *Crit Care*. 2013;17(5):R202. doi:10.1186/cc12896
21. Cosgrove SE. The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. *Clin Infect Dis*. 2006;42(Suppl 2):S82-S89. doi:10.1086/499406
22. Lam SW, Bauer SR, Fowler R, Duggal A. Systematic review and meta-analysis of procalcitonin-guidance versus usual care for antimicrobial management in critically ill patients: focus on subgroups based on antibiotic initiation, cessation, or mixed strategies. *Crit Care Med*. 2018;46(5):684-690. doi:10.1097/CCM.0000000000002953
23. Suberviola B, Castellanos-Ortega A, González-Castro A, et al. Prognostic value of procalcitonin, C-reactive protein and leukocytes in septic shock. *Med Intensiva*. 2012;36(3):177-184. doi:10.1016/j.medin.2011.09.008
24. Schuetz P, Maurer P, Punjabi V, et al. Procalcitonin decrease over 72 hours in US critical care units predicts fatal outcome in sepsis patients. *Crit Care*. 2013;17:R115. doi:10.1186/cc12787
25. von Dach E, Albrich WC, Brunel AS, et al. Effect of C-reactive protein-guided antibiotic treatment duration, 7-day treatment, or 14-day treatment on 30-day clinical failure rate in patients with uncomplicated gram-negative bacteremia: a randomized clinical trial. *JAMA*. 2020;323(21):2160-2169. doi:10.1001/jama.2020.6348

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 319-323, 2025
DOI: https://doi.org/10.32708/uutfd.1721258

ORIGINAL RESEARCH

Evaluation of Adult Patients Diagnosed with Idiopathic Membranous Glomerulonephritis: A Single-Center Retrospective Study

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ABSTRACT

Membranous glomerulonephritis (MGN), an immunocompetent nephropathy, is among the most prevalent causes of nephrotic syndrome. While spontaneous remission may occur, there is a risk of developing long-term end-stage renal failure. Treatment approaches for MGN lack full consensus; however, immunosuppressive therapies are critically important. This retrospective, cross-sectional study included 99 patients who visited our center between March 2007 and November 2015 and were diagnosed with MGN. Patient data were scanned and recorded retrospectively from the hospital information system. Of the 99 patients, 43 (43.4%) were female. The mean age of the patients was 46.7 years, with a mean follow-up duration of 18.5 months. A total of 71 patients (71.7%) presented with nephrotic proteinuria, while 57 patients (57.6%) had nephrotic syndrome. Immunosuppressive drugs were prescribed to 85 patients (85.9%). Among those receiving immunosuppressive therapy, 57 patients (79.9%) still exhibited proteinuria at the time of diagnosis. Remission was achieved in 63 patients (74.1%) who underwent immunosuppressive therapy, compared to 10 patients (71.3%) who received conservative treatment. Hypertension and IgG deposition may be associated with a poorer treatment response in patients diagnosed with idiopathic membranous glomerulonephritis (MGN). In summary, MGN is a common cause of nephrotic syndrome that can affect individuals of various ages and genders. Our study identified higher remission rates in patients treated with immunosuppressive therapy, which emphasizes the value of evaluating individual risk factors and selecting appropriate treatment strategies for effective disease management.

Keywords: Membranous glomerulonephritis. Immunosuppressive therapy. Remission.

İdiyopatik Membranöz Glomerülonefrit Tanısı Alan Yetişkin Hastaların Değerlendirilmesi: Tek Merkez Deneyimi

ÖZET

İmmünkompetan bir nefropati olan membranöz glomerülonefrit (MGN), nefrotik sendromun en yaygın nedenleri arasındadır. Spontan remisyon meydana gelebilse de, uzun vadeli son dönem böbrek yetmezliği gelişme riski vardır. MGN tedavi yaklaşımları konusunda tam bir fikir birliği yoktur; ancak immünosüpresif tedaviler kritik öneme sahiptir. Bu retrospektif, kesitsel çalışmaya, Mart 2007 ile Kasım 2015 tarihleri arasında merkezimizi ziyaret eden ve MGN tanısı alan 99 hasta dahil edilmiştir. Hasta verileri hastane bilgi sisteminden retrospektif olarak taranmış ve kaydedilmiştir. 99 hastanın 43'ü (%43,4) kadındı. Hastaların ortalama yaşı 46,7 yıl ve ortalama takip süresi 18,5 ay idi. Toplam 71 hasta (%71,7) nefrotik proteinüri ile başvururken, 57 hastada (%57,6) nefrotik sendrom vardı. 85 hastaya (%85,9) immünosupresif ilaç reçete edildi. İmmünosupresif tedavi görenler arasında 57 hastada (%79,9) tanı anında proteinüri devam ediyordu. İmmünosupresif tedavi uygulanan 63 hastada (%74,1) remisyon sağlanırken, konservatif tedavi uygulanan 10 hastada (%71,3) remisyon sağlandı. Hipertansiyon ve IgG birikimi, idiyopatik membranöz glomerülonefrit (MGN) tanısı alan hastalarda daha zayıf tedavi yanıtıyla ilişkili olabilir. Özetle, MGN, farklı yaş ve cinsiyetlerdeki bireyleri etkileyebilen nefrotik sendromun yaygın bir nedenidir. Çalışmamız, immünosüpresif tedavi gören hastalarda daha yüksek remisyon oranları tespit etmiş olup, bu durum, etkili hastalık yönetimi için bireysel risk faktörlerinin değerlendirilmesi ve uygun tedavi stratejilerinin seçilmesinin önemini vurgulamaktadır.

Anahtar Kelimeler: Membranöz glomerulonefrit. İmmunsupresif tedavi. Remisyon.

Date Received: 17.June.2025
Date Accepted: 5.August.2025

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Idiopathic membranous glomerulonephritis (MGN) is a chronic condition characterized by immune complex-mediated damage to the glomeruli and is one of the primary causes of nephrotic syndrome in adults. The hallmark of MGN is the deposition of subepithelial immune complexes along the glomerular basement membrane (GBM), which results in thickening that can be observed using light microscopy[1]. MGN can occur in all ethnic and age groups; however, it has the highest incidence in individuals during their fourth and fifth decades of life. It is rare in children and is the most common cause of nephrotic syndrome in adults[2-4].

The first pathological finding in MGN is the accumulation of subepithelial IgG and complement along the outer surface of the glomerular capillary wall, which appears histologically normal, as typically demonstrated by immunofluorescent staining. MGN begins with the formation of immune complexes between podocytes and the glomerular basement membrane (GBM). This phase is followed by alterations in podocytes, the deposition of new extracellular matrix between and around the immune deposits, and thickening of the GBM, known as membranous change. In some cases, there may also be focal glomerulosclerosis, tubular atrophy, and interstitial fibrosis, depending on the extent of podocyte damage[5,6].

Most patients present with nephrotic-range proteinuria, hypoalbuminemia, and edema. However, a subset may show subnephrotic proteinuria and microscopic hematuria. Initially, renal function is often preserved. Despite intraglomerular complement activation, serum complement levels are normal. Serological markers (e.g., antinuclear antibodies, ANCA, and rheumatoid factor) are absent. At the time of diagnosis, only 10–20% of patients have hypertension[7,8]. The clinical course of MGN is variable. Spontaneous remissions in proteinuria have been reported in 30% of patients. As the severity of proteinuria at presentation increases, the frequency of spontaneous remission appears to decrease. Patients with proteinuria less than 3.5 g/day, no erythrocytes in the urine, no hypertension, normal renal function, and no features suggestive of secondary systemic disease have a positive prognosis. Despite the favorable prognosis data, end-stage renal failure remains a significant cause of glomerulonephritis due to its high prevalence. The KDIGO 2021 guidelines have updated risk stratification and treatment decisions. These guidelines now include serologic testing for PLA2R/THSD7A, proteinuria levels, and trends in renal function[9,10]. Treatment options available encompass supportive therapy, calcineurin inhibitors, cyclophosphamide, and, more recently, rituximab[11].

This study aimed to evaluate the demographic, clinical, histopathological, and treatment outcomes of patients diagnosed with idiopathic MGN in our center and to identify predictors of response to therapy.

Material and Method

This study is a retrospective, cross-sectional descriptive analysis that included patients diagnosed with MGN who were referred to our center between March 2007 and November 2015. Patients with evidence of secondary causes, such as malignancy, autoimmune diseases, or infections, were excluded through comprehensive clinical, laboratory, and radiological evaluations. We reviewed and recorded various patient demographics and clinical data, including age, gender, smoking status, initial examination findings (such as blood pressure and edema), kidney biopsy results, and both initial and follow-up laboratory findings (Table I). These laboratory findings included urinalysis, proteinuria levels, serum urea, creatinine, albumin, immunoglobulin levels (IgG, IgM, and IgA), and complement levels.

Table I. Demographic and clinical characteristics of patients diagnosed with membranous glomerulonephritis.

Characteristic	Value
Number of patients	99
Average age (years)	46
Gender (female)	43(%43)
Clinical findings at diagnosis	
Hematuria	63(%63,6)
Edema	76(%76,8)
Hypertension	35(%35,3)
Nephrotic Syndrome	57(%57,6)
Laboratory findings at diagnosis	
Renal dysfunction (GFR<60 ml/min)	31(%31,3)
Hypertriglyceridemia	60(%60,6)
Hypercholesterolemia	78(%78,8)
Hypoalbuminemia	72(%72,7)

Treatment protocols were documented, categorizing patients based on their treatment regimens: those receiving only steroids, those receiving steroids in combination with cyclophosphamide, those receiving steroids with cyclosporine, and those managed with conservative treatment. Kidney biopsy findings were evaluated using light microscopy and immunofluorescence. Light microscopy assessed various parameters, including glomerular count, sclerotic glomerular count, basement membrane thickening, interstitial inflammation, fibrosis, vascular changes, and tubular atrophy. Immunofluorescence staining was performed on all samples using markers such as IgG, IgM, IgA, C3q, C1q, and fibrinogen.

Patients were divided into three age groups: ≤30 years, 31-60 years, and >60 years. Treatment responses were evaluated based on 24-hour urine protein levels following treatment. Patients were classified as achieving complete remission, partial remission, or no response to treatment. Complete remission was defined as a reduction in 24-hour urine protein excretion to less than 0.5 g/24 hours, while partial remission was defined as a decrease of more than 50% from the initial proteinuria level.

Statistical analysis

Descriptive statistical methods, including mean, median, frequency, standard deviation, and ratios, were utilized to analyze categorical variables. For groups of variables that did not exhibit a normal distribution, Pearson's chi-square test was employed. Statistical analysis was conducted using SPSS (IBM Corp. Release 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.), with differences deemed significant at a p-value of less than 0.05.

The chi-square test was utilized for comparing categorical variables, while continuous variables were analyzed using ANOVA. To reduce the likelihood of false positives from multiple comparisons, the Bonferroni correction was applied when appropriate.

Results

The study included 99 patients who were diagnosed with kidney biopsies between March 2007 and November 2015. All patients underwent the necessary examinations for their initial evaluation. Their ages ranged from 18 to 74 years, with a mean age of 46.7 ± 13.6 years. Specifically, 14 cases (14.1%) were in the 18–30 age group, 67 cases (67.7%) were aged 31–60 years, and 18 cases (18.2%) were over 60 years of age. Among the patients, 43 (43.4%) were female and 56 (56.6%) were male. Regarding smoking history, 32 patients (32.3%) reported having smoked, which included 26 males and 6 females. The average follow-up period for the patients was 18.5 months, with a minimum of 3 months and a maximum of 190 months.

The histopathological analysis of 93 patients who underwent kidney biopsy and pathology results at our center is shown in Table II. The immunofluorescence findings of the kidney biopsy are shown in Table III.

The histopathological analysis of 93 patients who underwent kidney biopsy, the immunosuppressive treatment protocols implemented for these patients, and the evaluation of treatment responses are detailed in Table IV. Among the patients, 85 (85.9%) received immunosuppressive treatment, with an average treatment duration of 12 months (3,100). Treatment responses were assessed based on 24-hour urine protein levels following the treatment. Complete remission was defined as a reduction in 24-hour urine protein excretion to less than 0.5 g/24 hours, while partial remission was characterized by a decrease of more than 50% from the baseline proteinuria level.

Table II. Histopatholological findings in renal biopsies (n=93)

Finding	n(%)
Basement membrane thickening	92 (98,9)
Interstitial inflammation	87 (%93,5)
Interstitial fibrosis	49(%52,6)
Mesangial proliferation	3 (%3,22)
Tubular atrophy	64(%68,8)
Vascular changes	40(%43)

Table III. Immunofluorescence staining results.

Markers	Positive n(%)
IgG	86(%92,4)
IgM	19(%20,4)
IgA	14(%15)
C3c	43(%46,2)
C1q	3(%3,2)
Fibrin	12(%12,9)

Table IV. Treatment modalities and treamtment outcomes.

Treatment Group	n	Complete remission	Partial remission	No response	p-value
Steroid Only	37(%37,4)	14(%37,4)	13(%35,1)	10(%27,5)	0,441
Steroid+Cyclophosphamide	36(%36,4)	15(%41,6)	14(%38,8)	7(%19,6)	0,429
Steroid+Cyclosporine	12(%12,1)	4(%33,3)	3(%25)	5(%41,7)	0,426
Conservative	14(%14,1)	2(%14,2)	8(%57,1)	4(%28,7)	0,264

Note: p-values reflect overall chi-square comparisons; Bonferroni correction was applied to account for multiple testing.

The effect of demographic and clinical data at the time of application on treatment outcomes is summarized in Table V. An analysis of the demographic data showed no statistically significant impact of age, gender, or smoking status on treatment responses. Likewise, the clinical data evaluated at the time of presentation revealed no statistically significant effects of hematuria, edema, or nephrotic syndrome on treatment response. However, the presence of hypertension at the time of application was found to have a statistically significant effect on treatment response (p=0.046) (Table V). A post-hoc chi-square analysis indicated that the difference was specifically between the group that experienced a partial response to treatment and the group that exhibited no response (p=0.031).

Table V. The effect of demographic data and clinical and laboratory data at the time of admission on treatment outcome.

	Complete remission	Partial remission	No response	p-value
Average age	48,34	44,34	48,03	0,395
Female	18(%41,8)	16(%37,2)	9(%21)	0,415
Male	17(%30,4)	22(%39,2)	17(%30,4)	0,415
Smoking	13(%40,6)	11(%34,4)	8(%25)	0,741
Hematuria	21(%33,3)	26(%41,3)	16(%25,4)	0,731
Edema	20(%30,7)	23(%35,4)	22(%33,9)	0,302
Hypertension	14(%40)	8(%22,8)	13(%37,1)	0,046*
Nephrotic syndrome	20(%35,1)	23(%40,3)	14(%24,6)	0,957
Renal dysfunction	11(%35,4)	14(%45,2)	6(%19,4)	0,620
Hypertriglyceridemia	23(%38,4)	21(%35)	16(%26,6)	0,561
Hypercholesterolemia	30(%38,5)	30(%38,5)	18(%23)	0,570
Hypoalbuminemia	27(%37,5)	28(%39)	17(%23,5)	0,858

Note: p-values reflect overall chi-square comparisons; Bonferroni correction was applied to account for multiple testing. *Statistically significant ($p<0.05$)

The effects of biopsy data on treatment outcomes are shown in Table VI. A statistically significant effect of IgG accumulation in immunofluorescence on response to treatment was detected ($p=0.027$). When examining which groups this difference originated from, it was seen to be between the group that responded partially to treatment and the group that did not respond ($p=0.007$).

Table VI. Effect of biopsy data on treatment outcome.

	Complete remission	Partial remission	No response	p-value
Mesangial proliferation	1(%33,3)	1(%33,3)	1(%33,3)	0,253
Basement membrane thickening	33(%35,8)	35(%38)	24(%26,2)	0,526
Interstitial inflammation	29(%33,3)	34(%39)	24(%27,7)	0,191
Interstitial fibrosis	18(%36,8)	19(%38,7)	12(%24,5)	0,944
Vascular changes	14(%35)	13(%32,5)	13(%32,5)	0,382
IgG	31(%36,1)	32(%37,2)	23(%26,7)	0,027*
IgM	5(%26,4)	8(%42,1)	6(%31,5)	0,406
IgA	6(%42,9)	6(%42,9)	2(%14,2)	0,752
C3c	13(%30,3)	16(%37,2)	14(%32,5)	0,438
C1q	2(%66,6)	1(%33,3)	0(%0)	0,893
Fibrin	4(%33,3)	4(%33,3)	4(%33,3)	0,851

Note: p-values reflect overall chi-square comparisons; Bonferroni correction was applied to account for multiple testing. *Statistically significant ($p<0.05$)

Additionally, age was categorized into three groups (≤30, 31–60, and >60 years) to assess treatment response. However, no statistically significant difference was observed among age categories.

Although hypertension and IgG deposition initially appeared to be associated with poor treatment response, these associations did not remain statistically significant after Bonferroni correction, underscoring the need for cautious interpretation of subgroup analyses.

Discussion and Conclusion

Despite advancements in diagnosis and treatment, idiopathic membranous glomerulonephritis (MGN) remains a major cause of end-stage renal disease. In our cohort, the average baseline creatinine level was within the normal range, consistent with the indolent progression of MGN. The majority of patients (57%) presented with nephrotic syndrome, in line with previous literature.

Although smoking is considered a risk factor for glomerular injury, our study did not observe a significant association between smoking status and treatment response[12,13]. This limitation may be attributed to the lack of data on smoking intensity and duration. Given the increased risk of malignancy in smokers with MGN, closer monitoring is warranted.

Histopathological findings were generally consistent with previous reports[14-19]. While IgG deposition appeared to correlate with reduced treatment response, no other biopsy features including interstitial fibrosis, tubular atrophy, or vascular changes showed significant predictive value. Subtyping of IgG or the inclusion of modern biomarkers like anti-PLA2R and THSD7A antibodies could have strengthened the pathological correlations[9,10]. Unfortunately, these tests were not routinely available during the study period.

