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Comparison of ethanolic and ethyl acetate fractions of Iraqi Medicago sativa for the treatment of urinary tract infection

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Abstract. *Urinary tract infections (UTIs), predominantly caused by uropathogenic Escherichia coli (UPEC), affect over 100 million people annually and are a leading cause of morbidity due to rising antibiotic resistance. Medicago sativa (alfalfa), a medicinal plant rich in phytochemicals, has shown antibacterial potential, yet its efficacy against UPEC in Iraq remains unexplored. This study investigates the antibacterial effects of M. sativa ethanolic and ethyl acetate fractions as potential alternatives to conventional antibiotics for UTI treatment.*

Methods. M. sativa was collected in Kirkuk, defatted with hexane, extracted with 85% ethanol, and fractionated into petroleum ether, chloroform, ethyl acetate, and ethanolic fractions. Phytochemical analyses, including Dragendorff's, Mayer's, and ppigh-performance liquid chromatography were performed. Urine samples from 85 UTI patients were cultured, yielding 30 UPEC isolates. Antibacterial activity was evaluated using the agar well diffusion method, with minimal inhibitory concentrations (MICs) determined via microplate serial dilution. Antibiotic susceptibility was tested using the Kirby-Bauer method against eight antibiotics. Data were analyzed using SPSS v26 (ANOVA, LSD).

Results. Most participants (56.7%) were under 40 years old, with females more affected. The ethanolic fraction demonstrated superior antibacterial activity, with a mean inhibition zone of 21.96 ± 1.9 mm at 75 mg/ml (p=0.001), compared to 17.32 ± 1.5 mm for the ethyl acetate fraction. High-performance liquid chromatography confirmed bioactive compounds, including gallic acid, quercetin, and myricetin. Meropenem exhibited 100% sensitivity, while cephalothin showed complete resistance.

Conclusions. M. sativa extracts, particularly the ethanolic fraction, exhibit significant antibacterial activity against UPEC, offering a promising alternative to antibiotics. Larger, multicenter studies are needed to validate these findings and explore clinical applications.

Keywords: *urinary tract infection, antibiotic, ethyl acetate fraction, M. sativa extract, ethanolic fraction.*

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

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Порівняння етанольної та етилацетатної фракцій іракської Medicago sativa для лікування інфекцій сечовивідних шляхів

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Резюме. *Інфекції сечової системи (ІСС), переважно спричинені уропатогенним Escherichia coli (UPEC), щороку вражають понад 100 мільйонів людей і є основною причиною захворюваності через зростання антибіотикорезистентності. Medicago sativa (люцерна), лікарська рослина, багата на фітохімічні сполуки, демонструє антибактеріальний потенціал, але її ефективність проти UPEC в Іраку досі не досліджена. Це дослідження спрямоване на вивчення антибактеріальних ефектів етанольної та етилацетатної фракцій M. Sativa, як потенційних альтернатив традиційним антибіотикам для лікування ІСС.*

Методи. M. sativa була зібрана в Кіркуку, знежирена гексаном, екстрагована 85% етанолом і фракціонована на фракції петролейного ефіру, хлороформу, етилацетату та етанолу. Проводили фітохімічний аналіз, включно з тестами Драгондорфа, Майєра та високоефективною рідинною хроматографією. Зразки сечі 85 пацієнтів з ІСС були культивовані, що дозволило визначити 30 ізолятів UPEC. Антибактеріальну активність оцінювали методом дифузії в агарі, а мінімальні інгібуючі концентрації (MIK) визначали шляхом серійного розведення в мікропланшетах. Чутливість до антибіотиків тестували методом Кірбі-Бауера проти восьми антибіотиків. Дані аналізували за допомогою SPSS v26 (ANOVA, LSD).

Результати. Більшість учасників (56,7%) були молодше 40 років та переважали жінки. Етанольна фракція показала вищу антибактеріальну активність із середньою зоною інгібування 21,96 ± 1,9 мм при 75 мг/мл (p=0,001) порівняно з 17,32 ± 1,5 мм для етилацетатної фракції. Високоефективна рідинна хроматографія підтвердила наявність біоактивних сполук, зокрема галовової кислоти, кверцетину та мірицетину. Меропенем мав 100% чутливість, тоді як цефалотин показав повну резистентність.

Висновки. Екстракти M. sativa, особливо етанольна фракція, демонструють значну антибактеріальну активність проти UPEC, пропонуючи перспективну альтернативу антибіотикам. Для підтвердження результатів і вивчення клінічного застосування необхідні більші багатоцентрові дослідження.

Ключові слова: *інфекція сечової системи, антибіотик, етилацетатна фракція, екстракт M. sativa, етанольна фракція.*

Introduction. Urinary tract infections (UTIs) are among the most common bacterial infections, significantly contributing to morbidity and mortality, second only to respiratory tract infections (RTIs). UTIs can affect individuals of any age but are more prevalent in women, with over 50% of women and approximately 12% of men experiencing a UTI in their lifetime [1]. Uropathogenic Escherichia coli (UPEC) is the primary cause of UTIs [2]. Asymptomatic bacteriuria, defined as bacterial proliferation in urine without urinary symptoms, is commonly due to commensal colonization [3]. It occurs in 1–5% of healthy premenopausal women, 4–19% of healthy older adults, and 15–50% of institutionalized elderly individuals [4].

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Medicinal plants play a critical role in developing innovative medications. In many rural communities, natural remedies remain preferred due to the efficacy of plant-derived drugs and concerns about the adverse effects of synthetic medications. Plants are a vital source of commercially used pharmaceuticals, with many synthetic drugs originating from natural plant compounds [5]. Antibiotics such as ciprofloxacin, trimethoprim, and sulfamethoxazole are commonly prescribed for UTIs, but high recurrence rates and increasing antibiotic resistance pose significant challenges [6]. Identifying novel antimicrobial agents is a global priority [7]. Pharmacological and phytochemical research supports the traditional use of herbs, offering the potential for clinical research and the development of new medications [5].

Medicago sativa (alfalfa), a perennial legume from the Leguminosae family, exhibits antibacterial properties due to its phytochemical constituents [8]. Multidrug-resistant pathogens, including E. coli, are a growing global threat, necessitating the development of new antimicrobial drugs [9]. This study investigates

the antibacterial efficacy of M. sativa extracts against UPEC in urine samples from Iraqi patients, highlighting its potential as a superior alternative to conventional antibiotics.

Materials and methods. *Plant Material.* The entire *Medicago sativa* plant, belonging to the Fabaceae family, was collected near Kirkuk, Iraq. The Pharmacognosy Department at the College of Pharmacy, Ashur University, verified the plant's identity. The study was approved by the local ethics committee of the Pharmacology Department at Baghdad College of Medicine (approval code: 178, dated 20/10/2021). In November 2021, during full bloom, the plant was harvested, cleaned, air-dried at room temperature in the shade, coarsely crushed, and weighed.

Extraction and fractionation. A 350-gram sample of powdered plant material was defatted with hexane, dried, and extracted using 85% ethanol. The resulting extract was fractionated into four fractions—petroleum ether, chloroform, ethyl acetate, and ethanolic—and dried using anhydrous sodium sulfate [10].

Preliminary qualitative phytochemical analysis. Chemical analyses were conducted on crude and fractionated M. sativa extracts to identify active constituents, including alkaloids, flavonoids, terpenoids, and steroids, following established methods [11].

Chemical tests:

- *Dragendorff's test (alkaloids):* A solution of potassium bismuth iodide, containing basic bismuth nitrate ($Bi(NO_3)_3$), tartaric acid, and potassium iodide (KI), produces an orange or reddish-brown precipitate upon contact with alkaloids, confirming their presence.
- *Mayer's test (alkaloids):* Two milliliters of alcoholic extract were mixed with 1–2 drops of Mayer's reagent (potassium mercury iodide in water). A white or creamy precipitate indicated alkaloids.
- *Lead acetate test (flavonoids):* One milliliter of 10% lead acetate solution was mixed with 2 ml of alcoholic extract. A yellowish-white precipitate confirmed flavonoids.
- *Salkowski test (terpenoids):* Two milliliters of organic extract or fractions were mixed with 2 ml of chloroform, evaporated to dryness, and treated with 2 ml of concentrated sulfuric acid. After heating for 2 minutes, a reddish-brown layer at the interface indicated terpenoids.
- *Liebermann-Burchard test (steroids):* Two milliliters of crude extract or fractions were mixed with 1 ml of chloroform, 2–3 ml of acetic anhydride, and 2 drops of concentrated sulfuric acid. A dark green color confirmed steroids.
- *H_2SO_4 test (steroids):* Two milliliters of extract or fractions treated with sulfuric and acetic acids developed a greenish color, indicating steroids.

High-performance liquid chromatography analysis of phenols and flavonoids in M. sativa extract. Phenols and flavonoids were analyzed using high-performance liquid chromatography (HPLC), not fast-liquid chromatography (FLC). A Nucleodur C18-DB column (50 x 4.6 mm, 3 µm particle size) was used under optimized conditions. The mobile phase consisted of a linear gradient of 0.05% trifluoroacetic acid (TFA) in deionized water (solvent A) and 0.05% TFA in methanol (solvent B).

Urine sample collection and culture. Urine samples were collected in sterile containers from 85 UTI patients at Medical City Hospitals, Iraq. After bacterial isolation, 30 samples were confirmed to contain *Escherichia coli.* The urine was inoculated onto agar plates and incubated aerobically at 37°C for 24 hours to isolate *E. coli.*

Antibiotic susceptibility testing. The susceptibility of bacterial isolates to six antibiotics – meropenem (10 µg), ceftriaxone (10 µg), levofloxacin (5 µg), ciprofloxacin (10 µg), nitrofurantoin (100 µg), and amikacin – was tested using the modified Kirby-Bauer disk diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates resistant to three or more antibiotics were classified as multidrug-resistant [12].

Evaluation of antibacterial activity. The ethanolic and ethyl acetate fractions of *M. sativa* were tested for their ability to inhibit Gram-negative bacteria, such as *E. coli*, using the agar well diffusion method [13, 14]. Pure *E. coli* colonies were grown on Mueller-Hinton Agar (MHA) at 37°C for 24 hours, suspended in sterile saline, and spread onto MHA plates. Three concentrations of plant extract were added to wells, with a 10 µg meropenem disk as a positive control and dimethyl sulfoxide as a negative control. Inhibition zones were measured after 24 hours at 37°C.

Minimal inhibitory concentration (MIC) determination. MICs were determined using a 96-well microplate. Each well received 100 µl of MHA, followed by 100 µl of plant extract in the first column. Serial dilutions were performed, discarding the final volume. A 50 µl bacterial suspension was added, and MIC was determined by the absence of turbidity [13].

Data analysis. Data were analyzed using IBM SPSS Statistics version 26. Descriptive statistics (means ± standard deviations, ranges, percentages, frequencies) were calculated for participant demographics, antibiotic susceptibility, inhibition zones, and HPLC retention times. One-way analysis of variance (ANOVA) with post-hoc least significant difference (LSD) tests was used to compare mean inhibition zones across Medicago sativa extract concentrations (25, 50, 75 mg/ml) and meropenem, with a significance level of $p=0.001$.

Results. *Bacterial development patterns in patients with UTI.* This study analyzed 85 urine samples from patients with UTI at hospitals in Baghdad, Iraq. Of these, 30 samples were confirmed to contain uropathogenic Escherichia coli (UPEC). The study participants, with a mean age of 38.0 ± 19.4 years, ranged from 20 to 72 years. The majority (56.7%) were under 40 years old, with 17 participants aged 20–39, 7 aged 40–59, and 6 aged 60–72. Females were more frequently affected than males.

Antibiotics susceptibility testing of UPEC isolates. Thirty UPEC isolates were tested against eight antibiotics: meropenem, amikacin, ceftriaxone, ciprofloxacin, levofloxacin, nitrofurantoin, trimethoprim, and cephalothin. Meropenem exhibited the highest sensitivity (100%), followed by amikacin (83.3%) and nitrofurantoin (80.0%). Ceftriaxone, ciprofloxacin, levofloxacin, and trimethoprim showed moderate sensitivity, while cephalothin was completely resistant (100%) (Table 1). These findings underscore the importance of antibiotic susceptibility testing for effective UPEC treatment.

Table 1

Antibiotic Susceptibility of UPEC Isolates

Drug	Sensitive, n (%)	Intermediate, n (%)	Resistant, n (%)
Meropenem	30 (100.0)	0 (0.0)	0 (0.0)
Amikacin	25 (83.3)	3 (10.0)	2 (6.7)
Ceftriaxone	10 (33.3)	1 (3.3)	19 (63.4)
Ciprofloxacin	14 (46.7)	0 (0.0)	16 (53.3)
Levofloxacin	14 (46.7)	0 (0.0)	16 (53.3)
Trimethoprim	16 (53.3)	0 (0.0)	14 (46.7)
Nitrofurantoin	24 (80.0)	3 (10.0)	3 (10.0)
Cephalothin	0 (0.0)	0 (0.0)	30 (100.0)

Phytochemical analysis of Medicago sativa extract. The crude extract of M. sativa contained alkaloids, flavonoids, terpenoids, and steroids, while the ethyl acetate fraction solely contained flavonoids, as indicated by the pre-eliminatory phytochemical study as shown in (Table 2).

Table 2

Phytochemical screening of crude extract and different fractions

Crude and fractions	Alkaloids	Flavonoids	Steroids	Terpenoids
Crude	+	+	+	+
F1	+	-	+	+
F2	+	-	+	+
F3	-	+	-	-

HPLC analysis. HPLC is a sensitive instrument for detecting low concentrations of phytochemicals in extracts. It allows for qualitative identifications by comparing retention periods with reference standards. HPLC analysis detects gallic acid, salicylic acid, caffeic acid, pyrogallol, quercetin, myricetin, naringin, and apigenin, as shown in Tables 3, 4, and Fig. 1 and 2.

Table 3

Standard HPLC results

Components	Retention Time	Area UV
Gallic Acid	2.33	32274
Pyrogallol	3.01	39079
Caffeic Acid	4.09	41169
Salicylic Acid	5.18	52672
Naringin	6.26	45870
Myrecetin	7.02	71387
Quercetin	7.92	47835
Apigenin	8.84	45761

Table 4

HPLC results of analyzed fractions with their retention

Fraction	Components	Retention Time	Area UV
Ethanol	Gallic Acid	2.363	24865
	Pyrogallol	3.04	25753
	Caffeic Acid	4.107	18919
	Salicylic Acid	5.183	20115
	Naringin	6.268	18261
	Myricetin	7.017	20899
	Quercetin	7.93	29338
	Apigenin	8.852	23199
Ethyl acetate	Gallic Acid	2.007	33379
	Pyrogallol	4.09	46455
	Caffeic Acid	5.163	25141
	Salicylic Acid	6.252	23256
	Naringin	6.992	16266
	Myricetin	7.902	43078
	Quercetin	8.828	25338

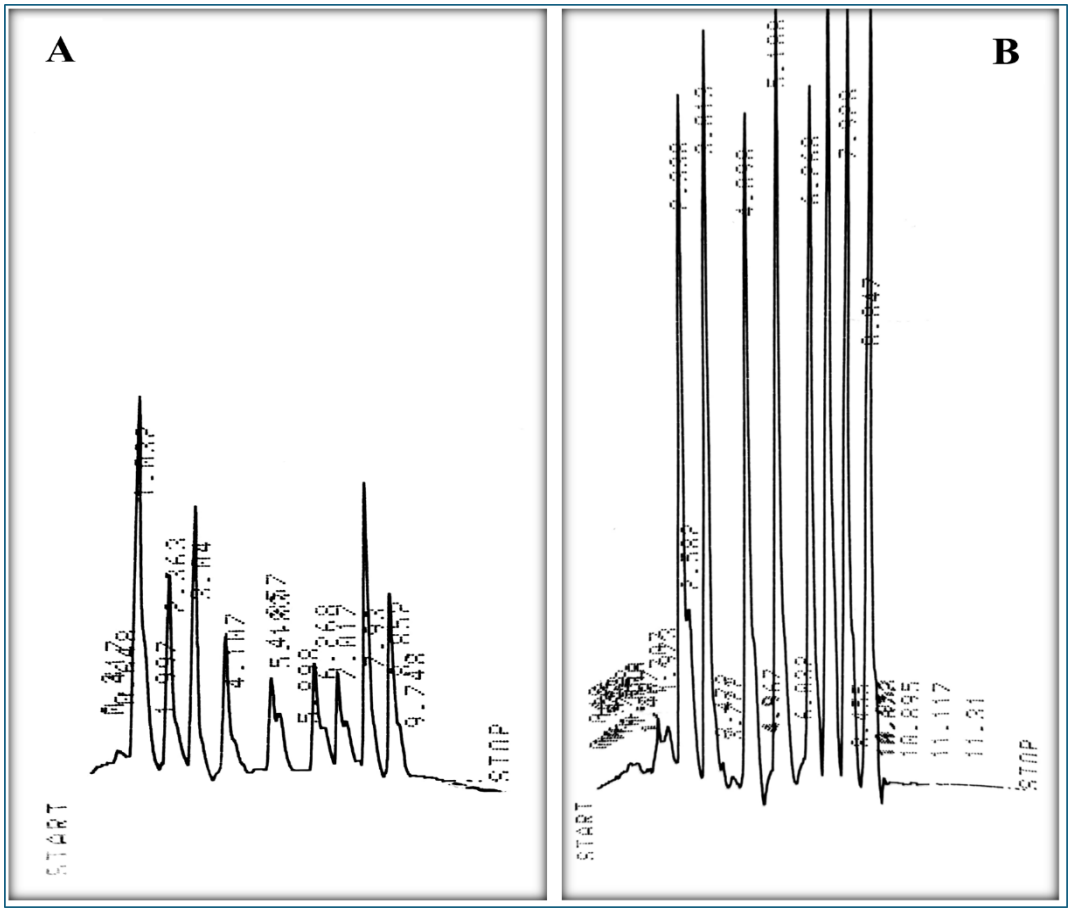

Fig. 1. HPLC chromatogram of the ethanolic fraction of Medicago sativa extract. (A) Sample; (B) Standards. Observed peaks and their retention times (min): Gallic Acid (2.363), Naringin (6.268), Myricetin (7.017), Quercetin (7.930), Apigenin (8.852).