Although hypertension and IgG deposition initially showed associations with poorer treatment response, these did not remain statistically significant after Bonferroni correction. This highlights the importance of cautious interpretation, particularly when multiple subgroup analyses are conducted.

Most patients (85.9%) received immunosuppressive therapy, with an overall response rate (complete or partial remission) of 74.1%. However, 26% failed to respond. New biomarker-focused approaches offer higher success rates with fewer side effects than immunosuppressive treatments by optimizing the treatment of high-risk patients. According to findings in the current literature, the widespread use of treatment options such as rituximab in MGN patients has improved treatment compliance and patient comfort[20,21].

The lack of significant differences between treatment groups may reflect the relatively small subgroup sizes. However, the overall remission rate is consistent with previous reports.

This study has several limitations. It was retrospective and single-centered, with missing data for some patients. The relatively short follow-up period (average 18.5 months) limited the ability to assess

long-term renal outcomes. In addition, treatment side effects were not systematically documented, preventing an evaluation of risk-benefit balance. This created a gap in analyzing the balance between treatment efficacy and side effects. Recent KDIGO 2021 guidelines emphasize individualized risk-based therapy, including the use of rituximab, which was not available during our study period but holds promise in refractory or high-risk cases.

Limitations include retrospective design, absence of biomarker data (PLA2R, THSD7A), and single-center setting. Nonetheless, the identification of hypertension and IgG positivity as adverse prognostic factors may aid clinical risk stratification.

In conclusion, while our study reinforces some established clinical and pathological features of idiopathic MGN, the absence of significant predictors after correction for multiple comparisons suggests a need for larger, biomarker-integrated studies. Incorporating modern diagnostics and risk stratification tools may help guide more individualized treatment approaches in the future.

Researcher Contribution Statement:
Idea and design: C.P, A.Y., A.E.; Data collection and processing: C.P, A.Y., A.E.; Analysis and interpretation of data: C.P, A.Y., A.E.; Writing of significant parts of the article: C.P, A.Y., A.E.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Approving Committee: Uludağ Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu
Approval Date: 29.11.2016
Decision No: 2016-20/ 23

References

1. Beck LH Jr, Salant DJ. Membranous nephropathy: Recent travels and new roads ahead. Kidney Int 2010;77:765-70.
2. Nangaku M, Shankland SJ, Couser WG. Cellular response to injury in membranous nephropathy. J Am Soc Nephrol 2005;16:1195-204.
3. Vaughan RW, Demaine AG, Welsh KI. A DQA1 allele is strongly associated with idiopathic membranous nephropathy. Tissue Antigens 1989;34:261-9.
4. Le Petit JC, Laurent B, Berthoux FC. HLA-DR3 and idiopathic membranous nephritis (IMN) association. Tissue Antigens 1982;20:227- 8.
5. Fervenza FC, Sethi S, Specks U. Idiopathic membranous nephropathy diagnosis and treatment. Clin J Am Soc Nephrol 2008;3:905-19.
6. Gluck MC, Gallo G, Lowenstein J, et al. Membranous glomerulonephritis: Evolution of clinical and pathologic features. Ann Intern Med 1973;78:1- 12.
7. Haas M. IgG subclass deposits in glomeruli of lupus and nonlupus membranous nephropathies. Am J Kidney Dis 1994;23:358-64.
8. Kuroki A, Shibata T, Honda H, et al. Glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis, membranous lupus nephritis, and idiopathic membranous nephropathy. Intern Med 2002;41:936-42.
9. KDIGO Clinical Practice Guideline for Glomerular Diseases. Kidney Int Suppl. 2021;11(3):S1–S576.
10. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med. 2014;371(24):2277–2287. doi:10.1056/NEJMoa1409354. PMID: 25470565.
11. Cattran D. Management of membranous nephropathy: when and what for treatment. J Am Soc Nephrol 2005;16:1188-94.
12. Rihova Z, Honsova E, Merta M, et al. Secondary membranous nephropathy-one center experience. Ren Fail 2005;27:397-402.
13. Hitoshi Y, Takashi T, Hitoshi S, Hiroshi S. Membranous nephropathy in Japan: analysis of the Japan Renal Biopsy Registry (J-RBR).Clin Exp Nephrol 2012;16:557–63.
14. Dumoulin A, Hill GS, Montseny JJ, Meyrier A. Clinical and morphological prognostic factors in membranous nephropathy: significance of focal segmental glomerulosclerosis. Am J Kidney Dis 2003;41:38-48.
15. Gupta R, Sharma A, Mahanta PJ, Jacob TG, et al. Focal segmental glomerulosclerosis in idiopathic membranous glomerulonephritis: a clinico-pathological and stereological study. Nephrol Dial Transplant 2010;25:444–9.
16. Unver S, Haholu A, Atasoyu EM, et al. A rare complication of idiopathic membranous nephropathy: crescentic transformation. Ren Fail 2008;30:573–5.
17. Koethe JD, Gerig JS, Glickman JL, et al. Progression of membranous nephropathy to acute crescentic rapidly progressive glomerulonephritis and response to pulse methylprednisolone. Am J Nephrol 1986;6:224–8.
18. James SH, Lien YH, Ruffenach SJ, et al. Acute renal failure in membranous glomerulonephropathy: a result of superimposed crescentic glomerulonephritis. J Am Soc Nephrol 1995;6:1541–6.
19. Singh A, Arif F, Mangat S, et al. Acute cellular crescentic glomerulonephritis in association with antiglomerular basement membrane disease and superimposed membranous nephropathy. Am J Kidney Dis 2004;43-8.
20. Kadatz M, Zimpelmann J, Dember LM, et al. Cost-effectiveness of rituximab versus cyclosporine in the treatment of membranous nephropathy: a model-based analysis of the MENTOR trial. Nephrol Dial Transplant. 2024;39(5):688–696. doi:10.1093/ndt/gfae084. PMID: 38621719
21. Fervenza FC, Appel GB, Barbour SJ, et al. Rituximab or Cyclosporine in the Treatment of Membranous Nephropathy. N Engl J Med. 2019;381(1):36–46. doi:10.1056/NEJMoa1814427. PMID: 31154981

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 325-330, 2025
DOI: https://doi.org/10.32708/uutfd.1677759

CASE REPORT

Concurrent Vismodegib and Hypofractionated Stereotactic Radiotherapy in a Patient with Recurrent Locally Advanced Basal Cell Carcinoma: Case Report*

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ABSTRACT

In this study, we present a patient with recurrent basal cell carcinoma (BCC) located in a high-risk area of the face who was treated with a combination of vismodegib and hypofractionated stereotactic radiotherapy (hSRT). Targeted therapies such as hedgehog pathway inhibitors play an important role in the treatment of advanced BCC, but the duration of response is limited due to resistance. The combination of hedgehog pathway inhibitors and radiotherapy has the potential to achieve a durable and effective therapeutic response, particularly in patients with locally advanced disease. A 68-year-old male patient had a recurrent mass localized to the nasal dorsum and extending to the medial canthus of both eyes, infiltrating the nasal and maxillary bones. Multiple biopsies taken from the lesion edges reported infiltrative BCC and basosquamous cell carcinoma foci. The patient responded well to vismodegib, but surgery was not considered. Considering the tolerance of the organs at risk, concurrent 4200 centigray/14 fractions hSRT was applied with the CyberKnife-M6 device. Acute grade 1 to 2 toxicity was observed during treatment. At the end of treatment, the mass regressed, ptosis in the left eye decreased, and the visual field expanded. Complete radiological and clinical response was achieved four months after treatment. Combination therapy was found to be effective in a patient with recurrent locally advanced BCC. The combination of vismodegib and hSRT should be evaluated in prospective studies.

Keywords: Basal cell carcinoma. Stereotactic radiotherapy. Vismodegib.

Lokal İleri Yinelemiş Bazal Hücreli Karsinom Tanılı Bir Olguda Eşzamanlı Vismodegib ve Hipofraksiyone Stereotaktik Radyoterapi: Olgu Sunumu

ÖZET

Bu çalışmada, vismodegib ve hipofraksiyone stereotaktik radyoterapi (hSRT) kombinasyonu ile tedavi edilmiş olan yüzün yüksek riskli bölgesinde yerleşmiş yinelemiş bazal hücreli karsinom (BHK)' lu bir hasta sunulmaktadır. Hedgehog yolu inhibitörleri gibi hedeflenmiş tedaviler, ileri evre BHK tedavisinde önemli bir rol oynar, ancak direnç nedeniyle yanıt süresi kısadır. Hedgehog yolak inhibitörleri ve radyoterapinin kombinasyonu, özellikle lokal ileri evre hastalarda kalıcı ve etkili bir tedavi yanıtı sağlama potansiyeline sahiptir. 68 yaşında bir erkek hastada, nazal dorsuma lokalize ve her iki gözün medial kantusuna kadar uzanan, nazal ve maksiller kemikleri infiltre eden nüks kitle mevcuttu. Lezyon kenarlarından alınan çoklu biyopsiler, infiltratif BHK ve bazoskuamöz hücreli karsinom odakları şeklinde raporlanmıştı. Vismodegib'e iyi yanıt veren hastaya cerrahi düşünülmemişti. Risk altındaki organların toleransı göz önüne alınarak CyberKnife-M6 cihazı ile eşzamanlı 4200 santigray/14 fraksiyon hSRT uygulandı. Tedavi sırasında akut derece 1-2 toksisite gözlendi. Tedavi sonunda kitle geriledi, sol gözdeki pitozis azaldı ve görme alanı genişledi. Tedaviden 4 ay sonra radyolojik ve klinik tam yanıt elde edildi. Tekrarlayan lokal ileri evre BHK'lu bir hastada kombine tedavi etkin bulundu. Vismodegib ve hSRT kombinasyonu ileri prospektif çalışmalarla değerlendirilmelidir.

Anahtar Kelimeler: Bazal hücreli karsinom. Stereotaktik radyoterapi. Vismodegib.

Date Received: 17.April.2025
Date Accepted: 20.July.2025

* Presented as a poster presentation at the "National Radiation Oncology Congress 2025" (April 2025, Cyprus)

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Keratinocyte carcinomas, which include basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are the most commonly diagnosed cancers worldwide[1]. BCC accounts for 65–80% of cases and has an incidence rate of 525 per 100,000 individuals[1, 2]. It is more frequently observed in fair-skinned males over the age of 65. Etiologic factors include ultraviolet radiation exposure, ionizing radiation, immunosuppression, chronic inflammation, hereditary syndromes, and family history[1]. Although treatment decisions are primarily based on the risk stratification of the patient and tumor characteristics, surgery remains the first-

line treatment. In low-risk cases, surgical treatment is usually sufficient, with reported 5-year recurrence rates of 2–3.5%[1]. For patients unsuitable for surgery or with specific tumor locations, non-surgical modalities such as cryotherapy, electrodessication and curettage, topical agents, photodynamic therapy, and laser treatment may be employed. However, these options present a wide range of 5-year recurrence rates, varying between 5% and 30%[1].

Radiotherapy (RT) can be considered an alternative to surgery for small tumors, and is also recommended for unresectable cases or when postoperative pathologic risk factors are present. Reported 5-year local control (LC) rates are 95% in early-stage and 56% in advanced-stage cases[3]. However, RT is contraindicated in the presence of connective tissue diseases and genetic predispositions, and is generally not recommended for patients under 60 years of age. Chemotherapy shows limited efficacy in aggressive, unresectable, or metastatic cases, with response rates below 30%[1]. Mutations in the sonic hedgehog (SHH) signaling pathway are identified in approximately 85% of patients with BCC[4]. Current treatment strategies involve molecular targeted therapy with hedgehog inhibitors (HHIs) such as vismodegib and sonidegib[5,6]. In the ERIVANCE study, 104 patients received vismodegib treatment for a median duration of 13 months, during which objective response rates of 48% in locally advanced and 33% in metastatic cases were reported[5]. However, the duration of response ranged between 7.6 and 9.5 months and was associated with the development of resistance. Furthermore, 17% of patients discontinued therapy due to adverse effects such as muscle spasms, alopecia, dysgeusia, weight loss, fatigue, and nausea.

The addition of RT to vismodegib has been proposed to overcome treatment resistance and enhance therapeutic response. In an experimental study by Hehlgans et al., vismodegib demonstrated radiosensitizing properties[7]. Case-based studies have reported meaningful clinical benefits with either sequential or concurrent combinations of HHIs and RT[8-10]. Barker et al. administered conventional RT at doses of 66–70 gray (Gy) in 33–35 fractions (fx) concurrently with vismodegib following 12 weeks of systemic therapy, which had already achieved a 63% response rate in 24 patients[11]. After RT, the response rate rose to 83%, with reported 5-year locoregional control, progression-free survival (PFS), and overall survival (OS) rates of 91%, 78%, and 83%, respectively.

A meta-analysis evaluating studies that applied hypofractionated RT using modern techniques reported favorable cosmetic outcomes in 80% of patients, and 5-year LC rates of 85%[12]. In periorbital, case-based studies, stereotactic radiotherapy achieved complete responses while preserving visual functions[13,14]. The new-generation CyberKnife-M6 (CK-M6) system, which enables non-isocentric real-time image-guided treatment delivery, offers a more comfortable option due to shorter treatment durations, and allows for better sparing of organs at risk[15].

In this report, we present a case of locally advanced periorbital BCC treated with concurrent vismodegib and CK-M6–based hypofractionated stereotactic radiotherapy (hSRT), with a significant therapeutic response at the end of treatment.

Case Report

A 68-year-old male patient had undergone four surgical procedures at an external center for a lesion diagnosed as SCC that had been present on the nasal dorsum for 35 years. In a positron emission tomography/computed tomography (PET/CT) scan performed in December 2020, skin-subcutaneous activity on the nasal dorsum was reported (SUVmax = 5.81). A biopsy from the same month revealed a 0.8 cm SCC with positive surgical margins, and the patient was followed up without further intervention.

In November 2023, cranial magnetic resonance imaging (MRI) showed a 3 × 2.5 cm centrally located mass on the nasal dorsum, extending prominently to the medial canthus of both eyes, infiltrating both nasal bones and the left maxillary bone (Figure 1A). Multiple biopsies obtained from the lesion margins—left eyebrow (1.5 cm), left lateral nose (1.4 cm), right lateral nose (0.8 cm), and right eyebrow (1 cm)—revealed infiltrative BCC and bazoSCC foci.

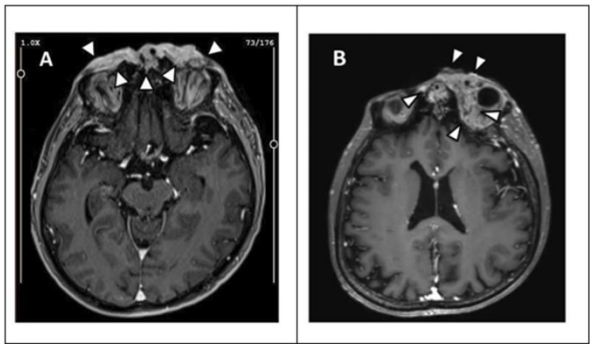

Figure 1: (A)
Axial contrast-enhanced T1-weighted cranial MRI from November 2023: " 3 × 2.5 cm multilobulated mass localized to the nasal dorsum, extending to the medial canthus of both eyes, infiltrating both nasal bones and the left maxillary bone, causing destruction and associated with widespread skin irregularity."
(B) *Axial contrast-enhanced T1-weighted cranial MRI from July 2024, prior to stereotactic radiotherapy.*

Concurrent vismodegib and RT

After receiving one cycle of docetaxel, cisplatin, 5-fluorouracil, and folinic acid, the patient was started on vismodegib 150 mg once daily, has been using it for 8 months. As a response was observed (Figure 1B), surgery was not considered, and the patient was referred to our department for RT.

At clinical examination in July 2024, a crusted lesion with mild discharge was noted at the midline of the nasal dorsum and glabella, measuring 5 cm in length, 3 cm in width, and 1 cm in height (Figure 2A). Neurological examination revealed reactive light reflexes bilaterally, preserved vision and ocular movements; however, the left eyelid was ptotic and could not fully open. The Karnofsky performance score was 80. The patient had no comorbidities, a 30 pack-year smoking history, and no significant family history. Informed consent was obtained for concurrent vismodegib and RT.

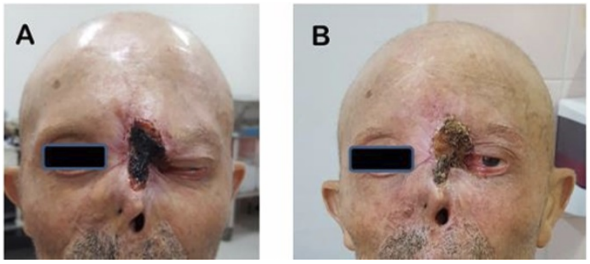

Figure 2: (A)
Pre-treatment: A 5 × 3 cm ulcerated lesion with mild discharge located at the midline of the nasal dorsum and glabella. ***(B)*** *Post-treatment: The lesion has regressed to 4 × 2.5 cm, and improvement in left eyelid ptosis is observed.*

The patient was immobilized with a non-invasive mask. Contrast-enhanced cranial MRI with 1 mm slice thickness was acquired and co-registered with the planning CT. A planning target volume (PTV) was created by adding a 10 mm margin to the gross tumor volume and adjusted according to anatomical landmarks. Taking organ at risk (OAR) tolerance into account, hSRT was planned at 4200 cGy in 14 fx. %95 of the PTV received 4200 cGy at the 87.7% isodose line. Fraction duration, coverage, homogeneity index, and conformity index were 19 minutes, 94.97%, 1.14, and 1.15, respectively. Biologically effective dose (BED) values were BED_{10} = 54.6 Gy for tumor tissue and BED_3 = 84 Gy for normal tissue. All OAR doses were within tolerance except for the maximum dose to the left eye. Dosimetric details are provided in Table I.

Table I. Dosimetric findings. GTV: Gross tumor volume, PTV: Planned target volume, fx= fraction.

Parameters	Volume max (cGy)	Mean (cGy)	Maximum (cGy)	Tolerance dose limits for 15 fx (Timmerman 2022)	
				Mean/ volume (cGy)	Maximum (cGy)
GTV (29.75 cm3)	4200	4405	4771		
PTV (62.85 cm3)	4200	4385	4789		
Right eye		799	3126	Mean ≤ 3300	3750
Left eye		2070	4233	Mean ≤ 3300	3750
Right lens		298	391		900
Left lens		509	637		900
Right optic nerve	884	959	1088	< 0.5 cm3 = 3900	4200
Left optic nerve	813	1400	2711	< 0.5 cm3 = 3900	4200
Chiasm	650	687	885	< 0.5 cm3 = 3900	4200
Brainstem	169	119	290	< 5 cm3 =4000	4400
Spinal cord	92	101	177	< 5 cm3 =3900	4200

Treatment was delivered on the CK-M6 system using real-time image guidance with imaging every 20–60 seconds (Figure 3). The total treatment duration was 19 days, with weekly physical examinations during RT. Acute Grade 1 ocular dryness and conjunctivitis, and Grade 2 erythema were observed and managed symptomatically. By the end of treatment, the lesion had regressed to 4 × 2.5 cm, left eye ptosis had resolved, and the visual field had improved (Figure 2B). In the follow-up cranial MRI performed in February 2025, four months after hSRT, complete response was achieved, with mild skin maceration observed at the treatment site (Figure 4).

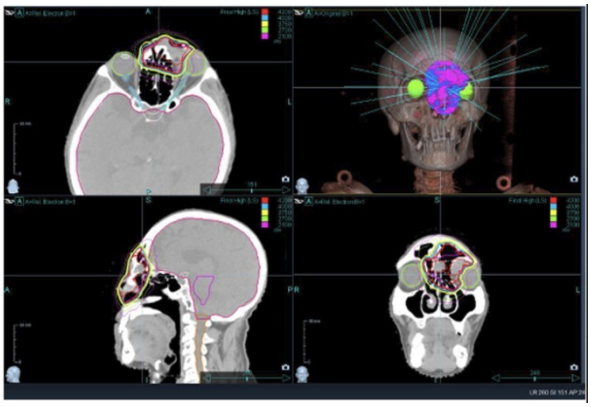

Figure 3:
Treatment plan (CyberKnife-M6)

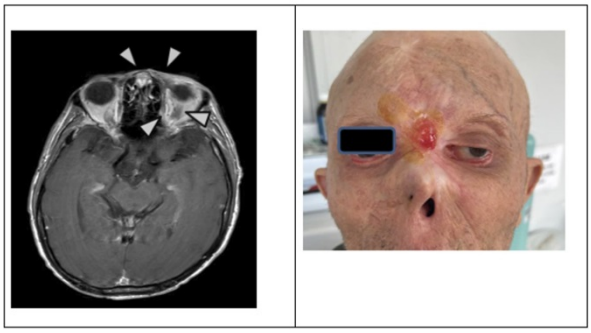

Figure 4:
At four months post-treatment, the left side of the figure shows cranial MRI demonstrating complete response, while the right side shows mild maceration in the glabellar region on clinical examination.

Discussion and Conclusion

This report presents the outcomes of concurrent hSRT and vismodegib treatment in a patient with locally advanced, recurrent BCC in the periorbital region who had previously undergone multiple surgeries.

Surgery is the gold standard in the treatment of BCC. In a randomized study comparing surgery and RT for tumors <4 cm, 4-year recurrence rates were 0.7% vs. 7.5%, respectively, with superior cosmetic outcomes reported for surgery (87% vs. 69%)[16]. On the other hand, a meta-analysis by Drucker et al. reported a local recurrence rate of 3.5% with RT, comparable to surgical outcomes[17]. Besides staging, tumor diameter, and histology, anatomical location has also been emphasized as a prognostic factor, with facial sites being considered moderate- to high-risk areas[18]. In periorbital tumors, due to medial canthus location, multiple recurrences, large tumor size, aggressive histology, and advanced age, orbital invasion occurs in 2–4% of cases, and approximately 5.5% of patients require orbital exenteration[19]. In cases requiring extensive surgery, incomplete resection, or medically inoperable locally advanced disease, RT serves as an effective alternative, allowing for preservation of organs and function.

RT for keratinocyte tumors can be delivered using various modalities such as electrons, orthovoltage/megavoltage photons, or brachytherapy[18]. In BCC, LC rates have been reported as 90% for primary tumors and 80% for recurrent cases[18]. To achieve 90% LC, it is recommended to deliver 45–60 Gy for tumors ≤2 cm and 64–70 Gy equivalent dose in 2 Gy fractions (EQD2) for tumors 2–3 cm in size[20]. Locke et al., in a cohort of 468 patients—mostly BCC—showed that tumors >1 cm had improved LC when treated with fraction doses >2 Gy and total doses >60 Gy[21]. Conversely, Silva et al. noted that when treating target volumes >5 cc, using fraction doses <4 Gy reduced late toxicity; however, LC decreased with every 5 Gy reduction in BED[22].

In locally advanced, metastatic, or nodal disease not suitable for surgery or RT, systemic therapy is recommended. Most sporadic BCCs harbor somatic mutations in the hedgehog pathway[4,23]. Randomized studies with HHIs like vismodegib and sonidegib have reported response rates of 60–70%, with adverse events seen in 25–32% of cases[5,6]. However, due to short response durations, combining systemic therapy with local modalities such as RT has been proposed to enhance efficacy[7,23]. In a preclinical study by Hehlgans et al., combined vismodegib and RT in cell cultures significantly reduced the expression of SHH target genes such as glioma-associated oncogene homologue (GLI1) and the apoptosis inhibitor survivin, suggesting a meaningful radiosensitizing effect[7].

Several case-based studies have explored the combination of vismodegib and RT. Gathings et al. reported outcomes in an 81-year-old patient with multiple BCCs and SCCs on the face, trunk, and extremities who received 10 weeks of vismodegib (150 mg/day), resulting in complete responses in most lesions. Partial responders were treated with 60 Gy intensity modulated RT or 40–48 Gy electron RT over seven weeks. At one-year follow-up, lesions showed complete response or marked regression, making surgical resection feasible[8]. In another study, Pollom et al. treated three lesions in two patients with recurrent periorbital BCC using conventional (50–66 Gy/33 fx) or hypofractionated (51 Gy/17 fx) RT concurrent with vismodegib. Clinical responses were observed with preserved visual function and PFS of 9–12 months[9]. Block et al. reported a 7 cm facial BCC involving skin, soft tissue, and buccal mucosa requiring extensive surgery. After four months of vismodegib (150 mg/day), >50% response was achieved, followed by concurrent 50 Gy/20 fx RT. The residual 1.5 cm lesion was locally resected and showed focal BCC with negative margins[10]. Another report described a patient with four separate lesions (left preauricular, trunk, lower extremity) treated with concurrent and adjuvant vismodegib for six months and 55 Gy/20 fx RT. All lesions achieved clinical complete response, one of which had pathological complete response, with no recurrence at 18 months[3].

Recent studies with larger cohorts further support the efficacy of combined treatment. Weissman et al. treated 12 patients who had responded to 2–3 months of HHI therapy with concurrent RT (55–60 Gy/25–30 fx). Complete response was observed in all patients, with a 40-month PFS rate of 89%. Grade 1–2 toxicity occurred in 75% of cases[23]. In another study, Barker et al. treated 24 patients with locally advanced BCC with 12 weeks of induction vismodegib, followed by concurrent conventional RT. No grade ≥3 toxicity was

reported. One-year locoregional control, and 1- and 5-year PFS rates were 91%, 100%, and 78%, respectively[11].

RT technique plays a crucial role in sparing surrounding tissues. Most studies employing concurrent vismodegib and RT have utilized conventional fractionation and conformal techniques[3,8,10,23]. Meta-analysis results suggest that hypofractionated regimens yield better cosmetic outcomes without compromising LC[12]. Commonly used regimens include 50 Gy/15 fx, 36.75 Gy/7 fx, and 35 Gy/5 fx, with BED_3 values ≤100 Gy recommended for optimal cosmetic outcomes. According to American Society for Radiation Oncology guidelines, BED_{10} values for curative-intent treatment are 70–93 Gy for conventional and 56–88 Gy for hypofractionated regimens, and 60–79 Gy vs. 56–70 Gy for postoperative RT, respectively[24]. Data on stereotactic RT are limited. In cases treated with Gamma Knife or CyberKnife, complete responses have been reported with single-fraction 15 Gy or 40 Gy in 10 fx, and a minimum of 18 Gy was suggested for durable LC in single-fraction settings[13,14].

In our case, to preserve visual function, treatment was planned in accordance with current guidelines using a 4200 cGy/14 fx hSRT regimen while accounting for OAR volume and maximum dose constraints[25]. BED equivalents were calculated as BED_{10} = 54.6 Gy and BED_3 = 84 Gy. No grade ≥3 toxicity was observed. In parallel with tumor regression, ptosis in the left eye improved and visual field expansion was noted. Follow-up cranial MRI at four months post-treatment confirmed complete response.

In conclusion, concurrent hSRT and vismodegib treatment using CK-M6 enabled effective therapy in a patient with locally advanced BCC without increasing toxicity. The impact of RT combined with HHIs on LC should be further validated through prospective, randomized trials.

Researcher Contribution Statement:
O.Ç: Case preparation, manuscript drafting, literature review, and final editing.
J.T: Assisted in case preparation and supported manuscript development.
F.G: Contributed by providing clinical data and technical details related to treatment delivery.
S.S: Initial identification of the case, major contribution to the discussion section, and critical revision of the manuscript.
Conflict of Interest Statement:
The authors of the article have no conflict of interest declarations.
Ethics Committee Approval Information:
Ethical approval was not required as this is a case report.

Refrences

1. Nagarajan P, Asgari MM, Green AC, Guhan SM, Arron ST, Proby CM, et al. Keratinocyte carcinomas: current concepts and future research priorities. Clin Cancer Res. 2019; 25(8):2379.
2. Aggarwal P, Knabel P, Fleischer AB, Jr. United States burden of melanoma and non-melanoma skin cancer from 1990 to 2019. J. Am Acad Dermatol. 2021, 85: 388–395.
3. Amini A, Freeman M, Melstrom L, Margolin KA, Parekh V, Abdulla FR, et al. Pathologic complete response with radiation and vismodegib in a patient with advanced basal cell carcinoma: A case report. Mol Clin Oncol. 2021, 14(3): 46.
4. Bonilla X, Parmentier L, King B, bezrukov F, Kaya G, Zoete V, et al. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. Nat Genet, 2016, 48(4): 398-406.
5. Sekulic A, Migden MR, Lewis K, Hainsworth JD, Solomon JA, Yoo S, et al. Pivotal ERIVANCE basal cell carcinoma (BCC) study: 12-month update of efficacy and safety of vismodegib in advanced BCC. J. Am Acad Dermatol. 2015; 72(6):1021.
6. Migden MR, Guminski A, Gutzmer R, Dirix L, Lewis KD, Combemale P, et al. Treatment with two different doses of sonidegib in patients with locally advanced or metastatic basal cell carcinoma (BOLT): a multicentre, randomised, double-blind phase 2 trial. Lancet Oncol. 2015;16(6):716-728.
7. Hehlgans S, Booms P, Gullulu O, Sader R, Rodel C, Balermpas P, et al. Radiation sensitization of basal cell and head and neck squamous cell carcinoma by the hedgehog pathway inhibitor vismodegib. Int J Mol Sci. 2018; 19:2485.
8. Gathings RM, Orscheln CS, Huang WW. Compassionate use of vismodegib and adjuvant radiotherapy in the treatment of multiple locally advanced and inoperable basal cell carcinomas and squamous cell carcinomas of the skin. J Am Acad Dermatol. 2014, 70(4):e88-e89.
9. Pollom EL, Bui TT, Chang AL, Colevas AD, Hara WY. Concurrent Vismodegib and Radiotherapy for Recurrent, Advanced Basal Cell Carcinoma. JAMA Dermatol. 2015, 151(9): 998-1001.
10. Block AM, Alite F, Diaz AZ, Borrowdale RW, Clarck JI, Choi M. Combination Trimodality Therapy Using Vismodegib for Basal Cell Carcinoma of the Face. Case Rep Oncol Med. 2015; 827608.
11. Barker CA, Dufault S, Arron ST, Lo AL, Algazi AP, Dunn LA, et al. Phase II, Single-Arm Trial of Induction and Concurrent Vismodegib With Curative-Intent Radiation Therapy for Locally Advanced, Unresectable Basal Cell Carcinoma. J Clin Oncol. 2024; 42(19):2327-2335.
12. Zaorsky NG, Lee CT, Zhang E, Keith SW, Galloway TJ. Hypofractionated radiation therapy for basal and squamous cell skin cancer: A meta-analysis. Radiother Oncol. 2017; 125:13-20.
13. Pontoriero A, Iatì G, Conti A, Minutoli F, Bottari A, Pergolizzi S, et al. Treatment of periocular basal cell carcinoma using an advanced stereotactic device. Anticancer Res. 2014, 34(2): 873-5.
14. Suzuki S, Yasukawa S, Tsuchimochi T, Inoue T. Gamma knife radiosurgery for basal cell carcinoma of the eyelid: report of a case. Acta Neurochir,2018, 160: 357-359.
15. Schüler E, Lo A, Chuang CF, Soltys SG, Pollom EL; Wang L. Clinical impact of the VOLO optimizer on treatment plan quality and clinical treatment efficiency for CyberKnife. J Appl Clin Med Phys, 2020, 21 (5): 38-47.
16. Avril MF, Auperin A, Margulis A, Gerbaulet A, Duvillard P, Benhamou E, et al. Basal cell carcinoma of the face: surgery or radiotherapy? Results of a randomized study. Br J Cancer. 1997, 76:100-106.
17. Drucker AM, Adam GP, Rofeberg V, Gazula A, Smith B, Moustafa F, et al. Treatments of primary basal cell carcinoma of the skin: A systematic review and network meta-analysis. Ann Inter Med. 2018, 169(7): 456-466.
18. Hennequin C, Rio E, Quero L, Clavere P. Radiation therapy of cutaneous cancers. Cancer Radiother. 2022, 26: 397-403.

19. Iuliano A, Strianese D, Uccello G, Diplomatico A, Teballi S, Bonavolonta G. Risk factors for orbital exenteration in periocular basal cell carcinoma. Am J Ophthalmol. 2012, 153(2): 238- 241.
20. van Hezewijk M, Creutzberg CL, Putter H, Chin A, Schneider I, Hoogeveen M, et al. Efficacy of a hypofractionated schedule in elecron beam radiotherapy for epithelial skin cancer: Analysis of 434 cases. Radiother Oncol. 2010, 95(2): 245-9.
21. Locke J, Karimpour S, Young G, Lockett MA, Perez CA. Radiotherapy for epithelial skin cancer. Int J Radiat Oncol Biol, Phys. 2001;51(3):748-755.
22. Silva JJ, Tsang RW, Panzarella T, Levin W, Wells W. Results of radiotherapy for epithelial skin cancer of the pinna: the Princess Margaret Hospital experience, 1982-1993. Int J Radiat Oncol Biol Phys. 2000; 47(2):451-459.
23. Weissman JP, Samlowski W, Meoz R. Hedgehog Inhibitor Induction with Addition of Concurrent Superficial Radiotherapy in Patients with Locally Advanced Basal Cell Carcinoma: A Case Series. Oncologist. 2021, 26(12):e2247-e2253.
24. Likhacheva A, Awan M, Barker CA, Bhatnagar A, Bradfield L, Brady MS, et al. Definitive and postoperative radiation therapy for basal and squamous cell cancers of the skin: Executive summary of an American Society for Radiation Oncology Clinical Practice Guideline. Pract Radiat Oncol. 2020, 10(1): 8-20.
25. Timmerman R. A story of hypofractionation and the Table on the Wall. Int J Radiat Oncol Biol Phys. 2022, 112(1): 4-21.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 331-339, 2025
DOI: https://doi.org/10.32708/uutfd.1653254

REVIEW

Independent Living Donor Advocate in Organ Transplantation: An Ethical and Application Guide

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ABSTRACT

Protection of Living Donor Rights was first legally recognised in Türkiye with the Regulation on Organ Transplantation Services published in 2022. This role aims to ensure that prospective living donors are evaluated in decision-making processes in line with voluntariness, informed consent, and ethical principles. This review study addresses the concept of Living Donor Rights Protector in terms of theoretical and legal aspects; the definition of this role, the appointment process, duties and responsibilities, and the qualifications that the role should have are examined in the light of the existing literature. As a result of the comprehensive literature review conducted by making use of international databases such as PubMed, Scopus, and Google Scholar, as well as Turkish legal and professional sources, it has been observed that academic studies in this field are limited and the need for guiding resources for practitioners continues. In this framework, the study aims to both contribute to the relevant literature and to provide a structural perspective for professionals who will take part in practice.

Keywords Living Donor Rights Protector. Living Donor Ethical Principles. Living Donor Rights. Psychosocial İnterview.

Canlı Verici Hakları Koruyucusu Rolüne Dair Tanımlayıcı Bir Derleme

ÖZET

Canlı Verici Hakları Koruyuculuğu, Türkiye'de ilk kez 2022 yılında yayımlanan Organ Nakli Hizmetleri Yönetmeliği ile yasal bir çerçeveye kavuşturulmuştur. Bu rolün amacı, canlı verici adaylarının karar verme süreçlerinde gönüllülük, bilgilendirilmiş onam ve etik ilkeler doğrultusunda değerlendirilmesini sağlamaktır. Bu derleme çalışması, Canlı Verici Hakları Koruyucusu kavramını teorik ve yasal yönleriyle ele almakta; söz konusu rolün tanımı, görevlendirme süreci, görev ve sorumlulukları ile sahip olması gereken nitelikler bağlamında mevcut literatür ışığında incelenmektedir. PubMed, Scopus ve Google Scholar gibi uluslararası veri tabanlarının yanı sıra, Türkçe yasal ve mesleki kaynaklardan yararlanılarak yürütülen kapsamlı literatür taraması sonucunda, bu alandaki akademik çalışmaların sınırlı olduğu, uygulayıcılar açısından ise rehber niteliğinde kaynaklara duyulan gereksinimin devam ettiği gözlemlenmiştir. Bu çerçevede, çalışma hem ilgili literatüre katkı sunmayı hem de uygulamada görev alacak profesyonellere yapısal bir bakış açısı kazandırmayı amaçlamaktadır.