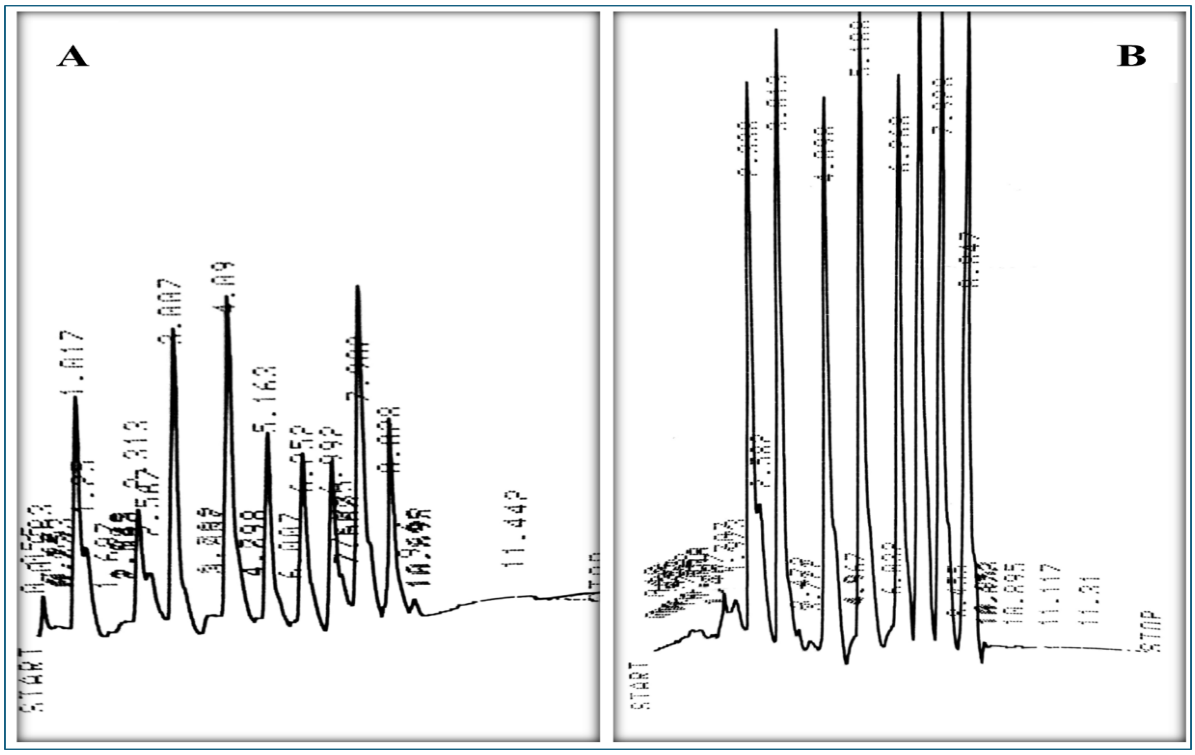

Fig. 2. HPLC chromatogram of ethyl acetate fraction: A.Sample, B. Standard. Peaks that were observed, Naringin= 6.992, Myricetin= 7.902, Quercetin= 8.828, and Gallic Acid, 2.007.

Antibacterial activity of different concentrations of M. sativa extracts against E. coli in comparison to meropenem. The sensitivity of uropathogenic Escherichia coli (UPEC) to Medicago sativa extracts, meropenem, and dimethyl sulfoxide (DMSO) was determined using the agar well diffusion method. Both ethanolic and ethyl acetate fractions exhibited growth inhibition zones, with higher concentrations of M. sativa extract demonstrating greater antibacterial activity (Fig. 3).

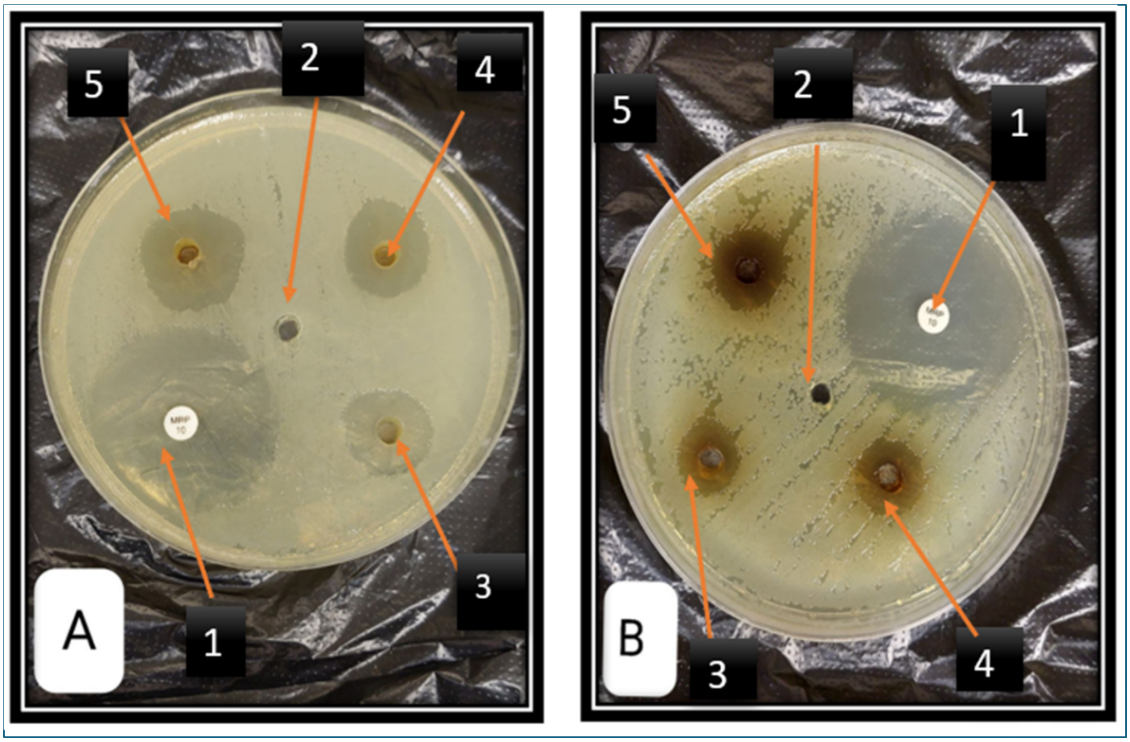

Fig. 3. Sensitivity of *Escherichia coli* to varying concentrations of *Medicago sativa* extracts. (A) Ethanolic fraction; (B) Ethyl acetate fraction. Treatments: (1) DMSO (negative control); (2) Meropenem (10 µg, positive control); (3) 25 mg/ml; (4) 50 mg/ml; (5) 75 mg/ml.

Meropenem produced a significantly larger mean inhibition zone compared to both the ethanolic and ethyl acetate fractions (p=0.001), as shown in Table 5 and Figure 4.

Table 5

Comparison of mean inhibition zones for *Medicago sativa* fractions and meropenem

Fraction/control, concentration (mg/ml)	Mean inhibition zone (mm, Mean ± SD)	P-value
Ethanol fraction	32.85 ± 2.7	0.001
Meropenem		
Ethyl acetate fraction	32.96 ± 2.6	0.001
Meropenem		

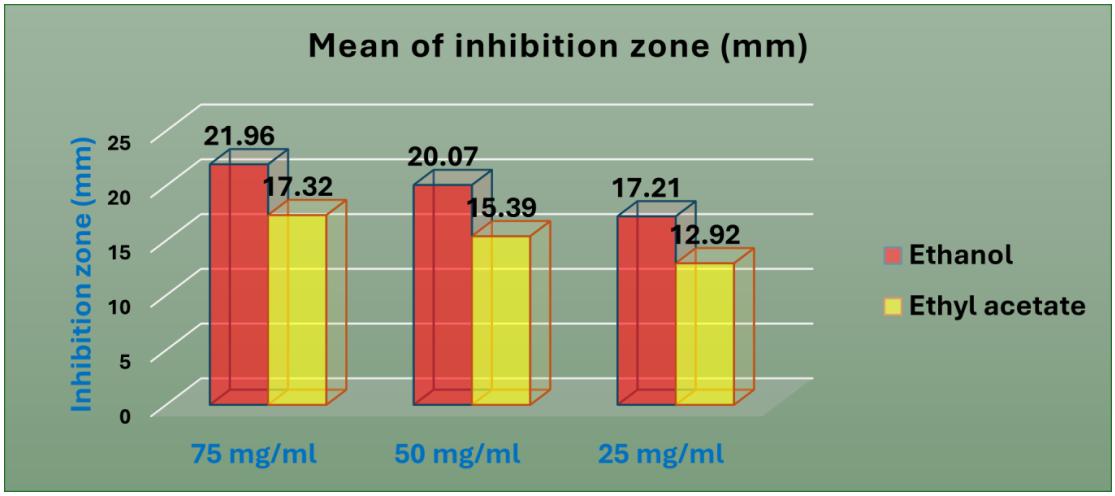

Fig. 4. Comparison of mean inhibition zones across varying concentrations of ethanolic and ethyl acetate fractions of *Medicago sativa* extracts.

Discussion. UTIs, affecting over 100 million people annually, are increasingly prevalent due to the rise of multidrug-resistant pathogens, leading to treatment challenges and higher mortality rates [15]. The overuse of antibiotics has driven the emergence of drug-resistant bacteria, necessitating novel antimicrobial agents [5]. Previous studies have identified diverse chemical constituents in Medicago sativa, with bioactive compounds contributing to its beneficial properties [16].

Phytochemical analyses revealed alkaloids, flavonoids, terpenoids, and steroids in the crude M. sativa extract, with only flavonoids detected in the ethyl acetate fraction, consistent with prior findings [16]. The ethanolic fraction also contained flavonoids, aligning with Grechana et al. [17]. High-performance liquid chromatography (HPLC) confirmed the presence of phenolic compounds, including gallic acid, caffeic acid, pyrogallol, salicylic acid, naringin, myricetin, quercetin, and apigenin, supporting previous research [18].

Flavonoids, a class of plant phenolic compounds, exhibit antibacterial properties by increasing bacterial membrane permeability, leading to leakage of intracellular contents and cell death. They also inhibit nucleic acid synthesis by interfering with DNA and RNA transcription, disrupt bacterial energy pathways by reducing ATP production, and prevent quorum sensing and biofilm formation, thereby reducing pathogenicity [19–22].

Quercetin exerts antibacterial effects by binding to DNA, causing strand cleavage, inhibiting DNA gyrase, and disrupting DNA replication. It also downregulates virulence genes, inhibits single-stranded DNA-binding proteins, and prevents biofilm formation by interfering with quorum sensing pathways, reducing bacterial adhesion and colonization [11, 23, 24]. Gallic acid inhibits bacterial growth by disrupting metabolic pathways and compromising membrane integrity, leading to intracellular leakage and cell death. It also prevents biofilm formation, reducing bacterial colonization in the urinary tract [25, 26].

Myricetin inhibits nucleic acid synthesis, cell envelope synthesis, and bacterial toxin production [27]. Apigenin disrupts bacterial membranes and inhibits cellular processes, including nucleic acid synthesis, cell envelope synthesis, biofilm formation, quorum sensing, and Ala-Ala synthetase. Naringin acts as a quorum-sensing inhibitor [27].

This study confirmed that females are more susceptible to UPEC infections due to their shorter and wider urethra, facilitating bacterial access to the bladder. Consistent with our findings, the highest UTI prevalence was observed in individuals aged ≤50 years (49.2%) and 50–69 years (57.3%) [28]. Women (77.3%) were more prone to UTIs than men (22.6%) [29].

Antibiotic susceptibility testing aligned with prior studies, showing high sensitivity to amikacin (83.3%) and nitrofurantoin (91.7%) for UTI treatment [30]. Previous research reported UPEC isolates were most susceptible to ciprofloxacin (86.5%), ofloxacin (75%), and nitrofurantoin (84.6%), supporting our results [31]. Gharavi et al. noted high sensitivity to nitrofurantoin (92.8%), imipenem (99.2%), amikacin (97.9%), and meropenem (97.2%) [32].

Medicago sativa (alfalfa) extract was found to inhibit *E. coli papC* and *rcsA* gene expression, reducing biofilm formation, consistent with our findings [33]. Meropenem, a broad-spectrum antibiotic, exerts its activity by covalently inhibiting penicillin-binding proteins (PBPs), which are peptidoglycan transpeptidases essential for bacterial cell wall integrity [34].

Prior studies reported resistance to cephalosporins and fluoroquinolones, with the highest susceptibility to tigecycline (100%), colistin (96.2%), amikacin (90.5%), fosfomycin (86.7%), and nitrofurantoin (84.9%), aligning with our results [29]. *E. coli* isolates exhibited complete resistance to cephalothin (100%), consistent with a reported resistance rate of 85.8% [35].

Medicago sativa extracts demonstrated significant antibacterial activity against *E. coli* at varying concentrations, with larger inhibition zones at higher concentrations (p=0.001) [36]. The ethanolic fraction showed the highest mean inhibition zones at 75 mg/ml (21.96 ± 1.9 mm), 50 mg/ml (20.07 ± 1.8 mm), and 25 mg/ml (17.21 ± 2.2 mm), with significant differences (p=0.001). The ethyl acetate fraction exhibited lower inhibition zones at 75 mg/ml (17.32 ± 1.5 mm), 50 mg/ml (15.39 ± 1.3 mm), and 25 mg/ml (12.92 ± 1.6 mm) (p=0.001). The ethanolic fraction's superior activity is likely due to its potent extraction solvent, consistent with Khan et al. [37].

The ethanolic extract of *M. sativa* also showed antibacterial activity against Gram-negative bacteria, including *Pseudomonas aeruginosa* and *E. coli*, at various concentrations, supporting its efficacy in UTI treatment [38, 39]. Additionally, *M. sativa* extract inhibited *Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Lactococcus lactis*, and *Bacillus licheniformis*, confirming its broad antibacterial properties [40].

In vitro tests demonstrated that *M. sativa* extracts exhibited significant antibacterial activity compared to meropenem (ethanolic fraction: 32.85 ± 2.7 mm; ethyl acetate fraction: 32.96 ± 2.6 mm; p=0.001). At 25 mg/ml, the ethanolic extract completely inhibited *E. coli* growth in broth media, with no growth observed on Mueller-Hinton agar. The ethyl acetate extract at 30 mg/ml exhibited bacteriostatic and bactericidal effects, as evidenced by no growth on Mueller-Hinton agar.

A recent study in India identified *E. coli, Enterococcus faecalis, Staphylococcus aureus*, and *Klebsiella pneumoniae* as primary UTI pathogens, with *E. coli* being the most common. *Tamarindus indica* and *Clitoria ternatea* outperformed ceftriaxone and piperacillin, reinforcing the potential of plant-based treatments [41]. Another study highlighted the efficacy of *Punica granatum, Aronia melanocarpa*, and *Cornus mas* for UTI prevention or combined antibiotic therapy, attributed to their tannins, steroids, terpenes, coumarins, flavonoids, and polyphenols [42].

The study has several limitations. First, the small sample size (85 urine samples) limits the generalizability of the findings to broader populations. Second, the study was conducted at a single center in Baghdad, Iraq, which may introduce regional bias and fail to account for geographic variations in UPEC strains or patient demographics. Third, the lack of in vivo testing restricts conclusions about the clinical efficacy and safety of *Medicago sativa* extracts for UTI treatment. Fourth, the study did not assess the potential synergistic effects between *M. sativa* extracts and conventional antibiotics, which could enhance therapeutic outcomes. Fifth, variability in plant material (e.g., differences in growth conditions or harvest timing) may affect the consistency of phytochemical profiles and antibacterial activity. Finally, the absence of long-term follow-up data limits understanding of the extracts' impact on UTI recurrence or resistance development. Larger, multicenter studies with in vivo models and diverse populations are needed to address these limitations and validate the findings.

Conclusions. This study demonstrates that Medicago sativa extracts, particularly the ethanolic fraction, exhibit significant antibacterial activity against UPEC, offering a promising alternative to conventional antibiotics for UTI treatment. The ethanolic fraction's superior efficacy, with inhibition zones of 21.96 ± 1.9 mm at 75 mg/ml (p=0.001), is attributed to bioactive compounds such as gallic acid, quercetin, and myricetin, which disrupt bacterial membranes, inhibit nucleic acid synthesis, and prevent biofilm formation. High resistance to cephalothin (100%) and moderate sensitivity to ciprofloxacin and levofloxacin underscore the need for novel treatments. Our results support the potential of M. sativa as a natural antimicrobial agent. Future research should focus on larger, multicenter trials to validate these findings and explore clinical applications, including synergistic effects with existing antibiotics.

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Author's contributions.

Ahmed Ibrahim Al-Yousif: ideation, approach, formal analysis, research, materials, data gathering, and composing the first draft.

Alaa Ghaith Ahmed: research, materials, data collection, and creation of the first draft.

Sheelan Amir Al-Darwish: writing, editing, data curation, formal analysis, research, resources, and technique.

Farah Fawzi: research, materials, and composing the first draft.