Anahtar Kelimeler: Canlı Donör Etik İlkeleri. Canlı Verici Hakları Savunucusu. Donör Hakları. Psikososyal Görüşme.

Living donor transplantation refers to the transfer of a healthy organ or part thereof from a suitable donor to a patient with end-stage organ failure.[1] Although living donor transplantation is not typically regarded as the primary treatment option, its prevalence has increased due to a persistent shortage of organ donations. This shortage can be attributed to various factors, including a lack of public awareness, insufficient training for healthcare professionals on the subject, diverse religious beliefs concerning organ transplantation, and varying levels of development across different countries.[2-3] Given the persistent shortage of organ donations, it can be inferred that this scarcity has contributed to the increasing reliance on living donors in recent years.

The growing number of potential living donors has highlighted the necessity for advocates dedicated to the rights of these individuals, ensuring their protection throughout the donation process. The 2022 Regulation on Organ Transplantation Services introduced the role of the Living Donor Rights Protector, defined as *"personnel who work to protect and defend the rights of living donor candidates in*

Date Received: 7.March.2025
Date Accepted: 2.July.2025

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medical and ethical terms" (author's translation from the Turkish Regulation on Organ and Tissue Transplantation Services, 2022). From this point forward, this role will be referred to as "the Protector."[1]

The role of the Protector was initially brought to the attention of the relevant ministry by the Transplant Advisory Committee following the death of a living liver donor in New York in 2002. Concurrently, the committee emphasized the necessity for the presence of the protector role at each transplant centre to facilitate the implementation of ethical principles in organ transplantation.[4]

The Protector is required to comply with the regulations and guidelines set by the Ministry in all transplant centres. Its primary purpose is to defend the rights of living organ donors. To fulfil this mandate effectively, the Protector should function autonomously from the recipient team, maintaining a level of independence that allows for unimpeded access to external authorities in instances of undue pressure.[5] This principle is explicitly articulated in Article 16 of the Regulation on Organ Transplantation Services, published in 2022.[1]

Protectors play a crucial role in safeguarding living donor candidates (hereinafter referred to as "candidates"). While the qualifications for this position are outlined in the 2022 Regulation on Organ Transplantation Services, the specific roles and responsibilities remain vaguely defined. Appointed with the approval of the Ministry, Protectors are in search of effective resources to enhance their function. This study aims to provide clarity on the relevant personnel and serve as a guiding reference.

Material and Method

This article is a literature-based narrative review based on the author's professional experience and institutional observations during her time working in an organ transplant centre in Türkiye. The aim of the study is to present the current state of the Protector role in the Turkish context, compare it with international approaches and propose a context-specific job description and workflow model.

In the literature review conducted for this purpose, international scientific databases such as PubMed, Scopus and Google Scholar, as well as relevant legal regulations and professional publications in Türkiye were examined. The keywords 'independent living donor advocate', 'donor advocacy in transplantation', 'living donor ethics' in English, and 'donor rights in organ transplantation', 'independent donor advocacy' in Turkish were used in the search process. Selected sources included international ethical guidelines, recommendations of the American and European transplant societies, professional publications, case studies, health legislation and ethical commentaries. Explanatory translations of Turkish legal texts were made by the author when necessary. In particular, the Regulation on Organ and Tissue Transplantation Services dated 2022 has been one of the main legal references of the study.

In addition, the author's professional observations on the institutional functioning of various transplant centres during his duty as a Protector formed the basis for the evaluations in the article. These observations do not contain personal data, are aimed at understanding organisational approaches and interpret general trends in practices. No systematic data collection, recording or direct interviews with individuals were conducted. Therefore, this study does not qualify as experimental or observational research and does not require ethics committee approval as it does not include human participant data.

This narrative review aims to provide a conceptual basis on the functionality of the Protector role and its applicability in Türkiye by integrating literature and organisational context.

Selecting a Living Donor Rights Protector

In Article 16 of the Regulation on Organ Transplantation Services, the training standards of the personnel that can be assigned are determined as follows:[1]

a) Mental Health and Diseases Specialist
b) Psychologist
c) Social Service Specialist
d) Organ and tissue transplant coordinator with a master's degree and certification in organ and tissue transplant coordinatorship

Article 16 of the Regulation on Organ Transplantation Services states that the appointment and dismissal of the Protector requires the approval of the Ministry.[1]

The Importance of a Living Donor Rights Protector

The Protector may gather additional information about the candidates to conduct an independent evaluation. This may include details such as alcohol consumption, substance use, aspects of personal life, irregular habits, and past psychiatric history.

In addition to these responsibilities, the Protector plays a vital role in combating organ trafficking and transplant tourism. By rigorously assessing each candidate's voluntariness and ensuring no financial or coercive incentives are involved, the Protector helps safeguard against exploitation of vulnerable individuals. This function aligns with the Istanbul Declaration on Organ Trafficking and Transplant Tourism (2008), which calls for a comprehensive ban on all forms of commercial organ trade and emphasizes the protection of at-risk populations.[6]

Core Values of the Living Donor Rights Protector

The core values of the Protector include independence, transparency, teamwork, advocacy, knowledge, and confidentiality. The Protector is required to think independently from the recipient and assist the candidate in understanding the donation process. It is important to assess the prospective candidate's voluntariness, and understanding of the procedure. Additionally, effective communication with the transplant team is essential to relay relevant information. While independence is crucial for the smooth functioning of the process, the Protector must also possess a thorough understanding of the transplant procedure. Being well-informed and maintaining meticulous and confidential records of interviews are fundamental components of the Protector role.[7-8]

Personality Traits of the Living Donor Rights Protector

Expected Personality Traits of the Protector:

- The Protector should have strong relationship-building skills and be able to share the collected data with the transplant team competently, within the framework of confidentiality.
- The Protector must respect professional ethical standards.
- The Protector should be able to empathize and remain unbiased.
- During interviews, the Protector should remain neutral and not express emotions or opinions to the candidates.
- The Protector should be accessible to candidates.
- The Protector must understand that their role is not an individual task and should be adaptable to teamwork.
- Due to the interdisciplinary nature of the Protector role (involving areas such as Religion, Medical Processes, Psychology, etc.), personnel in this position should possess a basic knowledge in these relevant fields.[9]

Overview of the Living Donor Rights Protector's Duties

- The Protector who meets with candidates should provide information regarding the psychosocial interview process and structure the informed consent procedure accordingly. For candidates who complete the consent process, the evaluation phase should be explained in detail, emphasizing the significance of psychosocial interviews in identifying potential coercion or undue influence. Moreover, it should be clearly communicated to the candidates that these interviews are conducted with strict confidentiality.
- During the evaluation process, candidates and their families should be assessed, and the families' level of vulnerability should be carefully measured. Ethical principles related to the transplantation process must be explicitly emphasized, and it should be clearly conveyed that the primary purpose of these interviews is to safeguard the candidates autonomy in the decision-making process.
- Candidates should be explicitly informed that organ trafficking constitutes a criminal offense, and their comprehension of this legal and ethical issue should be carefully assessed. Furthermore, candidates must be informed that the procedure is intended to serve the recipient's medical benefit, that the donated organ cannot be reclaimed under any circumstances, and that if the medical evaluation results are unfavourable, they will be deemed unsuitable for donation.
- Prioritizing candidates well-being, information should be provided about alternatives to living transplantation.
- Throughout the pre- and post-operative processes, the Protector acts as an advocate for the rights of candidates. For candidates who consent to donation, the Protector conducts adjustment and motivational interviews if any difficulties arise during the process. Additionally, when necessary, the Protector refers candidates to appropriate mental health services to ensure they receive adequate psychological support.[10]
- To enhance process awareness among candidates who consent to donation, the Protector conducts additional interviews, observes post-operative life standards, and takes early preventive measures in order to minimize the risk of potential victimization. After discharge, the Protector maintains regular follow-up meetings with donors at specified intervals and evaluates their psychosocial adaptation to post-donation life.
- If the candidate's complaints cannot be addressed by the transplant programs, the Ministry should be contacted to report the issue.
- The Protector should conduct individual interviews, record the sessions, prepare consent forms, and ensure the confidentiality and archiving of these documents.[11]
- It is important to interview the spouses of candidates who have concerns regarding family dynamics to assess the voluntariness of the decision-making process. Their concerns should be addressed with empathy and understanding.[12]
- To prevent potential conflicts of interest, the Protector, who operates autonomously, is required to communicate with and inform the Ministry, as the overseeing authority, if he/she encounters any challenges with current or forthcoming activities.[1-5-13]

- It is important to acknowledge that the risks faced by the candidate extend beyond the potential for mortality. Candidate who experience the loss of their recipients may encounter significant feelings of guilt during the post-operative period. Moreover, such losses can disrupt familial dynamics and hinder the attainment of a satisfactory quality of life. Therefore, it is essential to prepare for these eventualities by continuing support meetings with candidates and seeking assistance from the Department of Psychiatry when necessary.
- Counselling services should be implemented to facilitate the candidate's adjustment to their new lifestyle, and motivational sessions should be organized as needed to support this transition.

Living Donor Rights Protector's Right to Refuse

The Protector should have the authority to reject a candidate for organ donation in specific situations. If the candidate is unwilling, feels pressured to make a decision, belongs to a group with high social vulnerability, or does not have sufficient information about the donation process, they should not provide consent to donate.[5] To identify these scenarios, psychiatrists should be consulted before proceeding with the psychiatry outpatient clinic, and their findings should be communicated in detail.

If a negative aspect is detected during evaluations and approval is denied, the transplant team is expected to respond sensitively.

Selection of Interpreters for Living Donor Candidates

Evaluating candidates can be challenging due to cultural and language differences. When such differences arise, it is essential to utilize a professional interpreter rather than relying on family members or friends of the candidate. Selecting an interpreter from within the candidate's family may compromise the candidate's autonomy and hinder open communication. Questions that are easily asked in one's own cultural context may cause discomfort for candidates from different backgrounds. Moreover, candidates may feel uneasy when sensitive questions are posed in the presence of third parties. Even when a professional interpreter is used, their personal biases or emotions may still affect the interview. Additionally, gender preferences should be taken into account, as some candidates may be reluctant to communicate with an interpreter of the opposite sex.[12-14]

Living Donor Rights Protector's Psychosocial Interview Criteria

Psychosocial assessments should be conducted by a licensed social worker, psychologist, or psychiatrist who is knowledgeable about the transplantation process. The transplantation centre should establish a cooling-off period, which may range from 3 to 6 months. If surgery cannot be performed within this timeframe, the psychosocial interview should be repeated.[10]

Candidtes are required to be individuals with decision-making capacity who can make their choices without external influences that would compromise the principle of voluntariness. In other words, the candidate must be independent throughout the donation process. Several criteria can be used to assess the candidate's ability to make free decisions regarding the following:[15-18]

a) Whether he/she understands the risks involved,
b) Whether he/she is under the influence of his/her household or anyone else,
c) Whether he/she has clearly expressed his/her intention to be a candidate,
d) Whether he/she understands the possible consequences of being a candidate, as well as the potential impacts on the recipient.

Living Donor Rights Protector's Psychosocial Interview Goals

The goals of a psychosocial interview initiated with the candidate can be listed as follows:[9-10-12-19]

- Evaluate the psychosocial risks of the candidate, including their psychiatric history and connection with their social environment.
- Ensure the candidate understands the risks, benefits, and outcomes of the donation process.
- Assess the candidate's decision-making capacity and ability to cope with stress before and after the decision.
- Determine if the candidate is making a decision free from feelings of guilt, external pressure, or impulsivity.
- Assess the candidate's potential life conditions after donation.
- Plan for any support interventions the candidate may require.
- Identify any gaps in pre-donation education and refer the donor to therapeutic intervention if necessary.
- Evaluate the relationship between the donor and the recipient.
- Plan for the candidate's potential post-operative care.
- Investigate any findings that may indicate the candidate is acting against their own best interests.

In summary, the psychosocial interviews conducted by the Protector assess financial, social, and medical risks, provide information about essential medical

processes, and offer motivational support when necessary.[9]

If a thorough assessment cannot be completed in a single interview, a follow-up appointment should be scheduled. If any situations arise during the assessments that require social service intervention, appropriate counselling should be given, and the relevant unit should be consulted.

In Case the Candidate Changes Her/His Mind

Candidates have the right to change their mind during the screening process. They may decide not to proceed as candidates, claim they were pressured into attending the screening, or express a lack of willingness to donate. If this happens, candidates can request that this information be kept confidential. To protect candidates' reputations and ensure their safety, recipients can be informed that candidates are not medically suitable matches.[14-19]

Challenges Facing the Living Donor Rights Protector

Candidates were originally selected from family members. However, over time, advancements in medicine and the development of immunosuppressant regimens led to changes in transplantation guidelines. This shift allowed for a broader selection of candidates, enabling non-relatives to donate organs as well. Efforts to increase organ donation, such as organized events and advertisements aimed at large audiences on social media, have connected recipients with candidates who were previously unknown to each other. These changes have complicated the evaluation of a candidate's motivations.[10]

The biases of the transplant team could obstruct the role of the Protector. Instead of serving as a deterrent, evaluating the situation from an independent ethical perspective will contribute to a healthier implementation of the transplant program for both the individual and the institution.

Candidates who are giving to child recipients can present challenges for the Protector. In these situations, where the internal pressure on the candidate is greater than external pressure, it is essential to allow the donor time to come to a resolution. Additionally, psychosocial interviews should be conducted regularly to monitor the candidate's situation.

In situations where the recipient is their own child, candidates, such as mothers, may choose not to be included in the transplant queue. Some may even prefer to donate the organ themselves, regardless of how full the transplant queue is.[11] This scenario presents the Protector, who conducts the interview, with several internal dilemmas. How should this case be assessed? Should mothers with more than one child be encouraged to wait in the transplant queue? Should the mother be accepted as a candidate? These questions are complex and challenging to navigate. Consequently, the content of the interview should focus on raising awareness.

The decision to become a candidate is challenging due to the rules imposed by the patriarchal structure within extended families and the pressure exerted by elders in positions of authority over younger members of the household. Consent is required from the spouses of married candidates. The pressure from an oppressive family can affect both the candidate and their spouse, leading to disruptions in family dynamics.[19] If these issues are identified, it is advisable to interview the spouses separately.

Religious belief is known to influence the decisions of many candidates profoundly. For some, the act of giving is motivated by the hope of divine reward, while others view their donations as a direct offering to the creator. It is vital to honour and respect the beliefs and choices of these candidates. However, alternative transplant methods, such as cadaveric organ transplantation, dialysis, and medical treatments, should be thoroughly explained during interviews conducted with the candidate.

Candidates who wish to become donors for recipients facing imminent death may experience significant pressure. There may not be sufficient time to thoroughly evaluate these candidates.

In response to this situation, teamwork should be employed, and the process should be completed by obtaining more realistic results through the data gathered by the transplant team and psychiatric specialists.[12]

Young people who live healthy lives and do not have any chronic illnesses may struggle to understand the importance of their follow-up appointments, which can lead them to skip these visits. It is important to provide counselling for individuals who do not attend their follow-ups to help them understand the value of regular check-ins.

The rising number of patients on waiting lists has led to a greater focus on cross-donor organ transplantation. Recipient candidates who are unable to receive an organ from a willing donor can find suitability for transplantation through donor exchanges, provided they have obtained approval from their families. It is crucial to evaluate the relationships between donors and recipients carefully throughout this process.

Changing the recipient can cause anxiety, stress, and pressure for the candidate. In order to alleviate such emotional pressures, motivational interviews may be needed even if no explicit problem is detected and the process is entirely based on voluntary consent. However, since motivational interviews tend to be time-consuming, the Protector may face time constraints.

In this context, enhancing the overall efficiency of organ transplantation processes and minimizing the associated psychosocial impacts necessitate the development and implementation of more effective and structured strategies for conducting motivational interviews.

In an inter-institutional cross-transplantation program, there is a high probability that a patient may meet a candidate who does not have a Protector at their originating institution. In such cases, the process will need to start over, and the psychosocial suitability of the candidate will be assessed. This situation can be particularly challenging for a candidate who has not previously encountered it at their institution. Additionally, due to variations in procedures among different institutions, there may be inconsistencies in the volunteer status of candidates. These differing perspectives can lead to misunderstandings between the patient and the organ transplant centre.

Finally, another potential issue in cross-transplantation programs arises from religious beliefs. The candidate may want to know to whom they are donating an organ. If the recipient is perceived as a sinner according to the candidate's values, or if the candidate believes the recipient might misuse the healthy organ after transplantation, this could lead the candidate to reconsider their decision.

Such situations highlight the need for additional psychosocial support during the decision-making process for candidates.[21]

Discussion and Conclusion

There are varying perspectives on the responsibilities of the Protector, but a common theme in the studies is the importance of independence. While the Protector is expected to make independent decisions, they are also expected to demonstrate strength in teamwork. Challenges such as communication difficulties and a lack of knowledge are often viewed as personal obstacles for those in this role. A survey conducted by Steel et al. (2012) highlighted a significant lack of information regarding the Protector. It pointed out that there are no standardized criteria for their appointment, and each medical centre typically operates under different guidelines. Although postgraduate education is generally preferred for this role, individuals with less formal education may also take on these responsibilities. Unfortunately, many people assigned as Protectors often lack adequate training and tend to learn on the job by improvising with guidance from members of the transplant team.[4]

Eguchi et al. (2017) described an institutional initiative developed at Nagasaki University Hospital in Japan, known as the Donor Advocacy Team (DAT). This system was established to manage risks associated with serious, permanent, or fatal complications that may occur in living organ, tissue, or cell donors. The DAT model outlines a detailed set of procedures to ensure rapid institutional response, provides psychological and physical support to donors and their families, and delivers timely and transparent public communication following adverse events. Additionally, it offers support to healthcare professionals involved in transplantation and aims to strengthen the overall reliability and ethical standards of the transplant system. Although the DAT is not directly equivalent to the Protector role, it reflects a structured institutional approach to donor advocacy and safety within a non-Western healthcare setting.[22]

Hays et al. (2016) argued that specific knowledge and skills are necessary for Protectors to be effective.[9] To address this, comprehensive training programs should be developed for Protectors, followed by independent examinations to ensure that only qualified individuals are placed in the field.

Rudow et al. (2015) emphasized that psychosocial interviews should be conducted by a social worker, psychologist, or psychiatrist due to their familiarity with both the transplant process and the interview format.[10]

Additionally, Rudow et al. (2015), Hays et al. (2016), and Steel et al. (2012) highlighted the importance of a uniform training and application guide, which would greatly benefit candidates.[4-9-10]

Didem (2022) highlighted the importance of the Social Worker's role in the field of organ transplantation, noting that the involvement of Mental Health Professionals in organ donation activities yields positive outcomes.[23] Fisher (2004) pointed out that while there is a substantial amount of literature focusing on the post-operative roles of Mental Health Professionals, there are relatively few studies addressing the psychosocial processes that occur before and during transplantation. Didem (2022) concluded her study by suggesting that sharing the knowledge and experiences of Mental Health Professionals in organ transplantation could contribute significantly to academic development in the field and provide support for new professionals entering this area.[19-23]

Moreover, Robbins (2014) noted that various interventions exist due to the absence of a comprehensive implementation guide for the Living Donor Rights Protector, emphasizing that the protector role within this context is quite profound.[14]

The protection of the candidate is not merely a clinical concern but an ethical obligation central to all transplant programs. The World Health Organization's Guiding Principles on Human Cell, Tissue and Organ Transplantation (2010) emphasize that donation must always be voluntary and informed, with adequate

measures in place to prevent harm and exploitation. These principles call for transparency, equitable access, and the establishment of national oversight systems to ensure donor safety and dignity.[24]

Based on the issues discussed above and the findings from institutional experience and literature, the following recommendations have been made to support the development and institutionalization of the Protector role.

I. Based on the principle *primum non nocere* (first, do no harm), which is believed to have been first used in the 19th century and is attributed to the *Corpus Hippocraticum*, and considering that the fundamental purpose of the Protector is to safeguard candidates, efforts should be made to participate in campaigns and organizations aimed at increasing cadaveric donations, drawing attention to the importance of organ donation.

II. The Protector should not consist of a single individual; instead, it should be carried out by a team that includes professionals from various fields (such as a Psychiatrist, Psychologist, Social Worker, Nurse, Lawyer, and Religious Official).

III. After completing the psychosocial interviews, the Protector should present the evaluation results in an interdisciplinary committee.

IV. At least one post-surgical consultation should be conducted, followed by additional consultations at the third and sixth months after the procedure.

V. In addition to official one-time training sessions, continuous education programs should be implemented to establish up-to-date approaches, and the information gathered from the field should be evaluated to ensure the development of the current practice.[4]

VI. Candidates may not have sufficient paid leave rights for the post-operative period. To encourage organ donation, economic resources should be allocated by government channels, and cooperation should be established with employers to support candidaates.

VII. To safeguard the rights of candidates, an independent oversight body should be established, separate from transplant centres, also ensuring the safety and ethical standards of both candidates and relevant healthcare personnel. This body should provide a platform where candidates can report the pressures and challenges they face, upload interview forms, and report any ethical violations encountered throughout the process. The independent auditing mechanism will strengthen the protection of donor rights, contributing to more transparent and reliable outcomes.

VIII. To facilitate face-to-face meetings between the candidate and the Protector without third

IX. parties, a room equipped with standards that ensure autonomy and, if possible, video recording should be designed. This will enable the Protector to provide services more comfortably and allow the candidate to share their thoughts openly without concealing anything they wish to disclose.

X. The academic publication of protectors should be supported, and their participation in developmental activities such as conferences and educational events should be encouraged.

XI. Although it is still an emerging role with certain shortcomings, academic publications such as field-based studies and case presentations will guide the role and enhance its professionalism.

Limitations

This study has several limitations. First, it is based on field observations made by the author while officially serving as a Protector at an organ transplantation centre in Türkiye. These observations did not involve the collection of any personal data or direct interviews with individuals. Therefore, they were not systematically recorded, qualitative data analysis was not conducted, and ethical committee approval was not required. This situation may have limited the depth of information regarding some practices.

Second, there is a risk of social desirability bias in the external presentation of institutional processes. Observations and indirect institutional contacts indicate that some centers tend to portray their practices in a more idealized manner externally. Additionally, it has been understood that some healthcare workers hold reservations about the Protector role and that there are institutional concerns that this role might have a deterrent effect on donors. Such perceptions may have limited the transparency of institutions regarding their roles and operations.

Third, the literature used in this study is predominantly Western-centred. The limited availability of academic sources specific to the Turkish context reduces the generalizability of the findings in terms of cultural and structural diversity. However, this limitation is balanced by the study's original contribution of providing a field-based perspective on the current status of the Protector role in Türkiye.

Finally, the field observations are based on the practices of a limited number of centres. Therefore, the direct applicability of the proposed model to all

institutions may be limited. In the future, multi-centre, participatory, and regionally sensitive qualitative studies conducted with ethical committee approval could provide more in-depth contributions to this field.

Acknowledgments

We extend our heartfelt gratitude to our esteemed professors, Prof. Dr. Halil Sağlam and Prof. Dr. Aylin Bican Demir, who contributed to the realization of this study and created a supportive environment for all field personnel to conduct academic work. We also express our appreciation to all faculty members and healthcare professionals at Uludağ University Organ and Tissue Transplantation Centre for sharing their experiences regarding transplantation processes and supporting our development. Special thanks to Ahmet Arslan for his assistance in organizing this study, and to Lecturer R. Ayşe Güllülü and Social Work Specialists Recep Tiribolulu and Sinan Kaya for sharing their knowledge and expertise in planning psychosocial interviews.

Researcher Contribution Statement:
Idea and design: B.Ş., A.B.D.; Data collection and processing: B.Ş.; Analysis and interpretation of data: B.Ş., A.B.B., E.K.; Writing of significant parts of the article: B.Ş., A.B.D., E.K.

Support and Acknowledgement Statement:
We extend our heartfelt gratitude to our esteemed professors, Prof. Dr. Halil Sağlam and Prof. Dr. Aylin Bican Demir, who contributed to the realization of this study and created a supportive environment for all field personnel to conduct academic work. We also express our appreciation to all faculty members and healthcare professionals at Uludağ University Organ and Tissue Transplantation Center for sharing their experiences regarding transplantation processes and supporting our development. Special thanks to Ahmet Arslan for his assistance in organizing this study, and to Lecturer R. Ayşe Güllülü and Social Work Specialists Recep Tiribolulu and Sinan Kaya for sharing their knowledge and expertise in planning psychosocial interviews.

Conflict of Interest Statement:
The authors and their household members have no memberships, consultancies, expert roles, or company partnerships that could present a potential conflict of interest related to this study.

Ethics Committee Approval Information:
Non-applicable

References

1. Resmî Gazete. Organ nakli hizmetleri yönetmeliği. Resmî Gazete. December 9, 2022. [in Turkish]. http://www.resmigazete.gov.tr/eskiler/2022/12/20221209-3.htm. Accessed January 25, 2025.
2. Tay M. The importance of tissue and organ transplantation in Turkey. *Sağlık Akademisyenleri Dergisi.* 2016;3(3):106-110. [in Turkish] doi:10.5455/sad.13-1477691139
3. Yücetin L, Keçecioğlu N, Ersoy FF. Türkiye'de organ bağışı ve nakline bir bakış. *Dial Transplant Burns*. 2003;14(2):115-118. http://www.dty.org.tr/dty_files/TR/2003-14-02-00/PDF/01150005.pdf. Accessed January 25, 2025.
4. Steel J, Dunlavy A, Friday M, et al. A national survey of independent living donor advocates: The need for practice guidelines. *Am J Transplant.* 2012;12(8):2141-2149. doi:10.1111/j.1600-6143.2012.04062.x
5. Hays RE, LaPointe Rudow D, Dew MA, et al. The independent living donor advocate: A guidance document from the American Society of Transplantation's Living Donor Community of Practice (AST LDCOP). *Am J Transplant.* 2015;15(2):518-525. doi:10.1111/ajt.13001
6. Participants in the International Summit on Transplant Tourism and Organ Trafficking Convened by The Transplantation Society and International Society of Nephrology. The Declaration of Istanbul on organ trafficking and transplant tourism. *Clin J Am Soc Nephrol.* 2008;3(5):1227-1231. doi:10.2215/CJN.03320708.
7. Johnson B. A practical guide: Role of the independent living donor advocate: Protect or advocate or is it both? In: Steel J, ed. *Living donor advocacy.* 311-326. New York, NY: Springer; 2014. doi:10.1007/978-1-4614-9143-9_22
8. Sites AK, Freeman JR, Harper MR, et al. A multidisciplinary program to educate and advocate for living donors. *Prog Transplant.* 2008;18(4):284-289. doi:10.1177/152692480801800411
9. Hays R, Matas AJ. Ethical review of the responsibilities of the patient advocate in living donor liver transplant. *Clin Liver Dis (Hoboken).* 2016;7(3):57-59. doi:10.1002/cld.533
10. Rudow DL, Swartz K, Phillips C, et al. The psychosocial and independent living donor advocate evaluation and post-surgery care of living donors. *J Clin Psychol Med Settings.* 2015;22(2-3):136-149. doi:10.1007/s10880-015-9426-7
11. Perakslis ED, Knechtle SJ, McCourt B, et al. Doing it right: Caring for and protecting patient information for US organ donors and transplant recipients. *Patterns (New York).* 2023;4(4):100734. doi:10.1016/j.patter.2023.100734
12. Olbrisch ME, Benedict SM, Haller DL, et al. Psychosocial assessment of living organ donors: Clinical and ethical considerations. *Prog Transplant.* 2001;11(1):40-49. doi:10.1177/152692480101100107
13. Aguayo C, Knapik S, McDonough P, et al. *Kidney independent living donor advocacy training documentation manual*. NATCO, National Kidney Foundation's Council of Professional Living Donor Advocates, UNOS Living Donor Committee. Published January 2012. https://www.natco1.org/assets/1/6/ILDATManual_January2012_FINAL.pdf. Accessed August 6, 2024.
14. Robbins KC. The independent living donor advocate: An essential role for living kidney donation. *Nephrol Nurs J.* 2014;41(6):569-573, 586; quiz 574.
15. Biller-Andorno N, Agich GJ, Doepkens K, et al. Who shall be allowed to give? Living organ donors and the concept of autonomy. *Theor Med Bioeth.* 2001;22(4):351-368. doi:10.1023/a:1011842211016
16. Albert PL. Clinical decision making and ethics in communications between donor families and recipients: How much should they know? *J Transplant Coord.* 1999;9(4):219-224. doi:10.7182/prtr.1.9.4.75r22071pv3l1p06
17. Beauchamp TL, Childress JF. *Principles of biomedical ethics.* 4th ed. New York, NY: Oxford University Press; 1994.
18. Abecassis M, Adams M, Adams P, et al. Consensus statement on the live organ donor. *JAMA.* 2000;284(22):2919-2926. doi:10.1001/jama.284.22.2919
19. Fisher MS. Psychosocial evaluation interview protocol for living related and living unrelated kidney donors. *Soc Work Health Care.* 2004;38(1):39-61. doi:10.1300/J010v38n01_03
20. Smith MD, Kappell DF, Province MA, et al. Living-related kidney donors: A multicenter study of donor education, socioeconomic adjustment, and rehabilitation. *Am J Kidney Dis.* 1986;8(4):223-233. doi:10.1016/s0272-6386(86)80030-0

21. Kranenburg LW, Visak T, Weimar W, et al. Starting a crossover kidney transplantation program in The Netherlands: Ethical and psychological considerations. *Transplantation.* 2004;78(2):194–197. doi:10.1097/01.TP.0000129259.02340.F7
22. Tong A, Chapman JR, Kee T, et al. Perspectives of transplant professionals on the values, ethics, and challenges of living kidney donor evaluation in Asia. *Transplantation.* 2015;99(7):1386-1395. doi: doi:10.1097/TP.0000000000000534
23. Kaçar Adam D. Roles and duties of social workers in the organ transplant process: An intervention example based on case. *Gümüşhane Univ J Health Sci.* 2022;11(4):1715-1727. doi:10.37989/gumussagbil.1094357
24. World Health Organization. *WHO guiding principles on human cell, tissue and organ transplantation* [Internet]. Geneva: World Health Organization; 2010. http://www.who.int/publications/i/item/WHO-HTP-EHT-CPR-2010.01. Accessed May 15, 2025.

Uludağ Üniversitesi Tıp Fakültesi Dergisi
Journal of Uludağ University Medical Faculty
51 (2) 341-352, 2025
DOI: https://doi.org/10.32708/uutfd.1638089

REVIEW

The Relationship Between Dermatologic Diseases and Dementia; A Review

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ABSTRACT

In some inflammatory skin diseases such as bullous pemphigoid, psoriasis and atopic dermatitis, inflammation is not limited to the skin but spreads systemically. Systemic inflammatory processes can activate proinflammatory cytokines, stimulate neurons and microglia in the brain, and trigger neuroinflammation. Ultimately, some skin diseases may affect the course of dementia and worsen the prognosis. Dementia, which progresses with a decrease in cognitive functions, can also trigger some skin diseases, accelerate their formation, and increase their severity. For example; bullous pemphigoid and dementia are two complex disease groups with multifaceted interactions. The strongest association among bullous pemphigoid comorbidities is with neurological diseases. The effect on cognitive impairment in patients with atopic dermatitis begins in infancy. The risk of cognitive impairment increases in the first year, especially in herpes zoster patients with trigeminal nerve involvement. In delusional parasitosis, which is a delusional disorder, patients first apply to dermatologists, and delusional parasitosis accompanies Lewy body dementia, one of the dementia types. This review aims to summarize dermatological diseases associated with dementia, such as bullous pemphigoid, psoriasis, atopic dermatitis, herpes zoster, crusted scabies, and delusional parasitosis, and to provide suggestions based on these relationships that may provide dermatologists, neurologists, and psychiatrists with a new perspective on the management of dermatological findings in patients with dementia.

Keywords: Dementia. Dermatitis. Atopic. Herpes Zoster. Pemphigoid. Bullous. Psoriasis.

Dermatolojik Hastaliklar ve Demans İlişkisi; Bir Gözden Geçirme

ÖZET

Büllöz pemfigoid, psoriasis, atopik dermatit gibi bazı inflamatuar deri hastalıklarında inflamasyon sadece deriye sınırlı kalmayıp sistemik yayılım gösterir. Sistemik inflamatuar süreç proinflamatuar sitokinleri aktive ederek, beyindeki nöronları ve mikrogliaları uyarabilir, nöroinflamasyonu tetikleyebilir. Sonuçta bazı deri hastalıkları demansın seyrini etkileyebilir, prognozu kötüleştirebilir. Bilişsel fonksiyonlarda azalma ile giden demans hastalığıda birtakım deri hastalıklarını tetikleyebilir, oluşumunu hızlandırabilir, şiddetini artırabilir. Örneğin; büllöz pemfigoid ve demans çok yönlü etkileşimi olan karmaşık iki hastalık grubudur. Büllöz pemfigoid komorbiditeleri arasında en güçlü ilişki nörolojik hastalıklarladır. Atopik dermatitli hastalarda bilişsel bozukluk üzerindeki etki bebeklik döneminden itibaren başlamaktadır. Özellikle trigeminal sinir tutulumu olan herpes zoster hastalarında ilk 1 yıl için bilişsel bozukluk riski artmaktadır. Sanrısal bir bozukluk olan delüzyonel parazitoz hastalığında ise hastalar ilk dermatologlara başvurmaktadır ve delüzyonel parazitoz demans tiplerinden Lewy cisimcikli demanslara eşlik etmektedir. Bu derleme demans ile ilişkili dermatolojik hastalıklardan büllöz pemfigoid, psoriasis, atopik dermatit, herpes zoster, krutlu uyuz, delüzyonel parazitoz hastalıklarını özetlemeyi ve bu ilişkilere dayanarak demans hastalarındaki dermatolojik bulguların yönetiminde dermatologlara, nörologlara ve psikiyatristlere yeni bir bakış açısı kazandırabilecek öneriler sunmayı amaçlamaktadır.

Anahtar Kelimeler: Demans. Dermatit. Atopik. Herpes Zoster. Pemfigoid. Büllöz. Psöriyazis.

Date Received: 18.February.2025
Date Accepted: 20.July.2025

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Dementia is an acquired disease characterized by a decline in cognition involving one or more cognitive domains (learning, memory, language, executive function, attention, perceptual-motor, social cognition). In recent years, the prevalence of dementia in aging populations has been increasing at an alarming rate. Approximately 50 million patients worldwide have dementia and dementias due to various causes are becoming more common in the elderly [1]. Alzheimer's disease is divided into different

groups including dementia with Lewy bodies, frontotemporal dementia, vascular dementia and mixed dementia. Although each of the dementia subtypes has its own pathological findings such as abnormal protein accumulation, the common etiology shared among all these conditions is cerebrovascular dysfunction that occurs at some point in the disease process[2].

Dementia is associated with many dermatologic diseases. Dementia leads to the triggering, progression and exacerbation of some skin diseases; on the other hand, skin diseases negatively affect the course and prognosis of dementia[3-6]. Chronic inflammation is involved in some forms of dementia such as Alzheimer's disease and dermatologic diseases such as psoriasis, atopic dermatitis and bullous pemphigoid[7,8]. Chronic inflammation in the central nervous system and periphery may participate in the pathogenesis of diseases both by permeability changes in the blood brain barrier and by causing microglial activation changes[9,10]. First-generation antihistamines including diphenhydramine, chlorpheniramine and hydroxyzine, which are frequently used in the treatment of pruritus in dermatology, easily cross the blood-brain barrier, leading to sedation, anticholinergic side effects and dementia in the elderly[11].