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Research article

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Histomorphological assessment of T-2 toxin, ochratoxin A, and aflatoxin B1-induced renal damage in a rat model

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Abstract. *Fungal toxins are a prevalent cause of food contamination and can induce pathological changes in various organs of both humans and animals. This study aimed to investigate the histomorphological changes and immune response in rat kidneys exposed to aflatoxin B1, ochratoxin A, and T-2 toxin.*

Methods. A total of 44 albino rats were used, divided into four groups: three groups receiving different doses of toxins (24 mg/kg of aflatoxin B1, 64 mg/kg of ochratoxin A, and 25 mg/kg of T-2 toxin) for 20 days. Kidney samples were stained with hematoxylin-eosin, and picrofuchsin along with the Giemsa and May-Grünwald solutions.

Results. Histopathological analysis revealed specific changes, including vacuolization, necrosis with hemorrhagic foci, pyknosis, and inflammation in the renal tissue. A significant increase in mast cells and degenerative changes in renal tubular epithelial cells were also observed.

Conclusions. These findings suggest that ochratoxin A and aflatoxin B1 are potent nephrotoxins, causing severe damage to renal epithelial cells and their nuclei, while the T-2 toxin had a relatively less pronounced effect. This study highlights the detrimental effects of mycotoxins on kidney tissue, underscoring the need for further research on their prevention and mitigation to ensure food safety.

Keywords: *aflatoxin B1, ochratoxin A, T-2 toxin, mast cells, nephrotoxicity, food contamination.*

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Гістоморфологічна оцінка пошкодження нирок, спричиненого Т-2 токсином, охратоксином А та афлатоксином В1 у моделі щурів

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Резюме. *Грибкові токсини є поширеною причиною забруднення харчових продуктів і можуть викликати патологічні зміни в різних органах людини та тварин. Це дослідження мало на меті вивчити гістоморфологічні зміни та імунну відповідь у нирках щурів, підданих впливу афлатоксину В1, охратоксину А та Т-2 токсину.*

Методи. Дослідження провкдено на 44 альбіносних щурах, поділених на чотири групи: три групи отримували різні дози токсинів (24 мг/кг афлатоксину В1, 64 мг/кг охратоксину А та 25 мг/кг Т-2 токсину) протягом 20 днів. Зразки нирок фарбували гематоксилин-еозином, пікрофуксином, а також розчинами Гімзи та Май-Грюнвальда.

Результати. Гістопатологічний аналіз виявив специфічні зміни, включаючи вакуолізацію, некроз із геморагічними осередками, пікноз та запалення в нирковій тканині. Також спостерігалося значне збільшення кількості тучних клітин і дегенеративні зміни в епітеліальних клітинах ниркових канальців.

Висновки. Ці результати свідчать, що охратоксин А та афлатоксин В1 є потужними нефротоксинами, які спричиняють серйозне пошкодження епітеліальних клітин нирок та їх ядер, тоді як Т-2 токсин мав відносно менш виражений ефект. Це дослідження підкреслює шкідливий вплив мікотоксинів на ниркову тканину, наголошуючи на необхідності подальших досліджень щодо їх профілактики та пом'якшення для забезпечення безпеки харчових продуктів.

Ключові слова: *афлатоксин В1, охратоксин А, Т-2 токсин, тучні клітини, нефротоксичність, забруднення харчових продуктів.*

Introduction. Ensuring food safety is a paramount concern for both individuals and entities on a global scale. The growing importance of food supply availability and safety has become a significant issue for many countries, which are increasingly dependent on one another [1]. However, the health of both humans and animals is at risk due to the presence of mycotoxins—chemical toxins produced by fungi. These toxic fungal metabolites are naturally occurring in numerous foods and feed, and they can cause severe harm to public health, resulting in economic losses and safety issues worldwide due to the fungal contamination of agricultural commodities [2-7]. Approximately 25% of wheat worldwide is contaminated with mycotoxins, which have not been adequately studied for their complex structures and properties [8].

Mycotoxins, including aflatoxins, ochratoxins, and trichothecenes, are produced by various mold fungi such as Aspergillus, Fusarium, Penicillium, Claviceps, and Alternaria. These toxins can cause a range of acute and chronic health issues when ingested, including liver toxicity, nephrotoxicity, and immunosuppressive effects [9-14]. Of particular concern is the impact on kidney function, as mycotoxins have been shown to induce nephropathy in both animals and humans, though the specific renal effects of these toxins are not fully understood [2, 10, 14].

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Research has primarily focused on the general toxicological effects of mycotoxins, but studies specifically addressing their renal-specific impacts remain limited. While the effects of aflatoxins, ochratoxins, and trichothecenes on other organs have been studied in detail, fewer studies have investigated the histological and immunological responses of kidney tissues to these toxins. This gap in renal-specific research is particularly critical given the kidney's role in detoxifying harmful substances and its susceptibility to toxin-induced damage.

This study aims to address this gap by investigating the histomorphological and immunological response in rat kidneys following exposure to aflatoxin B1, ochratoxin A, and T-2 toxin. By examining these toxins' specific impacts on kidney structure and immune reactions, this study provides valuable insights into the renal toxicity of mycotoxins, contributing to a deeper understanding of their effects on kidney function and structure.

Materials and methods. Experimental design. Our study aimed to evaluate histomorphological changes in rat kidneys under the influence of specific mycotoxins, namely aflatoxin B1, ochratoxin A, and T-2 toxin. We

utilized a sample of 44 albino rats, aged between 3-4 months, with a body weight range of 150-200g. The animals were randomly divided into four groups (n=11 in each). The rats were subjected to a constant 12-hour light/dark cycle with 45–55% humidity conditions and were given a standard pellet diet and tap water ab libitum. The first group that served as a control group received a standard diet. While the second experimental group was fed a diet containing 24mg/kg of aflatoxin B1 for 20 days, the third group received a diet containing 64mg/kg of ochratoxin A for 20 days. Finally, the fourth third group was fed with T-2 toxin at 25mg/kg for 20 days. The doses were determined based on literature data, considering the concentrations of toxins commonly appearing in natural food crops and foodstuffs [15].

Each mycotoxin was first weighed using a high-precision balance. The powdered feed was thoroughly mixed with the prepared mycotoxins to provide its homogeneity. The toxins were dissolved in a small volume of solvent before mixing into the powdered feed. The size and weight of the pellets were standardized to ensure consistent dosing for each animal. After pellet preparation, the toxin concentrations in the final pellets were maintained consistent with the target doses. The number of pellets given to each rat was adjusted so that the total amount of toxin administered matched the calculated dose based on the animal's weight. This process was designed to minimize any potential variability in toxin exposure among the animals, ensuring consistent delivery to all subjects.

All experimental procedures have been performed according to the principles of the "International Recommendations on Carrying out of Biomedical Research with Use of Animals" (CIOMS, 2016), the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes (CE, 2005), the guidelines outlined in Directive 2010/63/EU [16], and approved by the National Center of Bioethics (Armenia).

Histological examination. During the experiment, the rats were euthanized using an intraperitoneal injection of ketamine hydrochloride (37 mg/kg) [17] immediately after the final doses of toxins were administered in the third week of the study. The kidneys were then removed and fixed using Bouin's solution and 10% formalin. The fixed tissues underwent standard histological processing, which included sequential dehydration with 70%, 80%, 90%, and 96% ethyl alcohol. Following dehydration, the tissues were cleared with xylene, embedded in paraffin, and coverslipped with DPX.

Paraffin sections approximately 5-6 μm thick were cut and stained using hematoxylin-eosin, picrofuchsin by Van Giesen, and Giemsa and May-Grünwald by Pappenheim [18]. The hematoxylin-eosin and picrofuchsin-stained preparations were utilized to examine the morphological changes in the rat kidneys due to mycotoxin exposure, including the sizes of the renal epithelial cell nuclei. Additionally, to assess the inflammatory responses to the mycotoxins, the number of mast cells within a standardized section area was counted across 50 fields of view. Cell counts were conducted on all sections from the studied animals and processed for microscopic reporting using magnifications of 200x, 400x, 600x, and 1000x. The microscope used was a B-293, equipped with an Optikam B5 Digital Camera (Model M-114, Italy). Images were recorded and analyzed using Optika Liteview software.

Data analysis. The numerical data obtained were analyzed using statistical methods to calculate the mean values, expressed as the mean ± standard deviation, with the assistance of Statistica 11 software. The significance of the differences between the mean values was determined using the Student's t-test (unpaired, two-tailed), with a p-value less than 0.05 considered statistically significant.

Results. Histopathological analysis. The present study aimed to investigate the effects of exposure to different mycotoxins on the kidneys in a rat model. It should be mentioned that the animals did not receive a high-fat diet to increase their weight, and as such, no weight data were collected. As a result, the toxin doses were not adjusted based on body weight.

The histomorphological examination of the aflatoxin B1-exposed rats revealed significant differences in both the ventromedial and dorsolateral zones of the kidneys compared to the control group (Fig. 1A-D).

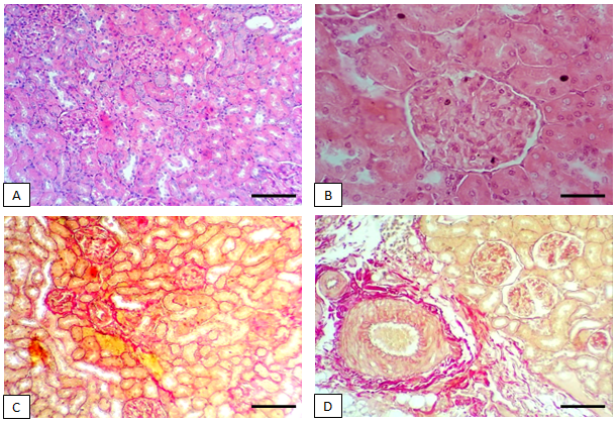

Fig. 1. Histological sections of the control rat kidneys (A-D). Glomerulus with proximal and distal convoluted tubules (A-ventromedial zone, B-dorsolateral zone), H&E (magnifications ×200 and ×400, scale bar = 50um). Blood vessels next to the renal corpuscle and tubular structures within the ventromedial zone (C). Cross-sectional view of connective tissue surrounding blood vessels in the dorsolateral zone, (D), picrofuxin, (magnifications ×200, scale bar = 75μm, 400, scale bar = 50μm).

Most of the cells in the kidneys of aflatoxin B1-exposed rats underwent pyknotic changes, and the proximal and distal tubes became more tightly spaced. Hemorrhagic foci were also observed, indicating functional inactivity of the kidneys after the daily intake of aflatoxin B1 (Fig. 2A-D).

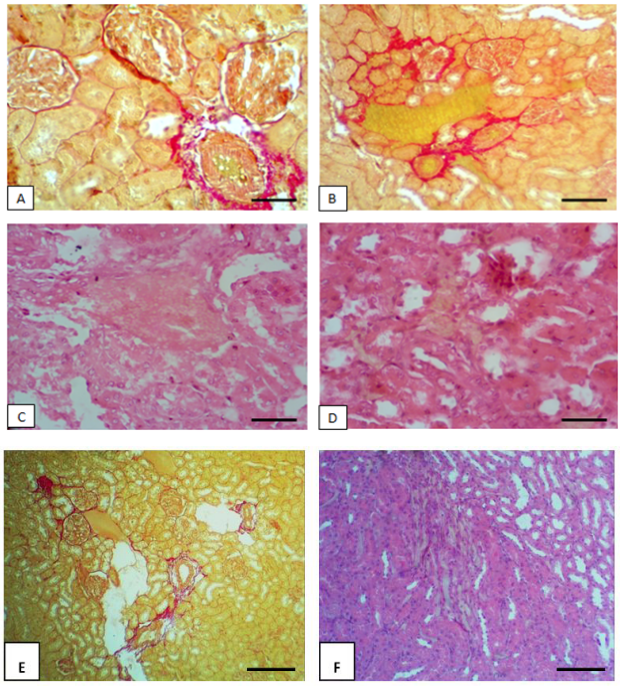

Fig. 2. Histological section of the kidney of a rat exposed with aflatoxin B1 (A), picrofuxin (magnifications ×400, scale bar = 50μm, (B), (magnification ×200, scale bar = 100μm). Hemorrhagic foci in the kidney of an aflatoxin B1-exposed rat (C, D), H&E (magnification ×400, scale bar = 50μm), (E), (picrofuxin, magnification ×200, scale bar = 100μm), (F), (H&E, magnification ×200, scale bar = 100μm).

Degenerative alterations were also evident in the kidneys of T-2 toxin-exposed rats, particularly in the renal tubules, where cells displayed widened margins, flattened structures, pyknotic changes, and cytoplasmic vacuolization (Fig. 4 A-D). Furthermore, a layer of heterochromatin was present in both the proximal and distal tubular cell nuclei, indicating decreased functional activity of the kidneys.

Similarly, the kidneys of rats exposed to ochratoxin A showed severe changes. These changes were characterized by degeneration of the epithelial cells in the renal tubules, including swelling and vacuolization, along with the accumulation of connective tissue around the affected areas. In some regions, necrosis of the tubules and atrophy of the glomerular capillaries were also observed (Fig. 3 A-D).

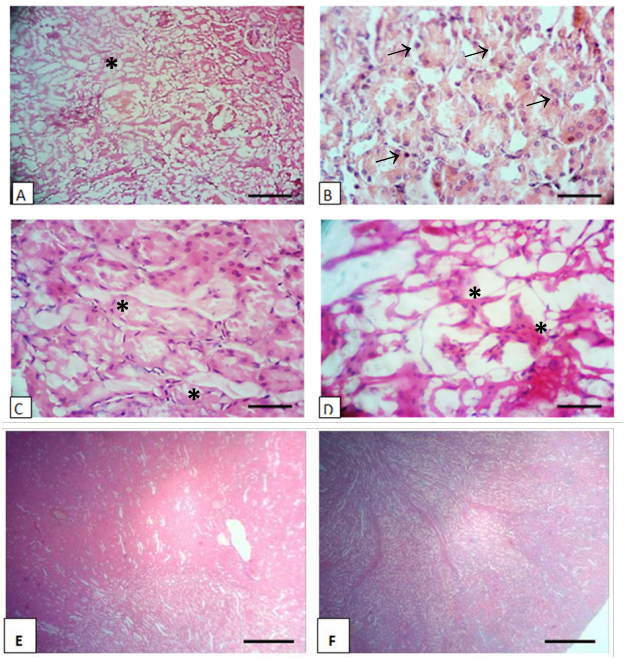

Fig. 3. Histological section of the kidney of an ochratoxin A-exposed rats. Pyknotic cells (arrows) of renal tubules and atrophy of glomerular capillaries. The swelling areas (asterisks) between the epithelial tubules and accumulated connective tissue elements, (A) (magnification ×100), (B,C,D) (H&E, magnification ×400, scale bar = 50μm), (E) (magnification ×40), (F) kidney of control group rats (H&E, magnification ×40).

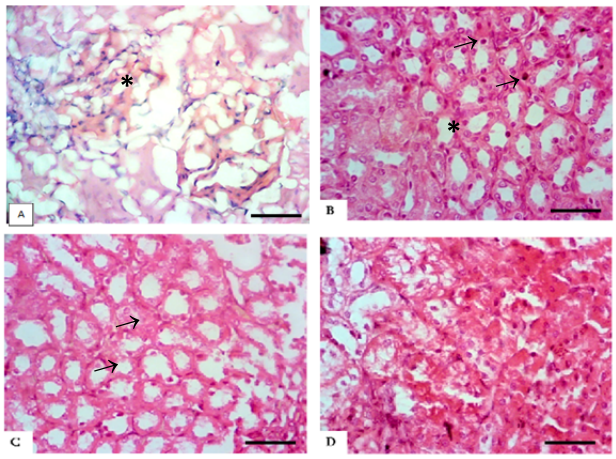

Fig. 4. Photomicrograph of T-2-toxin-exposed kidneys tissue sections. Degenerated renal corpuscles with pyknotic cells (arrows) and vacuolated cytoplasm (asterisk) (A). Proximal renal tubules have defected; cells are characterized by a vacuolated cytoplasm (asterisk) (B,C,D) (H&E, magnification ×400, scale bar = 50μm).

To assess the kidney's response to mycotoxin exposure, we examined the epithelial cells and their nuclei. Nuclear size was evaluated according to established criteria [19]. The results showed significant differences in the size of the epithelial cell nuclei in rats exposed to aflatoxin B1 and ochratoxin A, compared to the control group.

The diameter of cell nuclei was smaller in aflatoxin B1 and ochratoxin A-exposed groups compared to the control animals (p=0.001), whereas no significant alterations were observed in T-2 toxin-exposed rats (Table 1).

Table 1

Changes of epithelial cell nuclei in renal tubules of the rat kidneys with a daily intake of mycotoxins (n = 11/group)

Experimental groups	The diameter of the cell nuclei (μm) / mean±SD
Control	31.4±0.75
Aflatoxin B1	25.05±0.26*
Ochratoxin A	16.77±0.46*
T-2 toxin	33.21±0.63*

Significant difference (*p< 0.05) vs the control group

Consequently, the most remarkable changes were observed in ochratoxin A-exposed kidneys. Notably, the diameter of cell nuclei is smaller in aflatoxin B1-exposed and ochratoxin A-exposed groups compared to the control animals (Fig. 5).