Several studies have shown the impact of dementia on dermatologic diseases and how these diseases may worsen the prognosis of dementia[1,4,5,12]. Although the relationship between Alzheimer's disease and skin diseases among dementia types has been summarized, there are not enough articles on dermatoses common in all dementia subtypes. This review aims to summarize the dermatological diseases associated with dementia such as bullous pemphigoid, psoriasis, atopic dermatitis, herpes zoster, crusted scabies, delusional parasitosis and to provide recommendations that may provide a new perspective to dermatologists, neurologists and psychiatrists in the management of dermatological findings in dementia patients based on these relationships.

This review was prepared by searching Turkish and English peer-reviewed original research articles and case reports published in Google Scholar and PubMed databases until March 2025. The key terms dementia and dermatologic diseases were used during the search. Unlike the reviews in this field, in this study, dermatologic diseases that are common in patients with dementia were included by examining their pathogenesis and multifaceted interactions separately.

The relationship between dementia and dermatological diseases is summarized in Table I.

Dementia and Bullous Pemphigoid

Like dementia, bullous pemphigoid is another disease with increasing prevalence in the elderly population. The relationship between bullous pemphigoid and dementia has been the subject of many studies. In this section, the multifaceted the interaction of dementia and BP in terms of pathogenesis, morbidity and mortality in recent studies will be is summarized.

Bullous pemphigoid (BP) is the most common vesiculobullous disease among subepidermal autoimmune bullous diseases. This disease typically affects the elderly and is characterized by bullous lesions on a localized or generalized erythematous background accompanied by pruritus[13]. It may show various clinical presentations. For example, only excoriation, prurigo-like lesions, urticarial and eczematous lesions may be observed without bullous lesions. BP is an autoimmune bullous disease associated with circulating autoantibodies against the basement membrane hemidesmosomal proteins BP180 (BP antigen 2 or type XVII collagen) and BP230 (BP antigen 1)[14].

Histopathology plays an important role in the diagnosis of BP. In biopsy material obtained from a newly formed intact bulla, subepidermal bullae dominated by eosinophils and neutrophils, accumulation of eosinophils and neutrophils in the dermis, and eosinophilic infiltration along the dermoepidermal junction may be observed. In the diagnosis of autoimmune bullous diseases, immunofluorescence, immunofluorescence in saline-separated skin, ELISA and immunoblotting methods are needed in addition to routine histopathologic examination[15].

First-line treatment of BP includes topical or systemic corticosteroids. Immunomodulatory (azathioprine, mycophenolate mofetil, methotrexate, dapsone) and anti-inflammatory (tetracycline, nicotinamide, dapsone) drugs are used in cases of resistant disease unresponsive to topical treatments or to minimize the adverse effects of chronic corticosteroid treatment[16-18]. Other treatment modalities include intravenous immunoglobulin, plasma exchange and immunadsorption. Since long-term administration of corticosteroids may cause serious side effects, recent studies support the use of new biological agents such as rituximab, omalizumab and dupilumab in treatment by utilizing their ability to selectively inhibit autoantibody formation and inflammatory cascade[19]. Studies examining the clinical and demographic characteristics of patients diagnosed with BP and the treatment process with comorbidities are important in understanding the etiopathogenesis of the disease, in the management of BP patients, in determining the appropriate treatment for the patient and in preventing comorbidities that may develop.

Table I. The Relationship Between Dementia and Dermatologic Diseases

	Clinical presentation	Association with dementia	Pathogenetic interaction	Precautions
Bullous Pemphigoid	-Chronic itching -Erythematous urticarial plaques with bullae+/-	-BP patients are at high risk of dementia -The incidence of BP is high in patients with dementia. -The association of BP disease and dementia increases mortality risk	Cross-reaction of neuronal BP1 antigens with cutaneous BP1 -Blood brain barrier disruption and neuroinflammation	-BP patients should be screened for cognitive impairment at regular intervals for the development of dementia
Atopic Dermatitis	-Chronic, pruritic, recurrent erythematous lesions	-Individuals with atopic dermatitis are at increased risk of mild cognitive impairment and dementia	-Chronic inflammation, blood brain barrier disruption, astrogliosis and neurodegeneration, Increased expression of -IL-6-associated interleukin amyloid-beta precursor protein	- Elderly patients with atopic dermatitis should be evaluated for possible cognitive impairment
Herpes zoster	-Erythematous vesicular rashes accompanied by severe pain throughout the dermatome	-Involvement of the ophthalmic branch of the trigeminal nerve increases the risk of dementia in the first year -Early antiviral treatment is associated with a lower risk of dementia -Herpes zoster vaccine reduces the risk of dementia	-Reactivation of herpes zoster virus triggers the formation of misfolded oligomers, increased neuroinflammation with accumulation of amyloid plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein	-To be vigilant for mild cognitive impairment in elderly and immunosuppressed patients that may develop in the first year -Initiation of antiviral therapy quickly and at effective doses
Psoriasis	- Sharply circumscribed, erythematous plaques with silvery squames	- A 1.2-fold increased risk of Alzheimer's disease in patients with psoriasis - Psoriasis patients receiving systemic treatment have a lower risk of Alzheimer's disease than healthy controls	- Systemic inflammation - Decreased permeability of the blood brain barrier and neuroinflammation Involvement of -IL-12/IL-23 signaling in the development of amyloid-induced neurodegeneration	- Psoriasis patients should be monitored for mild cognitive impairment
Scabiyez	-Itching that wakes you up at night and increases in hot environments - Excoriated papules and silion between the fingers, wrists, axillary folds, periumbilical area, penis in men, areolar areas in women	- Dementia patients at high risk of crusted scabies	-Different clinical presentation in the elderly -51% asymptomatic -Delays in diagnosis due to late recognition by the caregiver	-More than the nationalization - Treatment refusal due to stigmatization, - Community information should be provided to prevent outbreaks
Delusional parasitosis	-Tactile hallucinations such as itching or the feeling that parasites are crawling on you	-Especially accompanies dementia with Lewy bodies		-Symptomatic treatment and psychiatric consultation
Drugs			-Blocking the common p40 subunit of -IL-12 and IL-23 reduces the number of Aβ plaques in Alzheimer's disease -Acitretin increases metalloproteinase ADAM 10 gene expression, inhibits Aβ peptide production and prevents the formation of amyloid plaques	- Systemic anti-inflammatory drugs reduce neuronal inflammation and systemic inflammation and ultimately reduce the risk of Alzheimer's disease

BP:Bullous Pemphigoid
IL:Interleukin
Aβ: Amyloid-beta
ADAM-10: metalloproteinase domain-containing protein 10

Many comorbidities have been described in BP patients, but the strongest association has been found between BP and neurologic diseases[20,21] BP is significantly associated with neurologic disorders. Having a neurologic disease for more than twelve months increases the development of BP 3-fold, especially in patients with dementia[20]. In recent studies investigating the relationship between Alzheimer's dementia and bullous pemphigoid, the risk of dementia in female BP patients over 80 years of age was found to be 40%[22].

Degenerative neuronal diseases may activate autoimmune factors against neuronal BP1 which may cross-react with cutaneous BP1 involved in BP

pathogenesis[1,20]. The presence of shared proteins between the central nervous system and the skin recognizes antigens in the central nervous system. This results in neuroinflammation and impaired permeability of the blood-brain barrier[1]. A recent study in BP patients found that high serum antibody levels against BP1 correlated with the presence of neurologic diseases[10,23].

The risk of Alzheimer's disease in BP patients is approximately 39%[22]. Decreased cognitive abilities and a higher risk of cognitive impairment in BP patients have been shown in many studies[24,25]. Given that the onset of dementia in BP is relatively insidious, it is often overlooked in clinical practice. Since dementia in BP may start as mild cognitive impairment, patients should be evaluated periodically in terms of cognitive impairment.

Due to the diversity of clinical presentations in BP, patients may be followed for long periods without a diagnosis of BP. In individuals with neurological disease, skin symptoms such as itching, erosion or eczema may go unnoticed by caregivers or the duration of symptoms may be easily ignored. On the other hand, in patients with neurological diseases such as dementia, the constant urge to scratch and the associated involuntary scratching may lead to increased mechanical trauma and rapid progression of skin lesions, thus leading to early onset of bullous lesions. The wide clinical diversity may lead to delayed diagnosis and increased morbidity and mortality.

In a meta-analysis examining the relationship between dementia and mortality in patients with BP, the accompaniment of dementia to BP was found to be associated with poor prognosis and increased the mortality rate almost 2-fold[27]. Therefore, caution should be exercised in patients with BP and concomitant dementia. Mortality may be increased by the neurologic diseases themselves and may be further increased by the autonomic dysfunction of patients with bullous pemphigoid; therefore, caution should be exercised in patients with bullous pemphigoid and concurrent dementia and stroke. When following both disease groups, it should be kept in mind that these diseases may present with atypical clinical forms in order to make an early diagnosis and prevent complications that may develop. Dermatologists should periodically screen BP patients for cognitive impairment in terms of dementia development. At the same time, patients with dementia presenting to the clinic should be evaluated in terms of atypical clinical presentations with pruritus and excoriation without bullae.

Dementia and Atopic Dermatitis

Atopic dermatitis is one of the most common chronic diseases in the world and is an inflammatory skin disease characterized by chronic, pruritic, recurrent erythematous lesions[8]. Although it is common in childhood, it may affect individuals of all ages. It shows a bimodal course, especially in early childhood and middle-aged individuals. Its association with systemic diseases recently suggests that it may be a systemic disease[27].

Although it has a very complex pathogenesis, it develops from a background of chronic inflammation triggered by genetic, immunologic and environmental factors. The mechanisms responsible for pathogenesis are microbiome alteration following barrier dysfunction, triggering immunologic changes as a result of allergen exposure and neuroinflammation involved in the formation of pruritus[28,29]. Th2 cells and related cytokines are predominant in atopic dermatitis[30].

The main symptom in the formation of the disease is pruritus, especially when flare-ups are not adequately controlled, cutaneous inflammation increases through mechanical stimulation, resulting in exacerbation of pruritus and disruption of the integrity of the skin barrier[27]. A vicious cycle of itching and scratching then develops[31]. It is reported that pruritus usually worsens at night and leads to severe sleep disturbances[31]. However, psychological distress and health problems are also increased, including anxiety, depression and attention deficit/hyperactivity disorder. However, it is not yet clear whether neuropsychiatric comorbidities in AD are caused by persistent and intense pruritus, poor sleep and stigmatization, or are direct consequences of proinflammatory cytokines produced by AD inflammation and accelerated neuroinflammation[32,33]. However, this condition has a significant impact on the quality of life of both patients and their families[34,35].

The main step in treatment is patient education, moisturizer application and repair of the barrier layer of the skin. Subsequently, itching is controlled and inflammation is suppressed. Various drugs, including topical and systemic therapies, can be used in treatment. Topical corticosteroids, calcineurin inhibitors and moisturizers are included[36]. Another topical drug that has completed a phase 3 study is the phosphodiesterase 4 inhibitor chrysabold[36]. In systemic treatment, antihistamines and immunomodulatory drugs (systemic steroid, cyclosporine, methotrexate, azathioprine, mycophenolate mofetil) are used[36]. Dupilumab, nemolizumab, mepolizumab, omalizumab are biological agents used in treatment[36].

Chronic inflammation, itching-scratching vicious cycle and resulting sleep disorders, stress and decreased quality of life are expected in atopic dermatitis. In a review of sleep and neurocognitive impairment, it was shown that quality sleep in infancy is important for neurocognitive development, and quality sleep in childhood and adolescence is important for the development of memory, language, sustained attention, encoding and consolidation of long-term memories, processing speed and executive functions[37-41].

Recent studies show that cognitive dysfunction, including all-cause dementia and mild cognitive impairment, is higher in the elderly population with atopic dermatitis than in the normal population[42,43]. In addition, a meta-analysis by Gwak et al. found an increased risk of all-cause dementia, especially Alzheimer's disease, in individuals with atopic dermatitis[44]. This relationship has been linked to many inflammatory hypotheses. Proinflammatory cytokines and chemokines are involved in the pathogenesis of both atopic dermatitis and Alzheimer's dementia[45,46]. Proinflammatory cytokines cross the blood-brain barrier and modulate neuroimmune pathways[47]. In addition, eotaxin, one of the chemokines, crosses the blood-brain barrier, stimulates microglia and generates reactive oxygen products in cells in the central nervous system and produces a cytotoxic effect on neurons[9]. As a result, cutaneous inflammation in atopic dermatitis may be thought to contribute to neurodegeneration in Alzheimer's disease by causing astrogliosis, microglia activation and release of inflammatory factors[48]. Furthermore, interleukin (IL)-6, a cytokine, is involved in the expression of amyloid-beta (Aβ) precursor protein[49]. The presence of circulating proinflammatory mediators and immune alterations may accelerate Aβ accumulation by impairing the Aβ clearance function of microglia in the brain[50,51]. However, prospective studies are needed to clarify the relationship between dementia and atopic dermatitis.

The finding that atopic dermatitis may be a potential risk factor for cognitive dysfunction and all-cause dementia in middle-aged and older adults should be a warning especially for dermatologists and patients with atopic dermatitis should be evaluated for possible cognitive impairment. When planning treatment, the effects of sleep disorders on long-term neurodevelopmental development should be taken into account, and it should be aimed to improve sleep quality and quality of life in addition to improving skin symptoms.

Dementia and Scabies

Scabies is an ectoparasitosis caused by Sarcoptes scabiei var. hominis. It can affect individuals of any age regardless of gender or race. Transmission from person to person can be direct (sexually, through close contact) or indirect (using personal belongings of scabies patients, using the same common living space). The diagnosis is easily made in most cases with anamnesis and typical clinical findings. The main complaint of the patients is itching that wakes them up at night and increases in warm environments. The presence of other itchy individuals in the family is another important finding supporting the diagnosis of scabies. The most common areas affected and excoriated papules are between the fingers, wrists, extensor faces of the extremities, axillary folds, sides of the trunk, periumbilical area, buttocks, penis in men and areolar areas in women[52]. Observation of gray-brown 0.5-1 cm comma-shaped tunnels caused by the mite is helpful in the diagnosis. Typical history and clinical findings constitute the main step in the diagnosis. Dermoscopy can be used to help the diagnosis. On dermoscopy, a linear segment corresponding to the cilium and characteristic small black triangular structures corresponding to the pigmented anterior part of the sarcopt at its end are seen and called jet sign or delta sign[53]. The most common form of scabies is classical scabies, which has two different clinical forms, classical scabies and crusty scabies, as well as forms with atypical clinical course. Scabies with crusty scabies is the most contagious rare form which is mostly seen in immunocompromised individuals and in patients with neurologic diseases that reduce scratching and itching, where the diagnosis can be easily missed[52]. It should be taken into consideration that the clinic may be atypical in patients with dementia and may progress to crusted scabies, which is highly contagious. Not delaying the diagnostic process and organizing an effective treatment in diagnosed patients constitutes an important step in preventing a large number of cases and preventing outbreaks. The increasing incidence of scabies, especially in recent years, constitutes an important public health problem.

In a study of elderly residents of nursing homes who were diagnosed with scabies by general practitioners and nursing home staff based on clinical symptoms, it was found that the time from the onset of clinical symptoms to the diagnosis of scabies was long and these patients were examined many times by general practitioners and misdiagnosed with other skin conditions such as eczema and received different treatments[12]. As a result, many of them were not diagnosed with scabies until other cases emerged, leading to an increased risk of transmission not only among patients but also among their caregivers and families, which can lead to outbreaks.

The signs and symptoms of scabies in the elderly differ from the classical symptoms. In a study by Cassel et al. 51% of patients diagnosed with scabies in

elderly people staying in a nursing home were asymptomatic and the majority of patients had a diagnosis of dementia[54]. In patients with dementia, itching-scratching action and expressive abilities decrease, it becomes very difficult to detect clinically and this situation brings with it an increase in the number of mites and progression to crusty scabies. Especially in dementia patients who require close contact care, the increase in the number of mites in crusty scabies and the very high level of contagiousness are effective in the formation of epidemics. Scabies outbreaks in healthcare facilities cause serious morbidity for other patients and staff as well as administrative burden[55]. Family physicians play an important role in the early diagnosis of patients in this group. In addition, the diagnosis of scabies brings stigmatization in society[56]. For this reason, especially family physicians may have difficulty in making a diagnosis in patients who do not show classical symptoms and whose relatives are asymptomatic. Relatives may not accept the diagnosis, refuse treatment and conceal it. Public information is also needed to prevent outbreaks and reduce cases.

Dementia and Herpes Zoster

Varicella zoster virus (VZV), a DNA virus, belongs to the alpha herpesviridiae family and is a highly contagious virus known as human herpes virus-3 (HHV-3). Primary infection occurs as varicella and is transmitted by direct lesion contact or droplet transmission. After primary infection, VZV settles in the cranial nerves and dorsal root ganglia and remains latent. In cases of advanced age, immunocompromised, stress and trauma, the virus reactivates and produces a clinical picture of herpes zoster characterized by erythematous, vesicular eruptions accompanied by severe pain along the dermatome innervated by the ganglion[57]. Thoracic dermatomes are most commonly involved, followed by cranial (most commonly the trigeminal nerve), lumbar and cervical involvement. The diagnosis is usually based on clinical findings. The diagnosis can be confirmed by Tzanck smear. Confirmatory tests include direct fluorescent antigen test, viral culture, polymerase chain reaction (PCR) to detect VZV DNA in skin lesion and organ samples[58]. It is recommended to start treatment within the first 72 hours after the onset of vesicular rash. Systemic acyclovir, valacyclovir, famciclovir and brivudine antiviral agents are used in treatment[57].

There are views emphasizing that involvement of cranial nerves is associated with dementia. Hypotheses on the mechanisms by which VZV infection causes dementia suggest that reactivation of the virus triggers the formation of misfolded oligomers, increasing neuroinflammation through the accumulation of amyloid plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein. VZV may also directly infect astrocytes and stimulate intracellular amyloid production and the aggregation of amyloid fibrils in the extracellular environment[5,59-61]. Another hypothesis is that herpes zoster causes nerve damage by causing cerebral vasculopathy and ischemia, especially when it involves the cranial nerves[62]. The results of studies investigating the relationship between herpes zoster and dementia vary[6]. Involvement of the ophthalmic branch of the trigeminal nerve increases the risk of dementia in the first year[63,64]. Recent studies emphasize that early initiation of antiviral treatment reduces the risk of dementia[65,66]. There are also studies emphasizing that the herpes zoster vaccine approved by the FDA in 2006 reduces the risk of dementia[67].

The results of studies examining the relationship between herpes zoster and dementia contradict each other. This may vary depending on the duration of herpes zoster diagnosis, the prevalence of skin symptoms, the time of initiation of antiviral treatment, the presence of concomitant immunosuppressive conditions, the severity of dissemination and inflammation. It needs to be supported by more comprehensive studies. Dermatologists should be especially careful about mild cognitive impairment that may develop within the first year in elderly and immunosuppressed patients diagnosed with herpes zoster and should emphasize the importance of rapid and effective initiation of antiviral treatment.

Dementia and Psoriasis

Psoriasis is one of the most common chronic autoinflammatory skin diseases. Clinically, the disease is usually characterized by sharply circumscribed, erythematous plaques with silvery scales. It is influenced by ethnicity, genetics and environmental factors; therefore, its prevalence varies between countries, with a prevalence of approximately 2-3% in the population. The most commonly affected areas are the knee, elbow, sacral region and extensor surfaces of the extremities. Scalp, joints and nails are also affected[68,69]. Psoriasis shows four different clinical patterns according to the morphologic appearance of the lesions; plaque, guttate, pustular and erythrodermic forms. According to their localization, they can be classified as scalp psoriasis, palmoplantar psoriasis, inverse psoriasis, nail psoriasis, etc.[70].

The pathogenesis of the disease is not known exactly. Genetic and environmental factors are thought to play a role together. It is a complex, immune-mediated disease in which T lymphocytes, especially Th1 lymphocytes, predominate and dendritic cells and cytokines (interleukin [IL] 23, IL-17 and tumor

necrosis factor [TNF]) play a central role. Typical clinical findings such as squamous, induration and erythema are the result of hyperproliferation and abnormal differentiation of the epidermis, inflammatory cell infiltrates and vascular dilatation[71,72]. The first step in treatment is the use of moisturizers, keratolytics and anti-inflammatory preparations. Topical treatment includes corticosteroids, vitamin D analogs, retinoids and calcineurin inhibitors. Another treatment option is phototherapy protocols such as narrowband UVB and PUVA. Advances in the pathogenesis of psoriasis have led to the use of targeted biologic agents. Systemic treatment includes methotrexate, psoretin, cyclosporine, fumarate, apremilast, immunomodulatory drugs and biological agents. Tumor necrosis factor alpha inhibitors (adalimumab, infliximab, etanercept), IL-17 inhibitors (secukinumab, ixekizumab), IL-23 inhibitors (ustekinumab, guselkumab, risankizumab) are biological therapies approved by the FDA in the treatment of psoriasis[73]. Improved pathogenesis and related treatment protocols have improved the quality of life of patients and reduced the development of comorbidities due to suppression of systemic inflammation. In recent studies, psoriasis patients receiving systemic treatment were found to have a lower risk of Alzheimer's disease compared to healthy controls[74].

The fact that psoriasis is proven to be a systemic inflammatory disease in terms of its predominantly cutaneous involvement and accompanying comorbidities has brought its relationship with dementia, which is quite common, to the agenda and recent studies have been conducted for this purpose. In a meta-analysis conducted in 2022, retrospective cohort studies, most of which were conducted in Asia, the United States or Europe, were analyzed and a 1.2-fold increased risk of Alzheimer's disease was found in psoriasis patients[75]. The results of many studies in the literature support this relationship between psoriasis and Alzheimer's disease[76-78]. However, there are studies showing an inverse relationship in the literature[79,80]. Differences in study results may vary depending on the duration of the disease, treatment protocols and ethnicity of the patients. In order to fully elucidate the relationship between psoriasis and dementia, existing studies need to be supported by more comprehensive studies in which disease severity, socio-demographic data, treatment methods used, and the presence of comorbidities that trigger inflammation are also examined.

In the presence of systemic inflammation, proinflammatory cytokines are activated, neurons and microglia in the brain are stimulated, ultimately contributing to the pathogenesis of Alzheimer's disease[81]. As a result of the decrease in the permeability of the blood brain barrier with increasing age, activated cells and proinflammatory cytokines in the periphery cross the barrier and neuroinflammation is triggered in systemic inflammatory diseases[81]. Furthermore, IL-12/IL-23 signaling, which plays a key role in the pathogenesis of psoriasis, has been suggested to play a role in the development of amyloid-induced neurodegeneration[82]. Suppression of systemic inflammation is important for neurocognitive functions. The use of systemic therapy in psoriasis patients has been associated with a lower risk of dementia[83,84]. Therefore, it is important to apply early inflammation suppressive treatments in psoriasis patients in order to prevent dementia.

Psoriasis patients may have early deterioration in verbal memory, executive functions and attention in the long term[85]. In recent studies, patients with psoriatic arthritis were investigated for mild cognitive impairment and a higher risk was found compared to healthy controls[86]. Similarly, a large-scale study found that problems related to the cognitive domain concerned 20% of patients[87]. Gisondi et al. reported that the incidence of mild cognitive impairment was higher in patients with chronic plaque psoriasis than in controls, suggesting an increased risk of developing Alzheimer's disease in patients with psoriasis[85]. Mild cognitive impairment is higher in patients with chronic plaque psoriasis than in the general population[85]. The relationship between skin and brain has implications at multiple levels (immunologic, psychological and endocrinologic). Therefore, dermatologists should be aware of mild cognitive impairment that may develop early in patients with psoriasis.

Conditions Associated with Dementia and Dermatologic Symptoms

Dementia is a highly prevalent condition in society, affecting an estimated 50 million people worldwide[1]. Although 70% FH is the most common type of dementia, vascular dementia, dementia with Lewy bodies, Parkinson's disease dementia and frontotemporal dementia are among the types of dementia[3]. Dementia-related psychosis, including delusions and hallucinations, tends to increase as the duration and severity of the disease increases, contributing to increased hospitalization, cognitive decline and caregiver burden. Although a variety of symptoms can occur in all types of dementia, visual hallucinations are particularly common in dementias with Lewy bodies (dementia with Lewy bodies and Parkinson's disease dementia).

Ekbom syndrome is a delusional disorder in which the body is infested with parasites or insects, which may be accompanied by tactile hallucinations, such as

itching or the sensation of parasites crawling on it. It usually occurs in women in the fifth decade[88]. Typically, symptoms include peeling and scarring of the skin as a result of efforts to eradicate the parasites[88]. The most commonly affected areas are the scalp, face, mouth, eyes, arms, breasts and genitalia[88]. To prove the presence of parasites, patients may bring a matchbox or other container into the doctor's office in which they place various dust particles, skin fragments, fibers, etc. This behavior is often referred to as "matchbox sign" and "specimen sign". It may occur due to primary, secondary and organic causes and may also accompany neurodegenerative diseases, especially dementia with Lewy bodies.

The relationship between dementia and delusional parasitosis is mostly in the form of case reports. In a case report in 2020, a 72-year-old patient who presented with the complaint of insects in his head and a patient with no previous personal or familial history of neuropsychiatric disorder was presented. In the detailed questioning of the patient, it was found that 2 years ago, he had difficulty finding an address, money management was impaired and he had difficulty using a smartphone. Two years later, he started to complain of "ants" crawling on his head. He claimed that an "anthill" was covering his scalp and started scraping and picking it off. This belief persisted despite being denied by his family. He also began to hear his name being called or the doorbell ringing several times a day, as well as sometimes hearing sounds that others could not hear, such as the whistling and humming of insects. He also had sleep and movement disturbances at night and depressive symptoms. The patient was diagnosed with dementia with Lewy bodies as a result of investigations[89]. Similarly, an 89-year-old female patient initially complained of oral senestopathy, followed by a belief that she had filaria infection in her nose and eyes, and was diagnosed with dementia with Lewy bodies after the onset of parkinsonism and cognitive impairment[90]. These cases emphasize to dermatologists that resistant parasitosis delusions are one of the findings of dementia and cognitive functions of patients with parasitosis should be evaluated.

In the two cases reported by Taomato et al. visual and tactile delusions were present with cognitive impairment. In both cases, mild cognitive impairment accompanied the onset of delusions. In case 1, the patient used insecticide to get rid of insects. In case 2, the patient had a history of self-immolation to get rid of insects. In addition, he had taken actions such as using insecticides and consulting many dermatologists[91].

In a 2020 case report from Turkey, a patient with delusional parasitosis who was followed up for 2 years with a diagnosis of dementia was presented. The patient was diagnosed with dementia 2 years ago and the presence of skin picking with the belief that he was infected with insects 1 year later was evaluated as secondary delusional parasitosis[92].

Patients self-mutilate in different ways, such as erosions, peeling, cuts, obsessive cleaning and even burning to remove parasites[93]. Due to the prevalence of skin manifestations, these patients consult a dermatologist rather than a psychiatrist[93]. Since patients do not accept the view that their delusions are not real, there is a significant delay in presentation to a psychiatrist and initiation of psychopharmacologic treatment[94]. In the approach to patients with delusional parasitosis admitted to the dermatology unit, it should not be tried to make them believe that insects do not exist, and after symptomatic treatment is organized for the skin lesions, a safe bond should be established with the patient and the patient should be appropriately referred to psychiatry.

Drugs Used in Dementia and Dermatologic Diseases

H1-antihistamine drugs are classified as first or second-generation antihistamines. First generation H1-antihistamines have anticholinergic effects[95]. Anticholinergic drugs block acetylcholine, a neurotransmitter in the central or peripheral nervous system. First generation antihistamines cross the blood brain barrier and cause drowsiness, sedation, somnolence and fatigue[95]. Drugs with anticholinergic activity include oxybutynin (urinary incontinence drugs), antidepressants, antiparkinsonian drugs and antihistamines. Although many studies have argued that antihistamines used to suppress allergic diseases are associated with the risk of dementia, a large-scale study conducted in 2018 found that antidepressant, urological and antiparkinsonian drugs with anticholinergic effects were associated with an increased incidence of dementia up to 20 years after exposure[96]. First generation H1-antihistamine drugs should be avoided especially in elderly people with dementia due to their side effects.

In particular, a significant reduction in the incidence of Alzheimer's disease has been found with systemic drugs used in the treatment of psoriasis (acitretin, methotrexate, cyclosporine and biological agents[74]. Anti-IL-12/23 p40 monoclonal antibody is a biological agent targeting the IL-23/T helper 17 axis used in the treatment of psoriasis. In recent studies, blockade of the common p40 subunit of IL-12 and IL-23 reduced the number of Aβ plaques in Alzheimer's disease and resulted in improvement in cognitive impairment in a mouse model[82,97]. In addition, the use of systemic anti-inflammatory drugs reduces the incidence of cardiovascular diseases in psoriasis patients. In a mouse model of Alzheimer's disease,

acitretin has been reported to increase gene expression of the metalloproteinase ADAM 10 (metalloproteinase domain-containing protein 10), inhibit Aβ peptide production and prevent the formation of amyloid plaques[98-100]. Systemic anti-inflammatory drugs reduce neuronal inflammation and systemic inflammation and ultimately reduce the risk of Alzheimer's disease[74]. However, the effects and mechanisms of these drugs on inflammation need to be supported by studies. Prospective studies in which diseases and comorbidities are considered separately are needed.

Conclusion

The fact that the skin and nervous system originate from a common germ and that the relationship between the skin and the brain is multifaceted with endocrinologic and immunologic interactions may explain why dermatologists are the first health professionals to encounter psychiatric and neurologic diseases. Many skin symptoms can be observed in patients with dementia. These symptoms may include nonspecific findings such as pruritus, xerosis, pressure sores, oral hygiene disorders, which increase with aging, as well as association with common dermatologic diseases such as psoriasis, BP, and atopic dermatitis associated with chronic inflammation and autoimmunity. Cognitive function assessments by dermatologists can help diagnose dementia at the level of mild cognitive impairment and treatment of skin diseases associated with systemic inflammation may prevent the development of dementia.

This review summarizes dermatologic manifestations and diseases in patients with dementia and provides guidance to dermatologists on the care of these patients. It also emphasizes the importance of a multidisciplinary approach to the management of dementia and suggests that skin diseases should be considered as part of this approach.

Researcher Contribution Statement:
Idea and design: Ç.H.;Data collection and processing:Ç.H., H.B.Y.; Analysis and interpretation of data: H.B.Y.; Writing of significant parts of the article: H.B.Y.
Ethics Committee Approval Information:
Ethics committee approval is not required.

References

1. Zhang H, Zhang D, Tang K, Sun Q. The Relationship Between Alzheimer's Disease and Skin Diseases: A Review. Clin Cosmet Investig Dermatol. October 2021;Volume 14:1551-60.
2. Raz L, Knoefel J, Bhaskar K. The neuropathology and cerebrovascular mechanisms of dementia. Journal of Cerebral Blood Flow & Metabolism. January 15, 2016;36(1):172-86.
3. Langan SM, Smeeth L, Hubbard R, Fleming KM, Smith CJP, West J. Bullous pemphigoid and pemphigus vulgaris--incidence and mortality in the UK: population based cohort study. BMJ. July 09, 2008;337(jul09 1):a180-a180.
4. Woo YR, Cho M, Han K Do, Cho SH, Lee JH. Increased risk of dementia in patients with atopic dermatitis: A nationwide population-based cohort study. Acta Derm Venereol. April 27, 2023;103:adv4557.
5. Li X, Sundquist J, Zöller B, Sundquist K. Dementia and Alzheimer's disease risks in patients with autoimmune disorders. Geriatr Gerontol Int. September 25, 2018;18(9):1350-5.
6. Weinmann S, Rawlings A, Koppolu P, Rosales AG, Prado YK, Schmidt MA. Herpes zoster diagnosis and treatment in relation to incident dementia: A population-based retrospective matched cohort study. PLoS One [Internet]. 01 January 2024 [cited 29 February 2024];19(1). Available from: https://pubmed.ncbi.nlm.nih.gov/38271405/
7. Künzli K, Favre B, Chofflon M, Borradori L. One gene but different proteins and diseases: the complexity of dystonin and bullous pemphigoid antigen 1. Exp Dermatol. 04 Jan 2016;25(1):10-6.
8. Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. Nat Rev Dis Primers. June 21, 2018;4(1):1.
9. Parajuli B, Horiuchi H, Mizuno T, Takeuchi H, Suzumura A. CCL11 enhances excitotoxic neuronal death by producing reactive oxygen species in microglia. Glia. December 2015;63(12):2274-84.
10. Messingham KN, Miller AD, Narayanan NS, Connell SJ, Fairley JA. Demographics and autoantibody profiles of pemphigoid patients with underlying neurologic diseases. journal of ınvestigative dermatology. Eylül 2019;139(9):1860-1866.e1.
11. Adelsberg BR. Sedation and performance issues in the treatment of allergic conditions. Arch Intern Med. March 10, 1997;157(5):494-500.
12. Hewıtt KA, Nalabanda A, Cassell JA. Scabies outbreaks in residential care homes: factors associated with late recognition, burden and impact. A mixed methods study in England. Epidemiol Infect. 08 May 2015;143(7):1542-51.
13. Lo Schiavo A, Ruocco E, Brancaccio G, Caccavale S, Ruocco V, Wolf R. Bullous pemphigoid: Etiology, pathogenesis, and inducing factors: Facts and controversies. Clin Dermatol. July 2013;31(4):391-9.
14. Yazıcı S, Bülbül Başkan E, Tunalı Ş, Aydoğan K, Sarıcaoğlu H. Retrospective analysis of the forty-six patients with bullous pemphigoid followed-up in our clinic. TURKDERM. September 23, 2016;50(3).
15. Erkin G. Histopathologic diagnosis of autoimmune bullous diseases. 2011;
16. Schmidt E, Sticherling M, Sárdy M, Eming R, Goebeler M, Hertl M, et al. S2k guidelines for the treatment of pemphigus vulgaris/foliaceus and bullous pemphigoid: 2019 update. JDDG: Journal der Deutschen Dermatologischen Gesellschaft. May 15, 2020;18(5):516-26.
17. Feliciani C, Joly P, Jonkman MF, Zambruno G, Zillikens D, Ioannides D, et al. Management of bullous pemphigoid: the European Dermatology Forum consensus in collaboration with the European Academy of Dermatology and Venereology. British Journal of Dermatology. April 2015;172(4):867-77.
18. Daniel BS, Borradori L, Hall RP, Murrell DF. Evidence-based management of bullous pemphigoid. Dermatol Clin. October 2011;29(4):613-20.