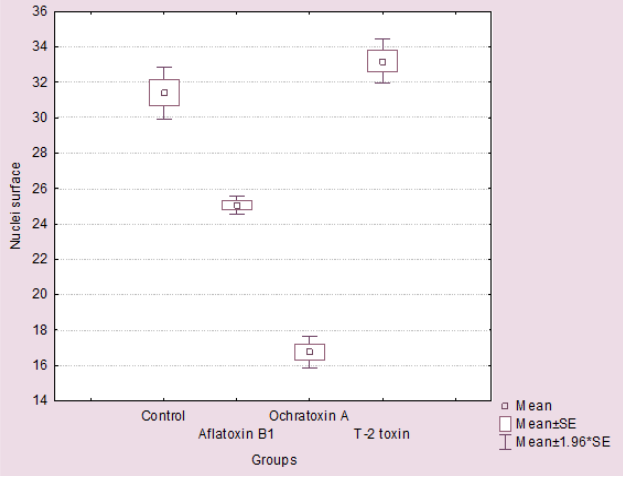

Fig. 5. Changes in sizes of the epithelial cell nuclei in aflatoxin B1, ochratoxin A, and T-2-toxin exposed rats' kidneys.

Based on the measurements obtained, it was observed that the average size of the renal epithelial nuclei decreased in animals that were exposed to aflatoxin B1 and ochratoxin A. This can be attributed to degenerative changes in the nuclei after the intake of these toxins, which testifies to their severe nephrotoxic effects.

Effects of mycotoxins on mast cells. Mast cells are a type of cell found in tissues that play an important role in maintaining local homeostasis by participating in inflammation, immunogenesis, blood coagulation, and circulation [20]. Exposure to mycotoxins has been linked to the activation of mast cells, and IgE antibodies to mycotoxins stimulate mast cells to release heparin, histamine, and pro-inflammatory cytokines [21]. The number of mast cells was counted to evaluate the immune responses of kidneys under exposure to mycotoxins. The quantitative changes in the number of these cells were compared between the control group and the mycotoxins-exposed animals.

Table 2 shows the number of mast cells in the kidneys of rats exposed to aflatoxin B1 and ochratoxin A significantly differs from the control group showing an increased number of mast cells. However, in animals exposed to T-2 toxin, the number of these cells did not demonstrate significant changes.

Table 2

Changes in the number of mast cells in control and mycotoxins-exposed rat kidneys (n = 11/group)

Experimental groups	Number of mast cells in each group/ (M ± SD)
Control	16.75 ± 2.3
Aflatoxin B1	17.75 ± 1.38*
Ochratoxin A	17.56 ± 0.26*
T-2 toxin	16.92 ± 0.33

Significant difference (*p< 0.05) *vs* the control group

The examination of animals that were exposed to mycotoxins revealed a considerable presence of degranulated mast cells, particularly around basement membranes (Fig. 6). These cells displayed a significant polymorphism, characterized by diverse granule sizes and densities in the cytoplasm, suggesting the presence of inflammatory reactions in the kidneys of mycotoxin-exposed rats. This reaction resulted in the destruction of the basal plates of the renal tubule walls.

Discussion. Recent medical literature suggests that the inhalation of mycotoxins may pose risks to human health. In animals, the ingestion of ochratoxin can lead to severe lesions, including glycogen accumulation, mitochondrial alterations, and extensive liver necrosis. Kidney damage is characterized by thickening of the glomerular basal membrane, lymphocytic infiltration of the interstitium, and IgG deposits in the glomeruli [22]. While most absorbed mycotoxins are excreted through urine, residues can accumulate in the liver, kidneys, and muscles, presenting a threat to both animal and human health [23].

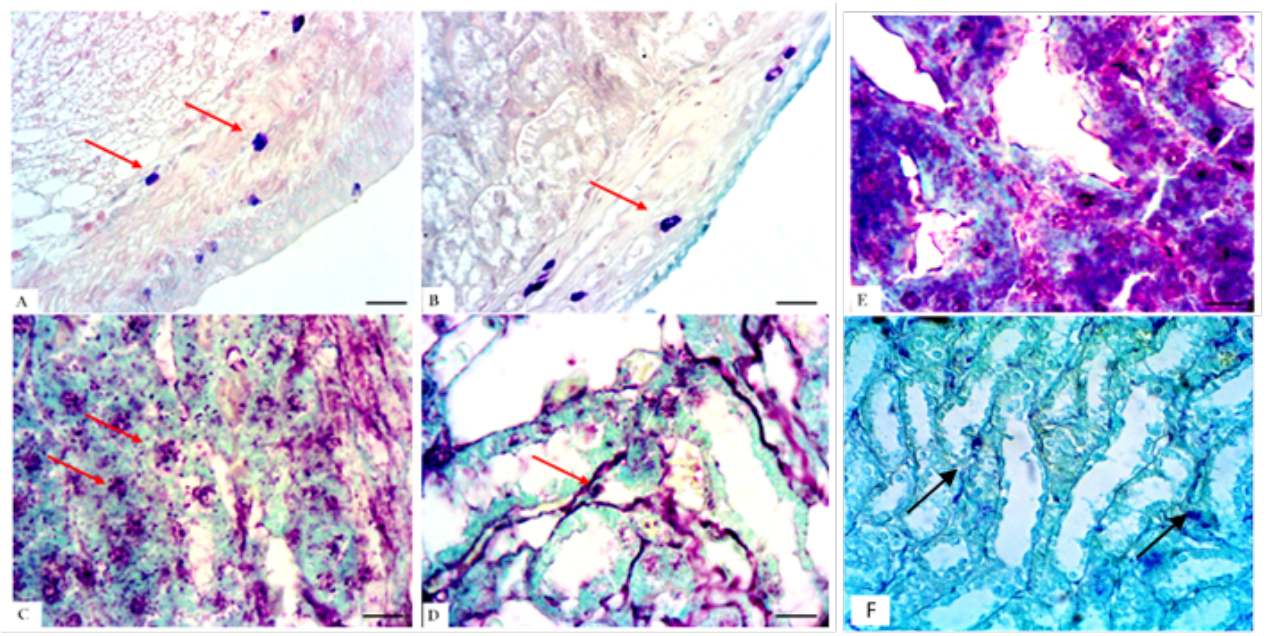

Fig. 6. Granulated mast cells in T-2 (A, B) and ochratoxin A (C) exposed rat kidneys. Degranulated mast cells (D, E) in aflatoxin B1 exposed rat kidneys (red arrows), Giemsa and May-Gr nwald staining (magnifications 400, scale bar = 50μm and x600, scale bar = 25μm). Mast cells in the control group (F), (magnifications 400, scale bar = 50μm).

The kidney is particularly susceptible to nephrotoxic attack, owing to its high blood flow and specialized metabolism. Environmental pollutants that target the kidney including mycotoxins can target the renal tissue as the majority of nephrotoxic compounds or their derivatives occur via the renal glomeruli and the proximal tubules [24]. The kidney and liver are predominantly affected, while immune cells in the thymus, spleen, and lymph nodes undergo structural and functional alterations, thereby influencing the function of immune organs. However, understanding of the mechanisms underlying ochratoxin A-induced immunotoxicity remains limited, particularly within the context of innate immunity [25]. The nephrotoxicity mechanism of mycotoxins involves multiple factors including oxidative stress, endoplasmic reticulum stress, mitophagy, inflammatory responses, and apoptosis, which are intertwined and interdependent [26]. This, in turn, can upregulate the proline dehydrogenase (PRODH) and pro-apoptotic factors (Bax, Caspase-3), and downregulate the apoptosis inhibitor Bcl-2 in mRNA and protein expression. Particularly, the ocharatoxin A may disrupt several cell functions, including cell proliferation, division, and signaling pathways. It also has a synergistic effect on other co-occurring mycotoxins [27]. Inflammation is one of the most important risk factors influencing the development of kidney disease. It has been reported that exposure to mycotoxins affects the transcription of the pro-inflammatory factor TNF-α and the production of the pro-inflammatory mediators [28-30]. This can contribute to the accumulation of inflammatory cells in the renal interstitium, thus creating an inflammatory infiltration composed of diverse immune cells. Prolonged inflammation eventually leads to chronic interstitial inflammation, tubular atrophy, and prominent fibrosis [31]. Mast cells in this case can contribute to the immune response as mycotoxins stimulate them to secrete pro-inflammatory cytokines and chemokines that activate the immune system leading to the stimulation of chronic neuroinflammatory symptoms [32-33].

Histopathological observations in visceral organs have highlighted the toxic effects of aflatoxin. Aflatoxin is primarily eliminated through the kidneys, and the accumulation of high concentrations of the toxin impairs excretory function, leading to congestion and pathological alterations. Aflatoxin-induced nephrotoxicity is believed to result from interference with the transport function in collecting tubular cells, along with diffuse impairment of proximal tubular function [34-36]. Most toxicological studies on mycotoxins have focused on exposure to a single toxin, without considering the potential interactions and combined effects—synergistic or antagonistic—that can occur in nature. Data on the toxic effects of mycotoxin mixtures are limited, and thus, the risks of exposure to multiple toxins remain uncertain [37].

In the present study, a significant decrease in the severity of histopathological and morphometric changes was observed in animals exposed to mycotoxins. Chronic administration of low doses of ochratoxin led to morphological and functional changes in the renal tubules, resulting in tubule tissue damage [38]. These findings suggest that simultaneous exposure to these toxins is likely, though the combined effects on human and animal toxicity remain unclear [39].

Among the mycotoxins studied, ochratoxin A exhibited the most pronounced nephrotoxic effects on the kidneys, while the effects of T-2 toxin were relatively

mild. In the kidneys of rats exposed to aflatoxin B1, the number of cells in the renal tubules was reduced. In contrast, in the kidneys of rats exposed to ochratoxin A, there were significant degenerative processes, marked by changes in renal tubule cells, the presence of numerous pyknotic cells, and cytoplasmic vacuolization. The number of cells in the kidneys of rats with various pathological alterations of the nuclei significantly increased following exposure to these mycotoxins. Additionally, the toxic effects of aflatoxin B1, ochratoxin A, and T-2 toxins were evident in changes in mast cells, which indicate an inflammatory response in the kidneys. The most pronounced immune responses were observed in rats exposed to ochratoxin A. These findings suggest that mycotoxins contribute to the progression of inflammation and cellular damage in kidney tissue [**40-42**].

Given the harmful effects of the studied mycotoxins, possible mitigation strategies, such as the use of dietary binders or detoxifying agents, are suggested. These agents could help alleviate the harmful effects of fungal metabolites and protect cells. Several studies have demonstrated that nutritional binders, such as bentonite clay, activated charcoal [**43-45**], and silymarin [**46**], can effectively adsorb aflatoxins and ochratoxins in the gastrointestinal tract, preventing their absorption and reducing systemic toxicity. These binders could be considered a preventive measure for individuals or animals at risk of exposure to contaminated food. Additionally, the application of certain antioxidants, such as vitamin C and E, and curcumin, may have potential protective effects against aflatoxicosis and ochratoxicosis. Evidence suggests that curcumin offers protective potential against tissue injury caused by certain drugs and environmental toxins. It has been shown to ameliorate aflatoxin B1-induced duodenal toxicity and liver injury by downregulating CYP450 enzyme activity and regulating hepatic long non-coding RNAs [**47-49**]. One of the most effective biological strategies to reduce the harmful effects of fungal toxins is the inclusion of probiotic yeasts and bacteria in the diet. Since probiotics can bind to toxins in the gastrointestinal tract, they can prevent toxin absorption and mitigate the effects on animal and human health [**50-52**].

Study limitations. This study has several limitations concerning the investigation of specific molecular biomarkers to further clarify the mechanisms of nephrotoxicity. The 20-day exposure period may not capture the long-term or chronic effects of mycotoxins on kidney health, which warrants further investigation over extended periods. The use of a rat model may not fully replicate human kidney toxicity, limiting the direct applicability of the findings to human health. Additionally, the study is restricted by the separate investigation of the mycotoxins instead of the combined version which should enhance our insights about the synergistic effects of those fungal toxins. Another key limitation is the variability in toxin administration. Despite our effort to provide the toxin's homogeneity in the feed, nevertheless, possible uneven mixing could have led to inconsistencies in exposure levels across animals. Albeit, these limitations may impact the reliability of the results, therefore future studies should provide more compelling findings into the interactions between multiple mycotoxins and warrant a better understanding of the collective effects of these toxins.

Conclusions. The present study reveals that prolonged exposure to mycotoxins results in nephrotoxic effects on the kidneys, causing cellular and histomorphological changes in the renal tubule walls. In cases of ochratoxicosis and aflatoxicosis, an elevated number of mast cells indicates inflammation in the kidneys. However, exposure to T-2 toxin produced only minor effects on kidney tissue and immune responses. The histopathological findings suggest that ochratoxin A and aflatoxin B1 are potent nephrotoxins, causing significant damage to epithelial cells and their nuclei, while the T-2 toxin had a less deleterious effect on kidney tissue. These findings contribute to a more precise understanding of the pathological effects of fungal toxins on renal histomorphology and their varied impact on kidney tissue.

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

Author contributions.

Anna Karapetyan: supervision, project administration, visualization;

Anna Karapetyan, Anna Grigoryan, Tamara Abgaryan: conceptualization, methodology, validation.

Tamara Abgaryan, Marietta Mkhitaryan, Lyudmila Niazyan, Ruzanna Adamyan: investigation, resources.

Anna Karapetyan, Anna Grigoryan, Marietta Mkhitaryan, Ruzanna Shushanyan: writing - original draft, writing - review & editing.

Data availability statement. The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

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Research article

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Retrospective analysis of statin use and arteriovenous fistula thrombosis in hemodialysis: Is there a dose-dependent effect?

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Abstract. *Arteriovenous fistula (AVF) thrombosis is a major vascular access complication in hemodialysis (HD) patients, contributing to increased morbidity. Statins, known for their pleiotropic effects, may reduce AVF thrombosis risk, but evidence on dose-dependent effects is limited. This study evaluated the association between statin use, dose intensity, and AVF thrombosis in HD patients.*
Methods. A multicenter, retrospective cohort study was conducted using data from 562 HD patients with native AVFs across 10 dialysis clinics (May 2021–April 2025). Patients were categorized by statin use (users vs. non-users) and dose intensity (moderate vs. high vs. none). The primary outcome was AVF thrombosis; death was treated as a competing event. Kaplan-Meier survival curves and Fine and Gray subdistribution hazard models, adjusted for age, diabetes, dialysis vintage, Kt/V, glucose, calcium, blood flow, and pre-HD cardiovascular disease, were used to assess thrombosis risk.
Results. Of 562 patients (median follow-up 59 months), 212 (37.7%) were statin users. AVF thrombosis occurred in 54 (9.6%) patients, with 11 (7.1%) in statin users vs. 43 (10.6%) in non-users ($p = 0.006$). Kaplan-Meier analysis showed lower thrombosis probability in statin users (log-rank $p = 0.001$), with high-intensity users having the lowest risk ($p = 0.004$). In the unadjusted Fine and Gray model, high-intensity statins were associated with reduced thrombosis risk (sHR 0.61, 95% CI 0.59–0.97, $p = 0.03$), with a significant dose-dependent trend ($p = 0.018$). The adjusted model showed no significant association (moderate: sHR 0.67, $p = 0.16$; high: sHR 0.57, $p = 0.26$).
Conclusions. Statin use, particularly high-intensity, may reduce AVF thrombosis risk in HD patients, with a dose-dependent trend in unadjusted analyses. However, adjusted results were non-significant, possibly due to limited events. Larger prospective studies are needed to confirm these findings and optimize statin therapy for vascular access preservation.
Key words: *hemodialysis, arteriovenous fistula, thrombosis, statins, risk, treatment.*

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Ретроспективний аналіз застосування статинів та тромбозу артеріовенозної фістули у пацієнтів, які лікуються гемодіалізом: чи існує дозозалежний ефект?

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Резюме. *Тромбоз артеріовенозної фістули (АВФ) є основним ускладненням судинного доступу у пацієнтів, які лікуються методом гемодіалізу (ГД). Статини, відомі своєю плейотропною дією, можуть зменшити ризик тромбозу АВФ, але докази щодо дозозалежних ефектів обмежені. Метою дослідження було оцінити зв'язок між застосуванням статинів, інтенсивністю призначеної дози та тромбозом АВФ у ГД пацієнтів.*

Методи. До цього багатоцентрового ретроспективного когортного дослідження залучено дані 562 пацієнтів з хронічною хворобою нирок V стадії та нативною АВФ, які лікувались методом ГД у 10 діалізних центрах з травня 2021–по квітень 2025. Пацієнтів класифікували за прийомом статинів (група статинів і група без статинів) та інтенсивністю призначеної дози (помірна проти високої проти жодної). Первинною кінцевою точкою був тромбоз АВФ; летальний випадок розглядався як конкуруюча подія. Для оцінки ризику тромбозу використовували криві виживаності Каплан-Мейєра та моделі суброзподілу ризиків Файна-Грея, скориговані з урахуванням віку, діабету, тривалості діалізу, Kt/V, рівня глюкози, кальцію, швидкості кровотоку та наявності серцево-судинних подій перед початком ГД.

Результати. Серед 562 включених у дослідження ГД пацієнтів 212 (37,7%) приймали статини. Протягом 5 років спостереження, тромбоз АВФ діагностовано у 54 (9,6%) пацієнтів, серед яких 11 (7,1%) у пацієнтів групи статинів та у 43 (10,6%) хворих без терапії статинами (p = 0,006). Аналіз Каплана-Мейєра продемонстрував нижчу ймовірність тромбозу у пацієнтів, які отримували статини (log-rank p = 0,001), з найнижчим ризиком за прийому інтенсивної дози (p = 0,004). У нескоригованій моделі Файна та Грея прийом високої дози статинів асоціювався зі зниженим ризиком тромбозу (sHR 0,61, 95% CI 0,59–0,97, p = 0,03), зі значною дозозалежною тенденцією (p = 0,018). Скоригована модель не виявила суттєвого зв'язку між дозою статинів та ризиком тромбозу АВФ (помірна доза: sHR 0,67, p = 0,16; висока: sHR 0,57, p = 0,26).