19. Cao P, Xu W, Zhang L. Rituximab, Omalizumab, and dupilumab treatment outcomes in bullous pemphigoid: A systematic review. Front Immunol. June 13, 2022;13.
20. Langan SM, Groves RW, West J. The Relationship between neurological disease and bullous pemphigoid: A population-based case-control study. Journal of Investigative Dermatology. March 2011;131(3):631-6.
21. Yu Phuan C, Yew Y, Tey H. Bullous pemphigoid and antecedent neurological diseases: An association with dementia. Indian J Dermatol Venereol Leprol. 2017;83(4):457.
22. Xie Z, Gao Y, Tian L, Jiang Y, Zhang H, Su Y. Pemphigus and pemphigoid are associated with Alzheimer's disease in older adults: evidence from the US Nationwide inpatient sample 2016-2018. BMC Geriatr. December 19, 2023;23(1):872.
23. Gambichler T, Segert H, Höxtermann S, Schmitz L, Altmeyer P, Teegen B. Neurological disorders in patients with bullous pemphigoid: clinical and experimental investigations. Journal of the European Academy of Dermatology and Venereology. September 04, 2015;29(9):1758-62.
24. Langa KM, Levine DA. The diagnosis and management of mild cognitive ımpairment. JAMA. December 17, 2014;312(23):2551.
25. Zhao W, Wang Y, Mao X, Payne AS, Feng S, Li W, et al. Detection of underlying dementia in bullous pemphigoid patients using cognitive evaluation tests: a multicenter case-control study. Ann Transl Med. November 2020;8(21):1397-1397.
26. Chen X, Zhang Y, Luo Z, Wu Y, Niu T, Zheng J, et al. Prognostic factors for mortality in bullous pemphigoid: A systematic review and meta-analysis. PLoS One. April 15, 2022;17(4):e0264705.
27. Küçük ÖS. Epidemiology and pathogenesis in atopic dermatitis. Turkey Clinics Journal of Dermatology. 2020;1-7.
28. Otsuka A, Nomura T, Rerknimitr P, Seidel JA, Honda T, Kabashima K. The interplay between genetic and environmental factors in the pathogenesis of atopic dermatitis. Immunol Rev. July 28, 2017;278(1):246-62.
29. Sidbury R, Khorsand K. Evolving concepts in atopic dermatitis. Curr Allergy Asthma Rep. July 29, 2017;17(7):42.
30. Kirchhof MG, Landells I, Lynde CW, Gooderham MJ, Hong C ho. Approach to the assessment and management of adult patients with atopic dermatitis: a consensus document. section ı: pathophysiology of atopic dermatitis and ımplications for systemic therapy. J Cutan Med Surg. November 15, 2018;22(1_suppl):6S-9S.
31. Schut C, Mochizuki H, Grossman SK, Lin AC, Conklin CJ, Mohamed FB, et al. Brain processing of contagious ıtch in patients with atopic dermatitis. Front Psychol. July 25, 2017;8.
32. Wan J, Wang S, Shin DB, Syed MN, Abuabara K, Lemeshow AR, et al. Neuropsychiatric disorders in adults with atopic dermatitis: A population-based cohort study. Journal of the European Academy of Dermatology and Venereology. March 26, 2024;38(3):543-8.
33. Farzanfar D, Dowlati Y, French LE, Lowes MA, Alavi A. Inflammation: A contributor to depressive comorbidity in ınflammatory skin disease. Skin Pharmacol Physiol. 2018;31(5):246-51.
34. Christensen RE, Jafferany M. Psychiatric and psychologic aspects of chronic skin diseases. Clin Dermatol. January 2023;41(1):75-81.
35. Kelly KA, Balogh EA, Kaplan SG, Feldman SR. Skin disease in children: effects on quality of life, stigmatization, bullying, and suicide risk in pediatric acne, atopic dermatitis, and psoriasis patients. Children. November 16, 2021;8(11):1057.
36. Alper S, Memet B. Systemic treatment in atopic dermatitis. Journal of Dermatology in Turkish Clinics. 2020;51-5.
37. Joseph D, Chong NW, Shanks ME, Rosato E, Taub NA, Petersen SA, et al. Getting rhythm: how do babies do it? Arch Dis Child Fetal Neonatal Ed. Jan 2015;100(1):F50-4.
38. Cameron S, Donnelly A, Broderick C, Arichi T, Bartsch U, Dazzan P, et al. Mind and skin: Exploring the links between inflammation, sleep disturbance and neurocognitive function in patients with atopic dermatitis. Allergy. January 19, 2024;79(1):26-36.
39. Svingos A, Greif S, Bailey B, Heaton S. The relationship between sleep and cognition in children referred for neuropsychological evaluation: a latent modeling approach. Children (Basel). February 28, 2018;5(3).
40. Astill RG, Van der Heijden KB, Van IJzendoorn MH, Van Someren EJW. Sleep, cognition, and behavioral problems in school-age children: A century of research meta-analyzed. Psychol Bull. 2012;138(6):1109-38.
41. Prehn-Kristensen A, Göder R. Schlaf und Kognition bei Kindern und Jugendlichen. Z Kinder Jugendpsychiatr Psychother. 01 Sep 2018;46(5):405-22.
42. Silverberg JI, Lei D, Yousaf M, Janmohamed SR, Vakharia PP, Chopra R, et al. Association of atopic dermatitis severity with cognitive function in adults. J Am Acad Dermatol. November 2020;83(5):1349-59.
43. Jackson-Cowan L, Silverberg JI. Longitudinal course of cognitive impairment in patients with atopic dermatitis. Arch Dermatol Res. January 28, 2023;315(6):1553-60.
44. Gwak Y, Kim S, Woo C, Shin K, Son E, Kim J, et. All. Assosiation between atopic dermatitis and dementia: Evidence from systematic review, metaanalyse and mendelian randomization. Acta-Dermato-Venerology January 2025; 3:105:adv41321
45. Lyubchenko T, Collins HK, Goleva E, Leung DYM. Skin tape sampling technique ıdentifies proinflammatory cytokines in atopic dermatitis skin. Ann Allergy Asthma Immunol [Internet]. 01 January 2021 [published 27 February 2024];126(1):46. Available from: /pmc/articles/PMC8782053/
46. Xiong Z, Thangavel R, Kempuraj D, Yang E, Zaheer S, Zaheer A. Alzheimer's disease: evidence for the expression of ınterleukin-33 and ıts receptor ST2 in the brain. Journal of Alzheimer's Disease. March 31, 2014;40(2):297-308.
47. Schut C, Mochizuki H, Grossman SK, Lin AC, Conklin CJ, Mohamed FB, et al. Brain processing of contagious ıtch in patients with atopic dermatitis. Front Psychol [Internet]. July 25, 2017 [cited February 27, 2024];8(JUL). Available from: /pmc/articles/PMC5524890/
48. Zhang ZG, Li Y, Ng CT, Song YQ. Inflammation in Alzheimer's disease and molecular genetics: Recent update. Arch Immunol Ther Exp (Warsz) [Internet]. October 24, 2015 [cited February 27, 2024];63(5):333-44. Available from: https://pubmed.ncbi.nlm.nih.gov/26232392/
49. Griffin WST, Barger SW. Neuroinflammatory cytokines-the common thread in Alzheimer's pathogenesis. US Neurol. 2010;6(2):19-27.
50. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer's disease. Nat Rev Neurosci. June 20, 2015;16(6):358-72.
51. Heneka MT, Carson MJ, Khoury J El, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol. April 2015;14(4):388-405.
52. Oba MÇ, Cesur SK, Tüzün Y. Scabies. Turkey Clinics Journal of Dermatology. 2021;13-8.
53. Gur TF. Basic Dermatology. Gürel MS, editor. Istanbul: Istanbul Medical Bookstore; 2023. 294-302 pp.

54. Cassell JA, Middleton J, Nalabanda A, Lanza S, Head MG, Bostock J, et al. Scabies outbreaks in ten care homes for elderly people: a prospective study of clinical features, epidemiology, and treatment outcomes. Lancet Infect Dis [Internet]. 01 August 2018 [a.venue 28 February 2024];18(8):894. Available from: /pmc/articles/PMC6060176/

55. Ozdamar M, Turkoglu S. A nosocomial scabies outbreak originating from immunocompromised transplant patients in Turkey: Upholstery as a possible cause. Transpl Infect Dis [Internet]. 01 August 2020 [a.yer 29 February 2024];22(4). Available from: https://pubmed.ncbi.nlm.nih.gov/32274845/

56. Mounsey KE, Murray HC, King M, Oprescu F. Retrospective analysis of institutional scabies outbreaks from 1984 to 2013: lessons learned and moving forward. Epidemiol Infect [Internet]. August 01, 2016 [published February 29, 2024];144(11):2462. Available from: /pmc/articles/PMC9150521/

57. Polat M, Karapinar T. Varicella Zoster Virus Infections. Turkey Clinics Journal of Dermatology. 2019;18-27.

58. Bader MS. Herpes Zoster: Diagnostic, Therapeutic, and Preventive Approaches. Postgrad Med. September 13, 2013;125(5):78-91.

59. Bronzuoli MR, Iacomino A, Steardo L, Scuderi C. Targeting neuroinflammation in Alzheimer's disease. J Inflamm Res [Internet]. November 03, 2016 [accessed March 01, 2024];9:199. Available from: /pmc/articles/PMC5098782/

60. Kagan BL, Jang H, Capone R, Teran Arce F, Ramachandran S, Lal R, et al. Antimicrobial properties of amyloid peptides. Mol Pharm [Internet]. April 04, 2012 [published March 01, 2024];9(4):708. Available from: /pmc/articles/PMC3297685/

61. Bubak AN, Como CN, Coughlan CM, Johnson NR, Hassell JE, Mescher T, et al. Varicella-Zoster Virus ınfection of primary human spinal astrocytes produces ıntracellular amylin, amyloid-β, and an amyloidogenic extracellular environment. J Infect Dis. March 16, 2020;221(7):1088-97.

62. Forbes HJ, Williamson E, Benjamin L, Breuer J, Brown MM, Langan SM, et al. Association of herpesviruses and stroke: Systematic review and meta-analysis. PLoS One. November 21, 2018;13(11):e0206163.

63. Schmidt SAJ, Veres K, Sørensen HT, Obel N, Henderson VW. Incident herpes zoster and risk of dementia. Neurology. August 16, 2022;99(7).

64. Warren-Gash C, Forbes HJ, Williamson E, Breuer J, Hayward AC, Mavrodaris A, et al. Human herpesvirus infections and dementia or mild cognitive impairment: a systematic review and meta-analysis. Sci Rep. March 18, 2019;9(1):4743.

65. Chen VCH, Wu SI, Huang KY, Yang YH, Kuo TY, Liang HY, et al. herpes zoster and dementia. J Clin Psychiatry. February 21, 2018;79(1):16m11312.

66. Bae S, Yun SC, Kim MC, Yoon W, Lim JS, Lee SO, et al. Association of herpes zoster with dementia and effect of antiviral therapy on dementia: a population-based cohort study. Eur Arch Psychiatry Clin Neurosci [Internet]. August 01, 2021 [published March 01, 2024];271(5):987-97. Available from: https://pubmed.ncbi.nlm.nih.gov/32613564/

67. Lehrer S, Rheınsteın PH. Herpes zoster vaccination reduces risk of dementia. In Vivo (Brooklyn). 2021;35(6):3271-5.

68. Gülekon A., Tüzün Y., Gürer S., Oğuz A. Psoriasis and similar dermatoses. Gülekon A., editor. C. 3rd Edition. Nobel Medical Bookstores; 2008. 745-764 s.

69. Langley RG, Ellis CN. Evaluating psoriasis with psoriasis area and severity index, psoriasis global assessment, and lattice system physician's global assessment. J Am Acad Dermatol [Internet]. October 01, 2004 [cited December 18, 2023];51(4):563-9. Available from: http://www.jaad.org/article/S0190962204010886/fulltext

70. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. Lancet [Internet]. July 21, 2007 [cited October 13, 2023];370(9583):263-71. Available from: http://www.thelancet.com/article/S0140673607611283/fulltext

71. Benhadou F, Mintoff D, Schnebert B, Thio HB. Psoriasis and microbiota: A systematic review. Diseases [Internet]. 02 June 2018 [published 10 October 2023];6(2):47. Available from: /pmc/articles/PMC6023392/

72. Fyhrquist N, Muirhead G, Prast-Nielsen S, Jeanmougin M, Olah P, Skoog T, et al. Microbe-host interplay in atopic dermatitis and psoriasis. Nat Commun [Internet]. 01 December 2019 [cited 10 October 2023];10(1). Available from: /pmc/articles/PMC6795799/

73. Alper S, Koç E. Turkey Psoriasis treatment guidelines. Turk Dermatol Venerology. 2021;

74. Kim M, Park HE, Lee SH, Han K, Lee JH. Increased risk of Alzheimer's disease in patients with psoriasis: a nationwide population-based cohort study. Sci Rep. April 15, 2020;10(1):6454.

75. Zingel R, Jacob L, Smith L, Konrad M, Kostev K. Association between psoriasis and dementia: a retrospective cohort study. J Alzheimers Dis Rep. January 19, 2023;7(1):41-9.

76. Zhao J, Li T, Wang J. Association between psoriasis and dementia: A systematic review. Neurología (English Edition). January 2024;39(1):55-62.

77. Charoenngam N, Rittiphairoj T, Ponvilawan B, Ungprasert P. Patients with psoriasis have a higher risk of dementia: A systematic review and meta-analysis. Indian J Dermatol Venereol Leprol. February 24, 2021;87:364.

78. Liu L, Chen S ting, Li H jin, Qiang Y, Sun X ying, Zhou Y qiong, et al. Association between psoriasis and dementia: Current evidence. Front Aging Neurosci. October 22, 2020;12.

79. Kridin K, Linder D, Shalom G, Piaserico S, Babaev M, Freud T, et al. Psoriasis and dementia: A cross-sectional study of 121,801 Patients. Acta Dermato Venereologica. 2020;100(15):adv00250.

80. Pezzolo E, Mutlu U, Vernooij MW, Dowlatshahi EA, Gisondi P, Girolomoni G, et al. Psoriasis is not associated with cognition, brain imaging markers, and risk for dementia: The Rotterdam Study. J Am Acad Dermatol. September 2021;85(3):671-80.

81. Xie J, Van Hoecke L, Vandenbroucke RE. The ımpact of systemic ınflammation on alzheimer's disease pathology. Front Immunol. January 06, 2022;12.

82. vom Berg J, Prokop S, Miller KR, Obst J, Kälin RE, Lopategui-Cabezas I, et al. Inhibition of IL-12/IL-23 signaling reduces Alzheimer's disease-like pathology and cognitive decline. Nat Med. December 25, 2012;18(12):1812-9.

83. Newby D, Prieto-Alhambra D, Duarte-Salles T, Ansell D, Pedersen L, van der Lei J, et al. Methotrexate and relative risk of dementia amongst patients with rheumatoid arthritis: a multi-national multi-database case-control study. Alzheimers Res Ther. 06 Dec 2020;12(1):38.

84. Huang KL, Yeh CC, Wu SI, Huang KY, Huang KY, Yang YH, Kuo TY, et al. Risk of dementia among ındividuals with psoriasis. J Clin Psychiatry. May 14, 2019;80(3).

85. Gisondi P, Sala F, Alessandrini F, Avesani V, Zoccatelli G, Beltramello A, et al. Mild cognitive ımpairment in patients with moderate to severe chronic plaque psoriasis. Dermatology. 2014;228(1):78-85.

86. Di Carlo M, Becciolini A, Incorvaia A, Beci G, Smerilli G, Biggioggero M, et al. Mild cognitive impairment in psoriatic arthritis. Medicine. March 19, 2021;100(11):e24833.

87. Sunkureddi P, Doogan S, Heid J, Benosman S, Ogdie A, Martin L, et al. Evaluation of self-reported patient experiences: ınsights from digital patient communities in psoriatic arthritis. J Rheumatol. May 2018;45(5):638-47.

88. Alves CJM, Martelli ACC, Fogagnolo L, Nassif PW. Síndrome de Ekbom secundária a transtorno orgânico: relato de três casos. An Bras Dermatol. August 2010;85(4):541-4.

89. Mendonça FJP de, Teixeira IA, Marinho V. Ekbom Syndrome associated with Lewy Body Dementia: A case report. Dement Neuropsychol. March 2020;14(1):83-7.

90. Ochiai S, Sugawara H, Kajio Y, Tanaka H, Ishikawa T, Fukuhara R, et al. Delusional parasitosis in dementia with Lewy bodies: a case report. Ann Gen Psychiatry. December 30, 2019;18(1):29.

91. Taomoto D, Kanemoto H, Satake Y, Yoshiyama K, Iwase M, Hashimoto M, et al. Case report: Delusional infestation in dementia with Lewy bodies. Front Psychiatry. November 10, 2022;13.

92. Okumus B, Hocaoglu C, Dilek N. A rarely known topic 'Delusional Parasitosis': A case report (tour). Journal of Clinical Psychiatry. 2020;23(1):111-5.

93. Reich A, Kwiatkowska D, Pacan P. Delusions of Parasitosis: An Update. Dermatol Ther (Heidelb) [Internet]. 01 Dec 2019 [a.yer 01 Mar 2024];9(4):631. Available from: /pmc/articles/PMC6828902/

94. Orsolini L, Gentilotti A, Giordani M, Volpe U. Historical and clinical considerations on Ekbom's syndrome. Int Rev Psychiatry [Internet]. August 17, 2020 [cited March 01, 2024];32(5-6):424-36. Available from: https://pubmed.ncbi.nlm.nih.gov/32436416/

95. Maeda T, Babazono A, Nishi T. Surveillance of First-Generation H1-Antihistamine Use for Older Patients with Dementia in Japan: A Retrospective Cohort Study. Curr Gerontol Geriatr Res. July 02, 2018;2018:1-6.

96. Richardson K, Fox C, Maidment I, Steel N, Loke YK, Arthur A, et al. Anticholinergic drugs and risk of dementia: case-control study. BMJ. April 25, 2018;k1315.

97. Rich P, Bourcier M, Sofen H, Fakharzadeh S, Wasfi Y, Wang Y, et al. Ustekinumab improves nail disease in patients with moderate-to-severe psoriasis: results from< scp>phoenıx</scp> 1. British Journal of Dermatology. February 18, 2014;170(2):398-407.

98. Han J, Lee J, Han K, Seo H, Bang C, Park Y, et al. Epidemiology and medication trends in patients with psoriasis: a nationwide population-based cohort study from korea. Acta Dermato Venereologica. 2018;98(4):396-400.

99. Tippmann F, Hundt J, Schneider A, Endres K, Fahrenholz F. Up-regulation of the α-secretase ADAM10 by retinoic acid receptors and acitretin. The FASEB Journal. June 14, 2009;23(6):1643-54.

100. Sodhi RK, Singh N. Retinoids as potential targets for Alzheimer's disease. Pharmacol Biochem Behav. May 2014;120:117-23.