Висновки. Застосування статинів, зокрема у високих дозах, асоційовано зі зниженням ризику тромбозу АВФ у ГД пацієнтів із дозозалежною тенденцією за результатами нескоригованого аналізу. Проте, після коригування на супутні фактори ці результати втратили статистичну значущість, що може бути зумовлено обмеженою кількістю подій. Для підтвердження цих даних та визначення оптимальної стратегії застосування статинів з метою збереження судинного доступу необхідні проспективні дослідження з залученням більшої когорти пацієнтів.

Ключові слова: *гемодіаліз, артеріовенозна фістула, тромбоз, статини, ризик, лікування.*

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Introduction. Arteriovenous fistulas (AVF) is the gold standard for vascular access in patients undergoing maintenance hemodialysis (HD), offering superior long-term patency and lower infection and complication rates compared to grafts and central venous catheters [1]. However, AVF thrombosis remains a major

clinical challenge, causing access failure, hospitalizations, the need for repeated interventions or surgical revisions, and contributing to increased all-cause and cardiovascular mortality [1, 2]. The identification of modifiable risk factors and prevention strategies to reduce the incidence of AVF thrombosis is, therefore, of critical importance to improving dialysis outcomes and preserving vascular access longevity.

Statins, widely prescribed for their lipid-lowering effects in cardiovascular disease (CVD) prevention [3-5], have also been shown to exert various pleiotropic effects, including anti-inflammatory, antithrombotic, and endothelial-stabilizing properties [6-8]. These mechanisms suggest a theoretical benefit of statins in maintaining AVF patency. Despite this biological plausibility, clinical evidence regarding the impact of statins on vascular access outcomes in HD patients remains limited and inconclusive [9-11].

Importantly, the role of lipid-lowering therapy in patients undergoing HD remains controversial. Major randomized controlled trials, such as 4D, AURORA, and SHARP, have demonstrated either modest or non-significant cardiovascular benefits of statin therapy in patients with end-stage kidney disease (ESKD) [12-14]. As a result, guidelines recommend selective and cautious statin use in this population, especially when initiated after the start of dialysis [15]. Although some recent large-scale observational studies have reported improved survival in statin users [16, 17], these findings do not specifically address vascular access outcomes and there is a general lack of data on the association between lipid-lowering therapy and the risk of AVF thrombosis in patients undergoing HD. Moreover, most available studies do not stratify results by statin dose and no consensus exists regarding whether higher-intensity statin therapy offers additional vascular access protection in HD patients.

Therefore, the present study aimed to evaluate the association between statin use and the risk of AVF thrombosis among patients receiving maintenance HD. Specifically, we sought to investigate whether higher statin doses are associated with a lower incidence of AVF thrombosis, independent of other known risk factors.

Methods. *Study design and setting*. This was a multicenter, retrospective cohort study conducted using data from a network of «Nephrocenter» dialysis clinics across six regions of Ukraine. The study included all patients receiving maintenance HD at 10 clinics, representing both urban and rural centers located in the Kyiv, Lviv, Odesa, Zaporizhzhia, Rivne, and Khmelnytskyi regions. The study period spanned from May 2021 to April 2025. Due to the retrospective design and use of de-identified patient data, the requirement for informed consent was waived by the institutional review board.

Study population. Adult patients (≥18 years) with ESKD receiving maintenance HD via AVF were eligible for inclusion. Patients were required to have been on HD for at least 3 months and to have complete medical records regarding medication history, vascular access events, and relevant clinical data. Other inclusion criteria were:

- Patients with a documented native AVF as the primary vascular access, used for at least 3 months, with no prior history of AVF failure or thrombosis before the start of follow-up.
- Availability of lipid profile data.
- Recorded information on statin use, including type, dose, and prescribing time (before or after HD initiation).
- Use of heparin anticoagulation during HD sessions, either as unfractionated heparin or low-molecular-weight heparin.

Exclusion criteria included:

- Use of arteriovenous grafts or central venous catheters as the primary vascular access.
- History of kidney transplantation during the study period.
- Incomplete records for statin use or lipid profiles.
- Known hypercoagulable disorders, active malignancy, use of antiplatelet or anticoagulant therapy on non-dialysis days, or the use of non-standard anticoagulation protocols during HD sessions (e.g., citrate anticoagulation or sessions without anticoagulation).

HD was performed using Fresenius 5008 dialysis machines, with high-flux dialyzers and bicarbonate-based dialysate. Standard treatment prescriptions involved three sessions per week, each lasting 4 hours, with blood flow rates typically ranging from 300 to 350 mL/min, adjusted individually based on vascular access function. The primary treatment goal was to achieve dialysis adequacy following international guidelines, targeting a single-pool Kt/V of ≥1.2. Dialysis adequacy and vascular access performance were regularly monitored as part of routine care.

Data collection. Data were extracted from electronic medical records across participating centers and included the following categories:

- Demographic and clinical variables: Age, sex, dialysis vintage at study enrollment, smoking and alcohol use status, presence of diabetes mellitus, history of cardiovascular disease (CVD) before HD initiation and during the dialysis period, CVD-related and non-CVD-related hospitalizations.
- Dialysis treatment parameters: Blood flow rate, ultrafiltration rate, and single-pool Kt/V values.
- Laboratory markers: Hemoglobin (Hb), C-reactive protein (CRP), serum calcium, phosphate, intact parathyroid hormone (iPTH), ferritin, and serum albumin levels.
- Lipid profile: Total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, and the atherogenic index of plasma (AIP).

- Statin use: Documented at the time of HD initiation or during follow-up, including the specific statin type and dosage.

Pre-HD CVD history was defined as any diagnosed non-fatal CVD event (e.g., myocardial infarction, stroke, heart failure, or coronary artery disease) recorded in the patient's medical history before HD initiation. Post-HD initiation CVD history was identified through medical records during the follow-up period and included new or recurrent events such as myocardial infarction, stroke, or heart failure. CVD-related hospitalization was defined as any admission with a primary diagnosis of a CVD condition, while non-CVD-related hospitalizations encompassed admissions for non-CVD causes (e.g., infections, gastrointestinal issues), as documented in hospital records.

To ensure data accuracy and completeness, patient records were cross-checked, and duplicate entries were removed. Patients with more than 5% missing data were excluded from the analysis. Data were collected at two time points: at the baseline and the last available follow-up before the study endpoint or completion.

Statin dosage classification. Statin use was determined based on electronic prescription records and confirmed by chart review. For baseline characteristics, the patients were classified into statins users and non-users. For dose-dependent analysis, the patients were divided into three groups: non-users, moderate-intensity-dose statin users, and high-intensity dose statin users. Statin intensity classification followed guidelines from the American College of Cardiology/American Heart Association (Table 1) [18].

Table 1

Statin Dosing and ACC/AHA Classification of Intensity

Statin	Moderate-intensity dosage	High-intensity dosage
Atorvastatin	10 to 20 mg	40 to 80 mg
Rosuvastatin	5 to 10 mg	20 to 40 mg
Simvastatin	20 to 40 mg	NA

Primary and secondary endpoints. The primary outcome was the incidence of primary AVF thrombosis in HD patients receiving statin therapy compared to those not receiving statins. AVF thrombosis was defined as a documented event of AVF failure due to clot formation requiring intervention (e.g., thrombectomy, angioplasty) or necessitating alternative vascular access. The secondary endpoint was the association of AVF thrombosis with statin dosage.

Statistical analysis. All statistical analyses were performed using MedCalc® Statistical Software version 23.1.3 (MedCalc Software Ltd, Ostend, Belgium), except for the Fine and Gray subdistribution hazard model, which was conducted using XLSTAT (Lumivero, 2025). Descriptive statistics were used to summarize patient characteristics across statin exposure groups. Continuous variables were assessed for normality using the Shapiro-Wilk test. Variables with normal distribution are presented as mean and standard deviation (M ± SD) and compared using the independent t-test or analysis of variance (ANOVA), as appropriate. Non-normally distributed data are presented as the median and interquartile range (Me [Q25–Q75]) and compared using the Kruskal-Wallis test. Categorical variables are presented as counts and percentages and compared using the chi-square (χ^2) test or Fisher's exact test, as appropriate.

Kaplan-Meier survival curves estimated the probability of AVF thrombosis, comparing statin users versus non-users and across dose intensity groups (non-users, moderate-intensity, high-intensity). Differences in survival distributions were assessed using the log-rank test, with pairwise comparisons adjusted for multiple testing (Bonferroni). Patients who died were censored at the time of death in the Kaplan-Meier analysis. To account for death as a competing risk, Fine and Gray subdistribution hazard models were employed, defining outcomes as thrombosis, death without thrombosis, or censored. Results are reported as subdistribution hazard ratios (sHR) with 95% confidence intervals (CI). Models were adjusted for age, diabetes, and variables differing between dose groups in descriptive analyses. A p-value < 0.05 was considered statistically significant.

Results. Baseline characteristics. A total of 680 patients receiving maintenance HD were initially screened for eligibility. After applying inclusion and exclusion criteria, 562 patients were included in the final analysis. The selection process is detailed in Fig. 1.

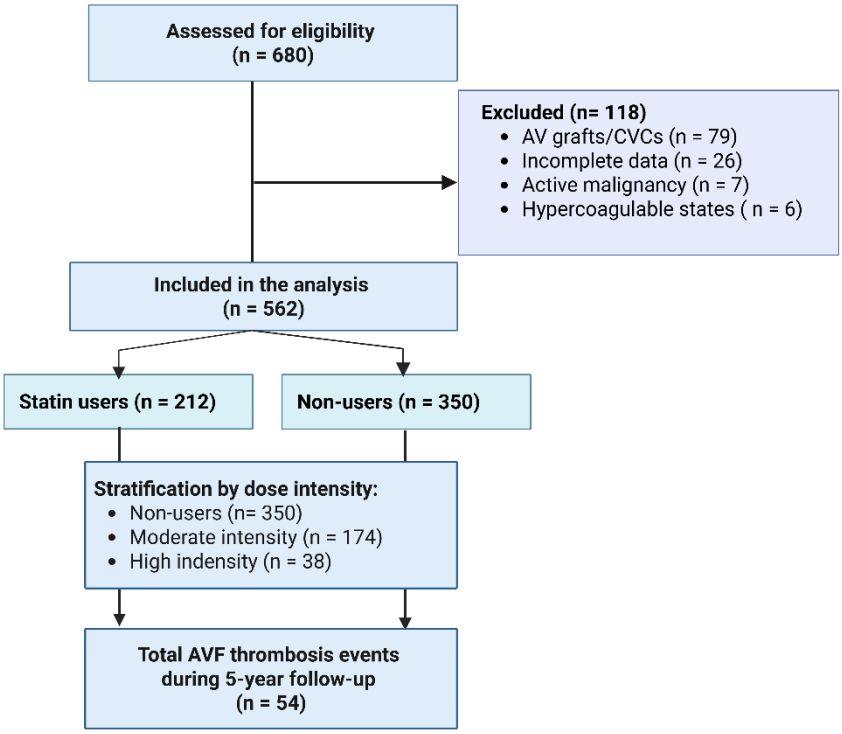

Fig. 1. The study flowchart.

Abbreviations: AVF, arteriovenous fistula, CVC, central venous catheter; HD, hemodialysis.

Of the 562 HD patients included, 212 (37.7%) were statin users and 306 (54.4%) were non-users at baseline or during follow-up. The median follow-up duration was 59 (47-60) months. Baseline demographic, clinical, and laboratory patients' characteristics stratified by lipid-lowering therapy are summarized in Table 2.

Table 2

Baseline characteristics of the study population according to statin use

Variable	All patients (n = 562)	Statin users (n = 212)	Non-users (n = 350)	p-value
Demographics				
Age, years	59 (60-66.2)	56.5 (48.4-66)	59 (50-67)	0.29
Male sex, n (%)	305 (54.3%)	104 (49.1%)	201 (57.4%)	0.06
Smoking status, n (%)	101 (17.9%)	33 (15.5%)	63 (18.0%)	0.16
Alcohol use, n (%)	49 (8.7%)	19 (8.9%)	30 (8.6%)	0.87
Clinical characteristics				
Diabetes, n (%)	109 (19,4%)	49 (23.1%)	60 (17.4%)	0.09
SBP (mmHg)	137 (130-150)	135 (130-150)	138 (132-150)	0.89
DBP (mmHg)	80 (80-90)	80 (80-90)	81 (78-90)	0.25
Pre-HD CVD history, n (%)	120 (21.3%)	18 (8.5%)	102 (29.1%)	<0.0001
CVD-related hospitalization, n (%)	99 (17.6%)	31 (14.6%)	68 (19.4%)	0.14
Non-CVD related hospitalization, n (%)	208 (37.0%)	82 (38.7%)	126 (36.0%)	0.68
Dialysis vintage (months)	47 (19.0-96.0)	53.5 (23.0-120.0)	43.0 (16.0-86.7)	0.002
Body mass index (BMI), kg/m	25.1 ± 5.1	24.8 ± 5.1	25.3 ± 5.4	0.28
Blood flow rate (mL/min)	291.2 ± 22.4	293.9 ± 24.7	289.6 ± 22.7	0.03
spKt/V	1.30 (1.2-1.43)	1.31 (1.2-1.46)	1.28 (1.13-1.42)	0.009

Continuation of Table 1

Variable	All patients (n = 562)	Statin users (n = 212)	Non-users (n = 350)	*p*-value
Total volume UF (mL)	1950 (400-2500)	2000 (350-2600)	1800 (400-2500)	0.27
Laboratory values				
Hemoglobin (g/dL)	101 (92-110.5)	103 (94-114)	100 (92-108)	0.01
CRP (mg/L)	5.39 (3.63-17.50)	6.49 (2.41-18.89)	5.24 (3.80-11.61)	0.57
Glucose (mmol/L)	5.20 (4.61-6.12)	5.06 (4.37-5.71)	5.34 (4.70-6.41)	0.008
Albumin (g/L)	39.6 (37.0-42.0)	39.7 (39.0-42.4)	39.0 (36.8-42.0)	0.15
Ferritin (ng/mL)	235 (84.5-545)	227.0 (78.2-550)	240.0 (86.1-542.0)	0.89
Calcium (mmol/L)	2.30 (2.19-2.42)	2.33 (2.23-2.46)	2.29 (2.17-2.41)	0.007
Phosphate (mmol/L)	1.62 (1.33-2.01)	1.67 (1.32-2.0)	1.60 (1.25-2.02)	0.32
iPTH (pg/mL)	320.7 (153.3-608.6)	314.9 (161.2-659.7)	331.6 (150.1-596.0)	0.53
Medications				
Erythropoiesis-stimulating agents, n (%)	548 (97.5%)	206 (97.2%)	342 (97.7%)	0.17
Iron therapy, n (%)	330 (58.7%)	125 (58.9%)	205 (58.6%)	0.73
Antihypertensives, n (%)	448 (79.7%)	174 (82.1%)	274 (78.3%)	0.28
ACEIs or ARBs, n (%)	260 (46.3%)	99 (46.7%)	181 (46.0%)	0.87
Calcium channel blockers, n (%)	245 (43.6%)	96 (45.3%)	149 (42.6%)	0.53
Beta-blockers, n (%)	197 (35.1%)	67 (31.6%)	130 (37.1%)	0.18
Alpha-blockers, n (%)	215 (38.3%)	87 (41.1%)	128 (36.6%)	0.31
Calcium-based phosphate binders, n (%)	221 (39.3%)	76 (35.8%)	145 (41.4%)	0.19

Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; HD, hemodialysis; iPTH, intact parathyroid hormone; SBP, systolic blood pressure; spKt/V, single-pool Kt/V; UF, ultrafiltration.

As shown in Table 1, there was no difference in the prevalence of diabetes mellitus between statin users and non-users in our cohort. However, statin users had a significantly lower prevalence of established CVD before HD initiation compared to non-users. They also exhibited a longer dialysis vintage, higher blood flow rate during HD sessions, higher dialysis adequacy, and higher hemoglobin and calcium levels. No significant differences were observed in other clinical markers or medication prescribing patterns between the groups.

Before HD initiation, only 62 of the 212 patients in the statin user group were taking statins, with the rest beginning treatment after HD initiation. Among statin users, rosuvastatin was the most common (56.1%), followed by atorvastatin (41.5%) and simvastatin (2.4%), with 82.1% receiving moderate-intensity doses and 17.9% high-intensity doses. At baseline, total cholesterol was lower in statin users, with no significant differences in LDL-C, HDL-C, triglycerides, or AIP. By the end of follow-up, statin users showed significantly lower total cholesterol, LDL-C, triglycerides, and AIP, and higher HDL-C compared to non-users, indicating more favorable lipid profile changes over time (Table 3).

Table 3

Statin types, doses, and lipid profile dynamics in statin users and non-users

Variable	Statin users (n = 212)	Non-users (n = 350)	*p*-value
Statin use characteristics			
Atorvastatin, n (%)	88 (41.5%)		
Rosuvastatin, n (%)	119 (56.1%)		
Simvastatin, n (%)	5 (2.4%)		
Moderate-intensity dosage, n (%)	174 (82.1%)		
High-intensity dosage, n (%)	38 (17.9%)		

Continuation of Table 1

Variable	Statin users (n = 212)	Non-users (n = 350)	*p*-value
Lipid profile dynamics			
Total cholesterol (mmol/L)			
Baseline	4.67 (3.78–5.39)	4.93 (4.14–5.77)	0.009
End of follow-up	4.43 (3.9–5.08)	5.07 (4.18–5.08)	<0.0001
LDL-C (mmol/L)			
Baseline	2.85 (2.23–3.56)	2.96 (2.31–3.86)	0.32
End of follow-up	2.68 (2.19–3.20)	3.10 (2.47-3.81)	<0.0001
HDL-C (mmol/L)			
Baseline	1.16 (0.89–1.41)	1.11 (0.91–1.37)	0.45
End of Follow-Up	1.21 (1.0–1.49)	1.05 (0.88–1.29)	<0.0001
Triglycerides (mmol/L)			
Baseline	1.8 (1.4–2.3)	1.9 (1.5–2.4)	0.22
End of follow-up	1.6 (1.2–2.1)	1.9 (1.4–2.5)	0.01
Atherogenic index of plasma			
Baseline	3.12 (2.50-4.08)	3.45 (2.45-4.54)	0.23
End of follow-up	3.23 (2.44-4.08)	3.56 (2.62-5.15)	0.01

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Statin use and AVF thrombosis. During the 5-year follow-up period, a total of 54 (9.6%) HD patients experienced AVF thrombosis events with 11 events (7.1%) in the statin user group and 43 events (10.6%) in the non-user group (2 = 6.4, p = 0.006). Additionally, 88 (15.7%) patients died with 27 (12.7%) in the statin user group, and 61 (17.4%) in the non-user group (2 = 2.1, p = 0.15).

Kaplan-Meier survival analysis revealed a significantly lower primary AVF thrombosis probability in statin users compared to non-users (Fig. 2).

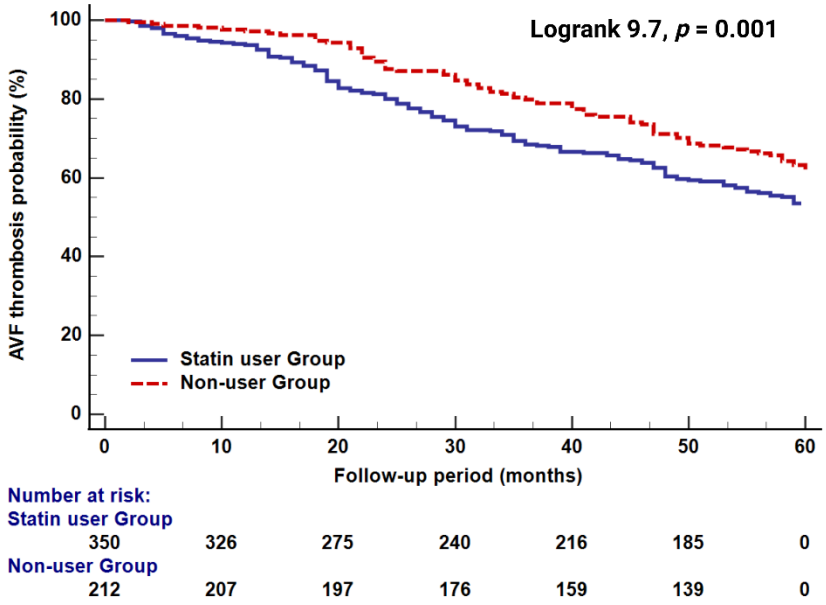

Fig. 2. Kaplan-Meier survival curves for time to AVF thrombosis in statin users vs. non-users.

Kaplan-Meier survival analysis stratified by statin dose intensity also revealed differences in AVF thrombosis-free survival (Fig. 3). Patients receiving high-intensity statins had the highest thrombosis-free survival, followed by those on moderate-intensity statins, with non-users showing the lowest survival (log-rank 10.90, p = 0.004).

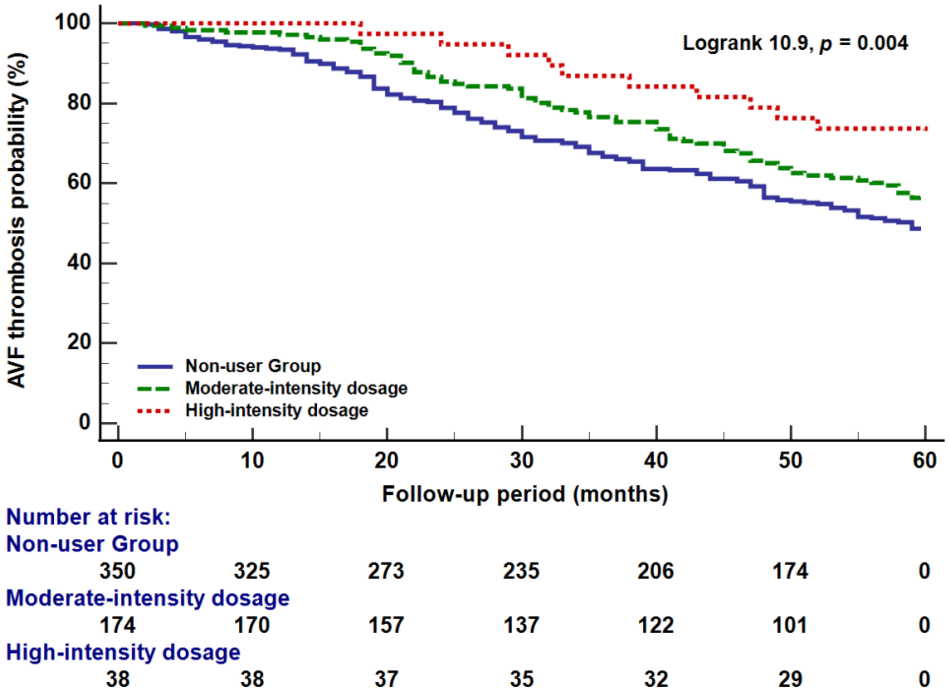

Fig. 3. Kaplan-Meier survival curves for time to primary AVF thrombosis by statin dose intensity.

Pairwise comparisons indicated lower thrombosis probabilities for moderate-intensity (p = 0.01) and high-intensity (p = 0.03) groups versus non-users, with no difference between high- and moderate-intensity (p = 0.52).

However, the Kaplan-Meier method does not account for death as a competing event, which occurred in 88 patients and may bias thrombosis risk. To address this, we employed the Fine and Gray subdistribution hazard model to estimate the effect of statin dose intensity on AVF thrombosis, accounting for competing mortality. In the Fine and Gray model, outcomes were defined as thrombosis, death without thrombosis, or censored. The unadjusted model showed a trend toward reduced thrombosis risk with increasing statin dose: moderate-intensity users had a subdistribution HR (sHR) of 0.87 (95% CI 0.72–1.18, p = 0.10), and high-intensity users had an sHR of 0.61 (95% CI 0.59–0.97, p = 0.03) compared to non-users. The overall association was significant (χ^2 = 8.01, df = 2, p = 0.018), supporting a dose-dependent effect.

Adjusting for age and diabetes, in addition to variables that differed significantly between groups in descriptive analyses (dialysis vintage, Kt/V, glucose, calcium, blood flow, and pre-HD CVD history), the Fine and Gray model did not determine statistically significant results for both moderate-intensity and high-intensity statin users compared to non-users (Table 4).

Table 4

Fine and Gray Subdistribution Hazard Models for AVF Thrombosis

Model	Exposure	HR (95% CI)	p-value
Unadjusted			
Dose Intensity	Moderate vs. Non-users	0.62 (0.30–1.08)	0.07
	High vs. Non-users	0.61 (0.59–0.97)	0.03
	Overall test	2 =8.01, df = 2	0.018
Adjusted			
Dose Intensity	Moderate vs. Non-users	0.67 (0.32–1.40)	0.16
	High vs. Non-users	0.57 (0.14–2.30)	0.26
	Overall test	2 =3.1, df = 2	0.21

Adjusted for age, diabetes, dialysis vintage, Kt/V, glucose, calcium, blood flow, and pre-HD CVD history.

HR = subdistribution hazard ratio; CI = confidence interval.

Discussion. This multicenter, retrospective cohort study evaluated the relationship between statin therapy and the risk of AVF thrombosis in patients undergoing HD, with a focus on the effect of statin dose intensity. Our findings suggest a dose-dependent protective effect, with Kaplan-Meier analysis showing a reduced probability of AVF thrombosis among statin users compared to non-users, and the lowest incidence in the high-intensity group. The unadjusted Fine and Gray model, accounting for the competing risk of death, further supported this trend, with a significant overall association driven by the high-intensity group. However, this association was attenuated after adjusting for confounders such as dialysis adequacy and pre-existing CVD, possibly due to limited events and unmeasured confounders.

The observed dose-dependent trend aligns with the biological mechanisms of statins, which extend beyond their lipid-lowering effects. Statins reduce vascular inflammation by decreasing C-reactive protein levels [19], inhibit platelet aggregation through antithrombotic pathways [7, 8], and improve endothelial function by upregulating nitric oxide production [8, 19, 20]. These mechanisms may mitigate the pro-thrombotic and inflammatory milieu often present in HD patients, where AVF thrombosis is driven by intimal hyperplasia, shear stress, and endothelial dysfunction [21, 22]. The more pronounced effect in the high-intensity group could reflect a greater suppression of these pathways, as higher doses achieve more significant reductions in inflammatory markers [19]. This suggests that the vascular benefits of statins in HD patients may be dose-dependent for maintaining AVF patency, a hypothesis that warrants further mechanistic studies.

Our findings are consistent with prior observational studies demonstrating a beneficial role of statins in AVF outcomes. Chang et al. [9] and Marinez et al. [23] reported improved AVF patency with statin use. Suh et al. specifically noted a reduction in AVF thrombosis in diabetic HD patients [24], aligning with our unadjusted results. In a recent meta-analysis, Bo-Jiang et al. found that AVF patency rates were significantly higher among statin-treated hemodialysis patients in the Asian population compared to controls [25]. Furthermore, consistent with our findings, Yamazaki et al. identified elevated LDL-C levels as an independent risk factor for reduced AVF primary patency [26]. In contrast, larger post hoc analyses [12] and a meta-analysis [10] have not demonstrated a significant association between statin use and AVF patency. This discrepancy may stem from methodological limitations in prior work, including a lack of dose stratification. When studies group all statins together without stratifying by type or dose, these specific benefits may be diluted, especially if low-intensity or less effective statins predominate in the study population [10, 23].

The clinical implications of our findings are twofold. First, the dose-dependent trend suggests that high-intensity statins could offer a targeted strategy to reduce AVF thrombosis. Clinicians might consider dose escalation in patients at high risk for AVF failure, balancing this against potential risks such as myopathy, which is more common in HD patients due to altered drug metabolism [27]. Second, the non-significant adjusted results highlight the need for personalized approaches, as the benefits of statins may vary based on patient-specific factors like dialysis adequacy or cardiovascular burden. Future studies should explore these interactions, potentially using machine learning to identify subgroups most likely to benefit from statin therapy. Moreover, larger prospective studies or RCTs are needed to confirm the dose-dependent effect, stratifying by individual statin types and doses to address the methodological limitations of prior works. Therefore, the negative or neutral findings in larger studies may reflect these methodological limitations, rather than a true absence of effect.

A key strength of our study is the use of the Fine and Gray models to account for competing risks, which is critical in HD populations with high mortality rates (15.7% in our cohort). However, our adjusted model's loss of significance underscores challenges in isolating statin effects in HD patients. This attenuation likely reflects the low number of events (54 total), reducing statistical power and widening confidence intervals. Additionally, HD patients have altered lipid metabolism [28] and a predominance of non-traditional risk factors such as chronic inflammation and oxidative stress [29], which may modify statins' effectiveness and were not fully captured in our dataset.

Several limitations must be considered when interpreting our findings. First, the retrospective design introduces potential selection bias and confounding. Although we adjusted for several variables that differed significantly between statin users and non-users, the influence of residual or unmeasured confounding cannot be completely ruled out. Second, the low number of events limited statistical power, particularly in the adjusted Fine and Gray model. Third, selection bias may have influenced the results. Patients prescribed statins may have been more likely to receive comprehensive cardiovascular care or closer monitoring, which could have contributed to better vascular access outcomes independent of statin therapy itself. Additionally, statin users had a significantly lower prevalence of pre-existing CVD in our cohort, which may reflect prescribing patterns not entirely accounted for in our adjusted models. Fourth, the majority of statin users received moderate-intensity therapy, limiting our power to detect a dose-response relationship between high-intensity statin use and AVF thrombosis risk. Moreover, simvastatin use was minimal, and no patients received combination lipid-lowering therapy, further narrowing the scope of our analysis. Finally, the study population was limited to patients from a single dialysis network in Ukraine, which may affect the generalizability of our findings to other regions or healthcare systems with different patient demographics, statin prescribing patterns, or dialysis practices. Future prospective studies and ran-

domized controlled trials are needed to validate these findings and clarify the potential role of statin therapy intensity in preserving AVF patency among patients undergoing HD.

Conclusions. Our study suggests a dose-dependent protective effect of statins against AVF thrombosis in HD patients, with high-intensity statins showing a significant unadjusted association. However, the loss of significance after adjustment and the limited number of events underscore the need for larger, prospective studies. Future research should focus on optimizing statin dosing and incorporating broader clinical variables to better understand the role of statins in improving vascular access outcomes in patients undergoing HD.

Author contributions.

Natalia Stepanova: conceptualization, methodology, formal analysis, visualization, writing - original draft;

Tetyana Ostapenko: investigation, writing - review & editing;

Valeriia Marchenko, Alina Holovanova, Mariia Lysii, Tetyana Kucher, Viacheslav Filonov, Victor Dzhur, Stetsenko Bohdan, Hanna Maroid, Nataliia Pavchak, Kateryna Rusyn, Oksana Rusyn, Bohdan Radiuk: data curation. All the authors reviewed the manuscript and approved it for publication.

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Quantifying the expression levels of *mexA* and *mexB* genes in response to the efflux pump inhibitor PAβN in ciprofloxacin-resistant *Pseudomonas aeruginosa* isolated from urinary tract infections

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Abstract. *Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, occurring in both community and healthcare settings. Pseudomonas aeruginosa, a Gram-negative opportunistic pathogen, is one of the five most significant nosocomial bacteria and a major contributor to UTIs. This study aimed to quantify the expression levels of efflux pump genes mexA and mexB in response to the efflux pump inhibitor phenylalanine-arginine β-naphthylamide (PAβN) in ciprofloxacin-resistant P. aeruginosa isolates.*

Methods. Fifty urine specimens were collected from UTI patients at various hospitals in Baghdad. Specimens were directly cultured by streaking on differential media. Five ciprofloxacin-resistant P. aeruginosa isolates were identified, with resistance confirmed using the disk diffusion method for antibiotic susceptibility. The broth microdilution method was employed to determine the minimum inhibitory concentration (MIC) of ciprofloxacin (CIP) alone and in combination with PAβN to assess PAβN's inhibitory activity. RNA was extracted and purified from the bacterial isolates, followed by reverse transcription and quantitative PCR to evaluate the expression of efflux pump-related genes. The expression levels of mexA and mexB were measured in the presence of the tested compounds using quantitative PCR.

Results. Antibiotic susceptibility testing revealed that the isolates were resistant to nearly all antibiotics tested, except piperacillin-tazobactam, which was effective against 64% of the isolates. None of the five selected isolates showed sensitivity to ciprofloxacin. The MIC for ciprofloxacin ranged from 31.25 to 62.5 mg/L, while the sub-MIC in the presence of PAβN was significantly reduced, ranging from 7.81 to 15.62 mg/L. The expression levels of mexA and mexB genes decreased significantly in three of the five isolates when exposed to PAβN and ciprofloxacin compared to ciprofloxacin alone, with expression levels reduced from 1.319 to 0.574, 0.159 to 0.008, and 194.0 to 4.9, respectively. However, two isolates exhibited overexpression of these genes.

Conclusions. The presence of PAβN significantly reduced ciprofloxacin MICs in most ciprofloxacin-resistant P. aeruginosa isolates in vitro. The expression levels of mexA and mexB genes decreased in most isolates when PAβN was used in combination with ciprofloxacin, suggesting that PAβN could enhance the efficacy of ciprofloxacin. These findings indicate that PAβN may be a promising adjunctive antimicrobial agent for treating UTIs caused by resistant P. aeruginosa.

Keywords: *Pseudomonas aeruginosa, efflux pumps, mexA gene, mexB gene, phenylalanine-arginine β-naphthylamide, multidrug resistance, urinary tract infections.*

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Салва А. Абдул Хусейн, Абдуламір М. Гаріб

Кількісне визначення рівнів експресії генів *mexA* та *mexB* у відповідь на інгібітор ефлюксних насосів PAβN у ципрофлоксацин-резистентних ізолятах *Pseudomonas aeruginosa*, виділених у пацієнтів з інфекцією сечової системи

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Резюме. *Інфекції сечової системи (ІСС) є однією з найпоширеніших бактеріальних інфекцій у світі. Pseudomonas aeruginosa, грамнегативний опортуністичний патоген, є одним із п'яти найзначніших нозокоміальних бактерій і основним чинником ІСС. Це дослідження мало на меті кількісно оцінити рівні експресії генів ефлюксних насосів mexA та mexB у відповідь на інгібітор ефлюксних насосів фенілаланін-аргінін β-нафтиламін (PAβN) у ципрофлоксацин-резистентних ізолятах P. aeruginosa.*

Методи. П'ятдесят зразків сечі було зібрано від пацієнтів з ІСС у різних лікарнях Багдада. Зразки безпосередньо культивували шляхом нанесення на диференційні середовища. Було ідентифіковано п'ять ципрофлоксацин-резистентних ізолятів P. aeruginosa, резистентність яких підтверджена методом дискової дифузії для визначення чутливості до антибіотиків. Метод мікродилюції в бульйоні використовували для визначення мінімальної інгібуючої концентрації (МІК) ципрофлоксацину (CIP) окремо та в комбінації з PAβN для оцінки інгібуючої активності PAβN. РНК екстрагували та очищали з бактеріальних ізолятів, після чого проводили зворотну транскрипцію та кількісну ПЛР для оцінки експресії генів, пов'язаних із ефлюксними насосами. Рівні експресії mexA та mexB вимірювали в присутності досліджуваних сполук за допомогою кількісної ПЛР.

Результати. Тестування на чутливість до антибіотиків показало, що ізоляти були резистентними до майже всіх протестованих антибіотиків, за винятком піперациліну-тазобактаму, який був ефективним проти 64% ізолятів. Жоден із п'яти відібраних ізолятів не виявив чутливості до ципрофлоксацину. МІК для ципрофлоксацину варіювала від 31,25 до 62,5 мг/л, тоді як суб-МІК у присутності PAβN значно знижувалася, варіюючи від 7,81 до 15,62 мг/л. Рівні експресії генів mexA та mexB значно знизилися в трьох із п'яти ізолятів при дії PAβN і ципрофлоксацину порівняно з ципрофлоксацином окремо, зі зниженням рівнів експресії з 1,319 до 0,574, з 0,159 до 0,008 та з 194,0 до 4,9 відповідно. Однак два ізоляти показали надмірну експресію цих генів.

Висновки. Присутність PAβN значно знизила МІК ципрофлоксацину в більшості ципрофлоксацин-резистентних ізолятів P. aeruginosa in vitro. Рівні експресії генів mexA та mexB знизились в більшості ізолятів при комбінованому використанні PAβN із ципрофлоксацином, що свідчить про те, що PAβN може підвищувати ефективність ципрофлоксацину. Ці результати вказують на те, що PAβN може бути перспективним допоміжним антимікробним агентом для лікування ІСШ, спричинених резистентними P. aeruginosa.

Ключові слова: *Pseudomonas aeruginosa, ефлюксні насоси, ген mexA, ген mexB, фенілаланін-аргінін β-нафтиламін, множинна резистентність, інфекції сечовивідних шляхів.*

Introduction. Urinary tract infections (UTIs) rank among the most prevalent bacterial infections worldwide, occurring in both community and healthcare settings. The primary microorganisms implicated in UTIs include *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae*, and *Streptococcus faecalis*, alongside various other microorganisms, including fungi and certain viruses [1, 2].

Pseudomonas aeruginosa is a gram-negative, opportunistic pathogen and is recognized as one of the five most significant nosocomial bacteria globally. It is a rod-shaped bacterium measuring 1–5 μm in length and 0.5–1.0 μm in width, non-spore-forming, and motile via one or two polar flagella [3]. This aerobic bacterium thrives in diverse environments, including soil, plants, and mammalian tissues. It can persist on medical devices and various surfaces by utilizing essential adhesion components such as flagella, pili, and biofilms.

P. aeruginosa is a major contributor to antibiotic resistance and hospital-acquired infections, commonly found on hospital floors, in operating rooms, and on surgical instruments. It is capable of surviving disinfectants and certain sterilization processes [4, 5]. This pathogen is often associated with healthcare-related infections such as ventilator-associated pneumonia (VAP), intensive care unit infections, central line-associated bloodstream infections, surgical site infections, urinary tract infections, burn wound infections, keratitis, and otitis media [6].

The increased mortality rate among patients suffering from these infections is largely attributed to *P.*

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aeruginosa's adaptability to environmental changes and its ability to rapidly develop resistance to antibiotics [7, 8]. The growing incidence of nosocomial infections caused by multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains of *P. aeruginosa* poses a significant challenge to antimicrobial therapy [9, 10].

Infections caused by *P. aeruginosa* are difficult to treat due to both intrinsic and acquired resistance mechanisms. Intrinsic resistance mechanisms include reduced outer membrane permeability, inducible β-lactamase production, and the activity of multidrug efflux systems [11]. *P. aeruginosa* possesses several multidrug efflux systems—MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM—which are major contributors to drug resistance in clinical isolates [12]. Based on this, the current study focuses particularly on these systems.

Among these, MexAB-OprM is regarded as the most crucial efflux pump due to its ability to expel a wide range of antibiotics, including fluoroquinolones, chloramphenicol, and certain β-lactams [13, 14].

Numerous natural compounds have shown the ability to inhibit efflux pumps, notably Phenylalanine-Arginine β-Naphthylamide (PAβN), a peptidomimetic compound recognized as a potent inhibitor of RND efflux pumps in gram-negative bacteria [15]. PAβN works through competitive inhibition, being preferentially expelled by efflux pumps instead of antibiotics, thus allowing antibiotics to accumulate inside bacterial cells. Additionally, PAβN diminishes quorum sensing (QS) signaling molecules without affecting bacterial viability and effectively inactivates virulence factors such as elastase, protease, pyocyanin, and bacterial motility [16].

The rise of antibiotic-resistant microorganisms has spurred research into novel therapeutic combinations that protect antibiotics from degradation by resistant bacterial enzymes [17]. Therefore, the present study aims to inhibit efflux pumps using chemical agents known as efflux pump inhibitors (EPIs), such as PAβN, to enhance the efficacy of antibiotics and increase the susceptibility of MDR isolates.

Material and methods. *Isolation and identification of Pseudomonas aeruginosa*. Fifty urine specimens were collected from UTI patients at various hospitals in Baghdad. The specimens were streaked onto blood agar, MacConkey agar, and cetrimide agar, and incubated aerobically at 37°C for 24 hours.

Morphological identification was conducted by gram staining and macroscopic examination. Confirmation of *P. aeruginosa* was achieved using the VITEK 2 Compact system and molecular detection methods [18].

Antibiotic susceptibility testing (Disc diffusion method). The Kirby-Bauer disc diffusion method was employed to assess the antibiotic susceptibility of bacterial isolates [19]. The inhibition zones were measured in millimeters and interpreted following the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. The antibiotics tested included: Piperacillin (100 µg), Ceftazidime (30 µg), Cefepime (30 µg), Ticarcillin-clavulanate (75/10 µg), Piperacillin-tazobactam (100/10 µg), Aztreonam (30 µg), Imipenem (10 µg), Meropenem (10 µg), Gentamicin (10 µg), Amikacin (30 µg), Levofloxacin (5 µg), and Ciprofloxacin (5 µg).

Estimation of Minimal Inhibitory Concentration (MIC) by broth microdilution method. The MIC of ciprofloxacin against P. aeruginosa was determined using the microtiter broth dilution method with resazurin dye, following CLSI guidelines [20].

- Bacterial suspensions were adjusted to 0.5 McFarland standard, then diluted in Mueller-Hinton broth to achieve an inoculum of approximately 1.5×10^5 CFU/ml.
- Ciprofloxacin solutions (1–512 µg/ml) were prepared from a 1024 µg/ml stock solution.
- Following 24 hours of incubation, 20 µl of resazurin dye was added to each well and incubated for 30 minutes.
- MIC values were visually determined as the lowest concentration where no color change (from blue to pink) was observed.

Assessment of the synergistic effect of the efflux pump inhibitor PaβN. The synergistic effects of PAβN and ciprofloxacin were evaluated by broth microdilution. Bacterial suspensions were incubated with ciprofloxacin in the presence and absence of 50 µg/ml PAβN. A 2 µL aliquot of the 5 mg/mL PAβN stock was added to each well simultaneously with the antibiotic.

MexA and MexB genes expression. Five *P. aeruginosa isolates* (PA8, PA20, PA44, PA45, and PA50) were selected based on their source (UTI patients), efflux pump activity, strong biofilm formation, and resistance to ciprofloxacin.

- Planktonic cells were cultured overnight in Mueller-Hinton broth at 37°C.
- The effects of ciprofloxacin alone and ciprofloxacin + PAβN on MexA and MexB expression were assessed at sub-MIC concentrations.
- RNA was extracted using the Direct-zol™ RNA MiniPrep Kit (ZYMO RESEARCH, USA) following the manufacturer's protocol.
- Gene expression was quantified by qPCR using 16S rRNA as the housekeeping gene.
- Reactions and thermocycler conditions are detailed in Tables 1 and 2.
- Fold changes in gene expression were calculated using the ΔΔCT method [21].

Molecular methods such as PCR are powerful tools for diagnosing most pathogenic microorganisms responsible for urinary tract infections [22–26].

Table 1

Components of qRT-PCR used in MexA and MexB gene expression analysis

Component	Volume (µL)	Final Concentration
TAKARA qPCR Master Mix (2X) Universal	10.0	2X
Forward Primer	0.4	0.2 µM
Reverse Primer	0.4	0.2 µM
Nuclease-Free Water	Up to 20.0	—
Template DNA	—	1 pg – 100 ng

Table 2

qRT-PCR thermocycling conditions used in this study

Step	Temperature (°C)	Time	Cycle(s)
Enzyme Activation	95	5 min	1
Denaturation	95	20 sec	40
Annealing	60	20 sec	
Extension	72	20 sec	

Result. Isolation of Pseudomonas aeruginosa. Among the urine samples collected, Pseudomonas aeruginosa was isolated from 28% of the samples, while the remaining 72% were negative for this bacterium.

Antibiotic sensitivity of Pseudomonas aeruginosa. The antibiotic susceptibility of P. aeruginosa to selected antibiotics is shown in Table 3. In general, the isolates exhibited resistance to most antibiotics tested, with the exception of piperacillin-tazobactam (TZP), which was effective against 64% of the isolates.

Table 3

Antibiotic sensitivity of Pseudomonas aeruginosa to selected antibiotics

Antibiotic	Resistant	Intermediate	Sensitive
Piperacillin	44 (88%)	4 (8%)	2 (4%)
Piperacillin-tazobactam	10 (20%)	8 (16%)	32 (64%)
Ticarcillin-clavulanate	46 (92%)	4 (8%)	0 (0%)
Amikacin	23 (46%)	0 (0%)	27 (54%)
Gentamicin	26 (52%)	1 (2%)	23 (46%)
Aztreonam	24 (48%)	5 (10%)	21 (42%)
Cefepime	28 (56%)	0 (0%)	22 (44%)
Ceftazidime	29 (58%)	2 (4%)	19 (38%)
Ciprofloxacin	27 (54%)	2 (4%)	21 (42%)
Levofloxacin	29 (58%)	2 (4%)	19 (38%)
Imipenem	25 (50%)	1 (2%)	24 (48%)
Meropenem	26 (52%)	0 (0%)	24 (48%)

Determination of ciprofloxacin MIC. The MIC of ciprofloxacin against P. aeruginosa was determined using the 96-well microtiter plate method. The MIC ranged from 62.5 mg/L to 125 mg/L, while sub-MIC values ranged from 31.25 mg/L to 62.5 mg/L.

Determination of Ciprofloxacin + PAβN MIC. When combined with the efflux pump inhibitor PAβN, the MIC of ciprofloxacin was significantly reduced, ranging from 31.25 mg/L to 15.62 mg/L. The sub-MIC concentrations were between 15.62 mg/L and 7.81 mg/L. These results are summarized in Table 4.

Table 4

MIC and sub-MIC of ciprofloxacin alone and in combination with PAβN

Isolate No.	ID	Ciprofloxacin MIC (mg/L)	Sub-MIC (mg/L)	CIP + PAβN MIC (mg/L)	Sub-MIC (mg/L)
1	8	125	62.5	31.25	15.62
2	20	125	62.5	31.25	15.62
3	44	125	62.5	31.25	15.62
4	45	125	62.5	31.25	15.62
5	50	125	62.5	15.62	7.81

MexA and MexB gene expression following treatment with ciprofloxacin and ciprofloxacin + PaβN. Five isolates were used to evaluate the expression of *MexA* and *MexB* genes under treatment with ciprofloxacin alone and in combination with PAβN. Expression levels are presented in Tables 5 and 6, respectively.

Table 5

Expression levels of MexA gene following ciprofloxacin and ciprofloxacin + PAβN treatment

No.	Isolate ID	Sub-MIC CIP	Sub-MIC CIP+PAβN	Control ΔΔCt	Fold Change (Control)	ΔΔCt CIP	Fold Change CIP	ΔΔCt CIP+PAβN	Fold Change CIP+PAβN
1	8	62.5	15.62	0	1.0	-0.4	1.32	0.8	0.57
2	20	62.5	15.62	0	1.0	-1.5	2.8	-4.4	21.1
3	44	62.5	15.62	0	1.0	2.65	0.16	6.85	0.008
4	45	62.5	15.62	0	1.0	-7.6	194.0	-2.3	4.9
5	50	62.5	7.81	0	1.0	0.19	0.9	-0.87	1.8

Table 6

Expression levels of MexB gene following ciprofloxacin and ciprofloxacin + PAβN treatment

No.	Isolate ID	Sub-MIC CIP	Sub-MIC CIP+PAβN	Control ΔΔCt	Fold Change (Control)	ΔΔCt CIP	Fold Change CIP	ΔΔCt CIP+PAβN	Fold Change CIP+PAβN
1	8	62.5	15.62	0	1.0	-5.55	46.85	-3.35	10.20
2	20	62.5	15.62	0	1.0	-1.0	2.0	-1.6	3.0
3	44	62.5	15.62	0	1.0	2.95	0.13	4.8	0.036
4	45	62.5	15.62	0	1.0	-6.5	90.5	1.5	0.3
5	50	62.5	7.81	0	1.0	0.67	0.6	-0.96	1.9

Figure 1 shows PCR results for 16S rRNA, MexA, and MexB.

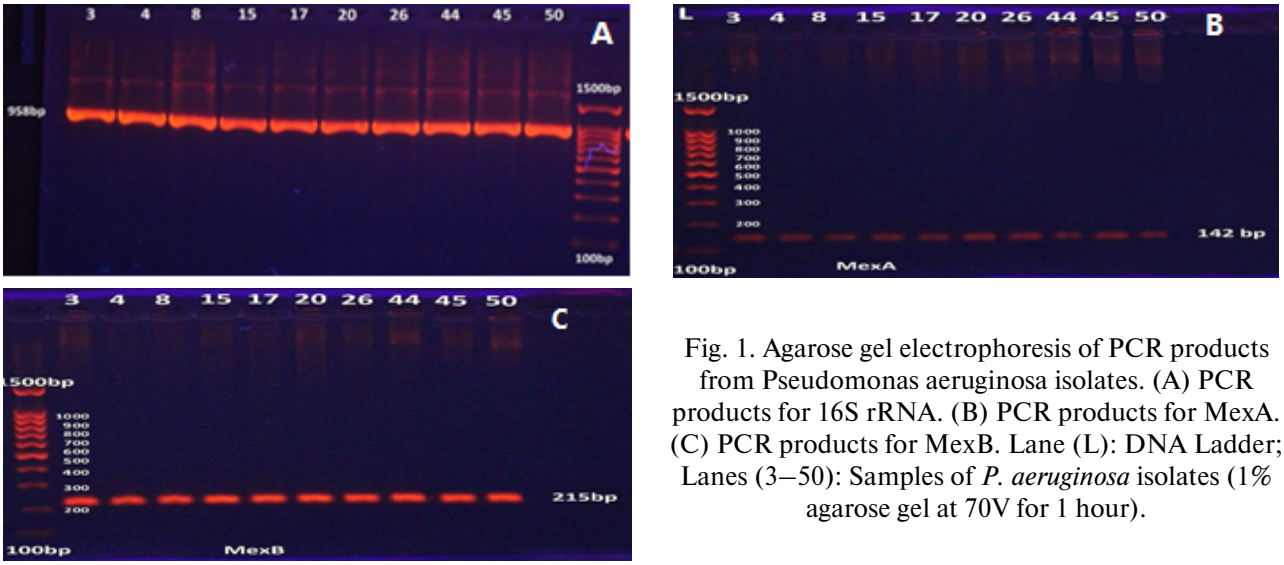

Fig. 1. Agarose gel electrophoresis of PCR products from Pseudomonas aeruginosa isolates. (A) PCR products for 16S rRNA. (B) PCR products for MexA. (C) PCR products for MexB. Lane (L): DNA Ladder; Lanes (3–50): Samples of *P. aeruginosa* isolates (1% agarose gel at 70V for 1 hour).

Discussion. *Isolation and identification of Pseudomonas aeruginosa*. The result of the isolation of *P. aeruginosa* showed that 28% of the total samples were positive. This finding aligns with several other studies that reported similar percentages of positive isolates, such as Mohamed et al. (2019) [27], who recorded 27%. However, our findings differ from those of Boushra et al. (2024) [28], who reported a higher percentage of 40%. This variation may be attributed to geographic, climatic, hygienic, and social factors [29].

Antibiotic sensitivity. Antibiotic sensitivity testing was performed using the Kirby-Bauer method. Our isolates appeared to be resistant to nearly all antibiotics tested, except Piperacillin-tazobactam, which was effective against 64% of the isolates. Notably, none of the five selected isolates in our study showed any sensitivity to ciprofloxacin, as shown in Table (3). The results were compared with other studies conducted both within and outside Iraq. These studies showed inconsistent results, which may be attributed to differences in clinical specimens, population-related social factors, and levels of exposure to antimicrobial agents, as reported by Ahmed Hasan et al. (2020) [30, 31].

Determination of MIC and sub-MIC. MIC determination was performed using the 96-well microtiter plate method for ciprofloxacin and its combination with PaβN. The MIC for ciprofloxacin alone ranged from 62.5 mg/L to 125 mg/L, indicating complete resistance of the isolates to ciprofloxacin. The sub-MIC values ranged from 31.25 mg/L to 62.5 mg/L, representing concentrations at which the bacteria could still grow [32, 33].

When ciprofloxacin was combined with PaβN, both the MIC and sub-MIC values decreased significantly. MIC values ranged from 31.25 mg/L to 15.62 mg/L, while sub-MIC values ranged from 15.62 mg/L to 7.81 mg/L. This suggests that the combination was more effective at inhibiting bacterial growth. These findings indicate that PaβN can suppress virulence factors, especially efflux pumps, in resistant *P. aeruginosa* strains, thereby reducing resistance and enhancing the effectiveness of ciprofloxacin [15, 16].

MexA and MexB gene expression. Gene expression data presented in Tables (5) and (6) showed that PaβN significantly reduced the expression levels of *MexA* and *MexB* genes in most isolates ($P \leq 0.05$). This supports the idea that combining ciprofloxacin with PaβN can reduce bacterial resistance and confirms the relationship between the overexpression of these genes and resistance to ciprofloxacin alone [16].

Our findings showed generally low MexA and MexB expression among the resistant isolates, which contrasts with the results of Abdolhosseini et al. [34], who did not use an efflux pump inhibitor. The reduction in gene expression in our study may support the hypothesis of synergism between PaβN and ciprofloxacin [35], indicating a potential bactericidal effect of the combination against *P. aeruginosa* [36].

Interestingly, isolates 2 and 5 exhibited different patterns in gene expression fold changes, suggesting possible mutations in regulatory genes that affect efflux pump expression, as suggested by Hadir et al. [37]. Due to a lack of references regarding such mutations, we recommend further research on isolates with this behavior.

Many recent studies have explored alternative antimicrobial strategies using chemical, biological, and physical approaches. Examples include the use of methanolic extracts of *Cladophora glomerata* and *Spirulina platensis* as antimicrobial agents [38, 39], *Lactobacillus acidophilus* supernatants against *P. aeruginosa* [26], essential oils of *Mentha spicata* to inhibit *Proteus mirabilis* biofilms [40], nickel oxide nanoparticles [41], nano-zirconium oxide particles combined with selected antibiotics against *E. coli* and *K. pneumoniae* [42], and even the application of audible sounds and magnetic fields on methicillin-resistant *Staphylococcus aureus* [43].

This study faced several limitations. First, the time constraints for sample collection affected the outcomes. Second, there were delays in receiving the PaβN compound from the supplier. Third, although real-time PCR was used to analyze gene expression, the reliance on conventional PCR and electrophoresis for some procedures limited precision.

Conclusions. In conclusion, the addition of PaβN led to a significant reduction in ciprofloxacin MICs in most ciprofloxacin-resistant *P. aeruginosa* isolates in vitro, indicating its potential as a promising antimicrobial adjuvant. The gene expression analysis revealed a clear decrease in *MexA* and *MexB* expression when ciprofloxacin was combined with PaβN in most tested isolates. This suggests that additional regulatory factors, such as mutations or plasmid loss, may influence gene expression in the presence of PaβN. Our study raises important questions about antibiotic combination therapies and highlights the need for further research to enhance antimicrobial strategies.

Ethical statement. This study was approved by the Ethics Commission of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq (Reference No. 30 / 7289, dated 30/11/2023).

Conflicts of interest. The authors declare no conflicts of interest.

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Data availability statement. The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions. Both authors contributed equally to the development of the research plan, statistical analysis of results, and manuscript writing.

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The prognostic and diagnostic value of fibroblast growth factor 23 in patients undergoing hemodialysis

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Abstract. *Fibroblast growth factor 23 (FGF23) is a phosphotropic hormone secreted by osteoblasts and osteocytes into the systemic circulation. It exerts its effects on the kidneys, parathyroid glands, heart, and bones. FGF23 is a critical phosphaturic hormone that, alongside parathyroid hormone (PTH), regulates phosphate reabsorption and calcitriol ($1,25(OH)_2D$) synthesis in the kidneys. The present study aimed to evaluate the diagnostic and prognostic significance of fibroblast growth factor 23 in patients undergoing hemodialysis.*

Methods. A total of 88 patients were examined in this cross-sectional study. The cohort comprised 36 women (40.9%; 95% CI 30.64–51.18) and 52 men (59.1%; 95% CI 48.82–69.36), with a mean age of 55.81 ± 13.14 years. Group 1 consisted of 69 patients with stage 5 chronic kidney disease (CKD) receiving renal replacement therapy via hemodialysis, while Group 2 included 19 patients with stage 3 CKD.

Results. FGF23 levels were elevated in 67 patients (97.1%; 95% CI 91.87–99.72) in Group 1, with a median (Me) of 1258.32 pg/mL (interquartile range [IQR] 169.46–1338.46). In Group 2, FGF23 levels were elevated in 18 patients (94.7%; 95% CI 80.58–100), with a median of 150.5 pg/mL (IQR 74.22–929.12). A significant difference was observed between the groups ($p < 0.05$). The median duration of hemodialysis in Group 1 was 15 months (IQR 8–36). In Group 1, correlation analysis revealed weak associations between FGF23 and phosphorus ($r = 0.13$; $p > 0.05$), total calcium ($r = 0.04$; $p < 0.05$), ionized calcium ($r = 0.02$; $p < 0.05$), and parathyroid hormone ($r = 0.08$; $p > 0.05$). Significant correlations were found between FGF23 and creatinine ($r = 0.41$; $p < 0.005$), urea ($r = 0.33$; $p < 0.005$), urine volume ($r = -0.75$; $p < 0.005$), and hemodialysis duration ($r = 0.57$; $p < 0.005$). Regression analysis for predicting residual urine volume based on FGF23, creatinine, urea, and hemodialysis duration yielded an R^2 of 0.7369, F-statistic of 92.45 ($p < 0.0001$), standard error of residuals of 5.843, and residual degrees of freedom of 66.

Conclusions. The weak correlations between FGF23 and calcium-phosphorus metabolism indicate that FGF23 is not a suitable diagnostic marker for mineral and bone disorders (CKD-MBD) in patients undergoing hemodialysis. However, FGF23 is a significant predictor of residual urine volume in hemodialysis patients, as demonstrated by the regression analysis. The model, incorporating FGF23 and hemodialysis duration, explains 73.7% of the variation in urine volume, highlighting its strong prognostic capability. These findings underscore the clinical significance of FGF23 as a biomarker for assessing residual renal function in dialysis patients.

Keywords: *fibroblast growth factor 23, chronic kidney disease, hemodialysis, calcium-phosphate metabolism, diuresis.*

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Прогностична та діагностична значимість фактора росту фібробластів 23 у пацієнтів, які лікуються методом гемодіалізу

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Резюме. *Фактор росту фібробластів 23 (ФРФ23) є фосфотропним гормоном. Він секретується остеобластами та остеоцитами в системний кровообіг і реалізує свій вплив у нирках, паращитовидних залозах, серці та кістках. ФРФ23 є критичним фосфатуричним гормоном, який, разом з паратиреоїдним гормоном (ПТГ), регулює рециркуляцію фосфатів та синтез кальцитріолу (1,25(OH)2D) в нирках. Метою цієї роботи було встановити діагностичну та прогностичну значимість фактора росту фібробластів 23 у пацієнтів, які лікуються гемодіалізом.*

Методи. До цього одномоментного дослідження було залучено 88 пацієнтів. Серед обстежених хворих було 36 (40.9%; 95% ДІ 30.64-51.18) жінок та 52 (59.09%; 95% ДІ 48.82-69.36) чоловіків, середній вік – 55.81±13.14 років. 1 групу склали 69 пацієнтів з ХХН 5 стадії, які знаходяться на замісній нирковій терапії методом гемодіалізу, 2 групу 19 пацієнтів з ХХН 3ст.

Результати. Рівні ФРФ23 були підвищені у 67 (97.1%; 95% ДІ 91.87-99.72) в 1 групі Me 1258.32 (169.46; 1338.46) пг/мл, у той час у групі 2 рівень ФРФ23 був вище норми у 18 хворих (94.74%; 95% ДІ 80.58-100) Me 150.5 (74.22; 929.12). Було виявлено достовірну різницю між групами ($p<0.05$). Тривалість проведення гемодіалізу становила (місяцях) Me 15 (8; 36). В 1 групі було проведено кореляційний аналіз між ФРФ23 та фосфором($r=0.13$; $p>0,05$), Са (заг)($r=0.04$; $p<0.05$), Са (іон)($r=0.02$; $p<0.05$), паратгормоном($r=0.08$; $p>0.05$). Також в 1 групі встановлено кореляційні зв'язки між ФРФ23 та креатиніном($r=0.41$; $p<0.005$), сечовиною($r=0.33$; $p<0.005$), об'ємом діурезу($r=-0.75$; $p<0.005$), тривалістю гемодіалізу($r=0.57$; $p<0.005$). Результати регресійного аналізу для прогнозування кількості залишкового діурезу в залежності від ФРФ23, креатиніну, сечовини, тривалості гемодіалізу: $R^2=0.7369$. F-статистика: 92.45 ($p<0.0001$). Стандартна помилка залишків: 5.843. Ступені свободи залишків: 66.

Висновки. Слабкі кореляції ФРФ23 з кальцієво-фосфорним обміном роблять його непридатним діагностичним маркером порушень КФО у пацієнтів на програмному гемодіалізі. ФРФ23 є значущим предиктором об'єму діурезу у пацієнтів на гемодіалізі згідно з результатами регресійного аналізу. Модель, яка включає ФРФ23 та тривалість гемодіалізу, пояснює 73.7% варіації діурезу, що свідчить про її високу прогностичну здатність. Це підкреслює клінічну значимість ФРФ23 як біомаркера для оцінки залишкової функції нирок у пацієнтів на діалізі.

Ключові слова. *Фактор росту фібробластів 23, хронічна хвороба нирок, гемодіаліз, кальцієво-фосфорний обмін, діурез.*

Introduction. Fibroblast growth factor 23 (FGF23) is a phosphotropic hormone. It is secreted by osteoblasts and osteocytes into the systemic circulation and exerts its effects on the kidneys, parathyroid glands, heart, and bones. FGF23 is a critical phosphaturic hormone that, along with parathyroid hormone (PTH), regulates phosphate recirculation and calcitriol (1,25(OH)2D) synthesis in the kidneys [1]. In the parathyroid gland and kidneys, FGF23 suppresses PTH secretion and reduces the production of active vitamin D. By acting on the epithelial cells of the proximal tubules, FGF23 reduces the surface expression of sodium/phosphate cotransporters, thereby decreasing renal phosphate reabsorption. The cumulative physiological effect of FGF23 is to increase renal phosphate excretion and decrease systemic phosphate levels. However, in chronic kidney disease (CKD), phosphate excretion and the renal effects of FGF23 are reduced due to the loss of functional renal mass and decreased α-Klotho expression, leading to high serum phosphate and FGF23 levels. Starting from the early stages of CKD, serum FGF23 levels progressively increase to maintain normal phosphate levels, but cannot promote renal phosphate excretion as nephron function and sensitivity to FGF23 decrease. In patients with end-stage renal disease (ESRD) who are dependent on renal replacement therapy, FGF23 levels can reach 1000 times the normal range. Elevated FGF23 levels are associated with various pathological conditions, including secondary hyperparathyroidism, osteoporosis, cardiac remodeling, and increased risk of cardiovascular mortality in CKD patients [2].

FGF23 contributes to maintaining phosphate, calcium, 1,25(OH)2D, and PTH homeostasis. Additionally, factors such as iron deficiency, erythropoietin, and inflammation play a significant role in FGF23 synthesis and degradation [3].

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In patients with CKD, a strong association is observed between elevated FGF23 levels and an increased risk and progression of vascular calcification [4]. FGF23 is considered a potential prognostic biomarker for vascular calcification and cardiovascular events in this population [5]. The consistent correlation between high FGF23 levels and the risk of vascular calcification in CKD underscores the need to understand the underlying mechanisms in this high-risk group.

Currently, the use of FGF23 for assessing calcium-phosphate metabolism in hemodialysis patients is not standard practice due to methodological limitations. The lack of standardized measurement methods and established reference values significantly complicates the interpretation of FGF23 levels. Furthermore, the utility of FGF23 for assessing residual kidney function in this patient category remains insufficiently studied and warrants further investigation. Therefore, the present study aimed to determine the diagnostic and prognostic significance of fibroblast growth factor-23 in patients undergoing hemodialysis.

Materials and Methods. This cross-sectional study involved 88 patients with chronic kidney disease (CKD), including 69 patients with stage 5 CKD undergoing renal replacement therapy with hemodialysis (Group 1). A comparison group of 19 hospitalized patients with stage 3 CKD was also selected (Group 2). The study was conducted in accordance with international standards regarding the coordinated participation of respondents, the ethical component of research, and the collection of biomaterial (Helsinki Declaration of the World Medical Association – «Ethical Principles for Medical Research Involving Human Subjects» and «Universal Declaration on Bioethics and Human Rights» (UNESCO)). The study protocol was approved by the local ethics committee of Danylo Halytsky Lviv National Medical University (protocol number 3 dated March 18, 2024). All patients signed a written informed consent to participate in the study.

Among the examined patients, there were 36 (40.9%; 95% CI 30.64-51.18) women and 52 (59.09%; 95% CI 48.82-69.36) men, with mean age 55.81±13.14 years. The inclusion criteria for patients in the study were: being on renal replacement therapy with hemodialysis for at least 6 months, age from 18 to 85 years, patient consent to participate in the study, and ability to adequately cooperate during the study. Exclusion criteria: patient refusal to participate in the study, age <18 years, information about acute infectious processes of any etiology, oncological diseases, and mental disorders.

In all patients, the diagnosis of CKD was established in accordance with the order of the Ministry of Health of Ukraine No. 593 dated 02.12.2004 (as amended by the order of the Ministry of Health of Ukraine No. 384 dated 24.05.2012) [6] and according to the recommendations of the Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group 2024 [7].

All patients underwent a standard examination, which included general clinical, biochemical, and instrumental methods of examination. Biochemical parameters were determined in the laboratory of St. Panteleimon Hospital 1TMU in Lviv. The glomerular filtration rate (GFR) was calculated using the CKD-EPI (2021) formula to identify and select individuals with stage 3 chronic kidney disease (CKD) for the comparison group. PTH and FGF23 levels were determined by enzyme-linked immunosorbent assay (ELISA). To determine FGF23, a reagent kit from FineTest (China) was used. Reference values were 0-25 pg/mL for healthy serum. The assay demonstrated a sensitivity of 9.375 pg/mL, indicating its capacity to detect low concentrations of FGF23. The specificity of the assay was confirmed by its ability to specifically recognize FGF23, with no observed cross-reactivity with other analogous molecules. This high specificity ensures the accurate quantification of FGF23 without interference from related substances.

The «RStudio» program was used to calculate statistical indicators. General statistical indicators were calculated in «Microsoft Excel» using built-in formulas. The frequency of qualitative indicators was presented as absolute (n) and relative (%) values, as well as a 95% confidence interval (CI) in the form «n (%; 95% CI)». When analyzing quantitative data, the distribution of values was determined using the Shapiro-Wilk test. For quantitative data with a normal distribution, the results were presented as mean and standard deviation (M±SD). For quantitative data with a non-normal distribution, the median and 25-75 quartiles (Me (Q25-Q75)) were used. To compare two independent samples, the non-parametric Mann-Whitney U test was used. Spearman's rank correlation coefficient was used to analyze the correlation between quantitative indicators. The backward stepwise regression method was used to determine the prognostic significance. The statistical significance of the correlation coefficients was established. The critical significance level (p) for testing statistical hypotheses in this study was set at 0.05.

Results. The results of the study yielded the following data. FGF23 levels were elevated in 67 (97.1%; 95% CI 91.87-99.72) patients in Group 1, with a median (Me) of 1258.32 (169.46; 1338.46) pg/mL, while in Group 2, FGF23 levels were above normal in 18 patients (94.74%; 95% CI 80.58-100), with a Me of 150.5 (74.22; 929.12). A significant difference was found between the groups ($p<0.05$).

Since the primary function of FGF23 is to maintain phosphate, calcium, and parathyroid hormone metabolism, all patients underwent an assessment of calcium-phosphate metabolism (CPM) parameters. To determine renal excretory function, creatinine and urea levels, as well as urine output, were analyzed (Table 1).

Table 1

CPM and Renal Function Parameters in CKD Patients

Parameter	Group 1	Group 2	p
Phosphorus, mmol/L	1.61 (1.22; 2.11)	1.06 (0.87; 1.17)	<0.0001
Total Calcium, mmol/L	2.6 (2.44; 2.78)	2.37 (2.27; 2.45)	<0.0001
Ionized Calcium, mmol/L	1.31 (1.2; 1.39)	1.17 (1.13; 1.24)	0.0001
Parathyroid Hormone, pg/mL	201 (118; 314)	56 (44; 133)	0.0001
Creatinine, µmol/L	783 (540; 971)	158 (150; 169)	<0.0001
Urea, mmol/L	22.6 (18.8; 27.4)	11.1 (10; 11.95)	<0.0001
Diuresis, mL/kg/day	9.1 (2.6; 20.77)	30.6 (28.3; 33.85)	<0.0001

The duration of hemodialysis was (in months) Me 15 (8; 36). To assess the effect of FGF23 on CPM in Group 1, a correlation analysis was performed between FGF23 and phosphorus (r=0.13; p>0.05), total Ca (r=0.04; p<0.05), ionized Ca (r=0.02; p<0.05), and parathyroid hormone (r=0.08; p>0.05). Post-hoc power analysis for the non-significant correlations indicated insufficient power (power = 0.1892 for phosphorus; power = 0.1101 for PTH). The estimated sample sizes required to detect such weak associations with 80% power at an alpha of 0.05 were 456 and 1027 observations, respectively.

Further, to clarify the effect of FGF23 on renal excretory function, a correlation analysis was performed between FGF23 and creatinine, urea, urine output, and hemodialysis duration in Group 1 (Fig. 1-4).

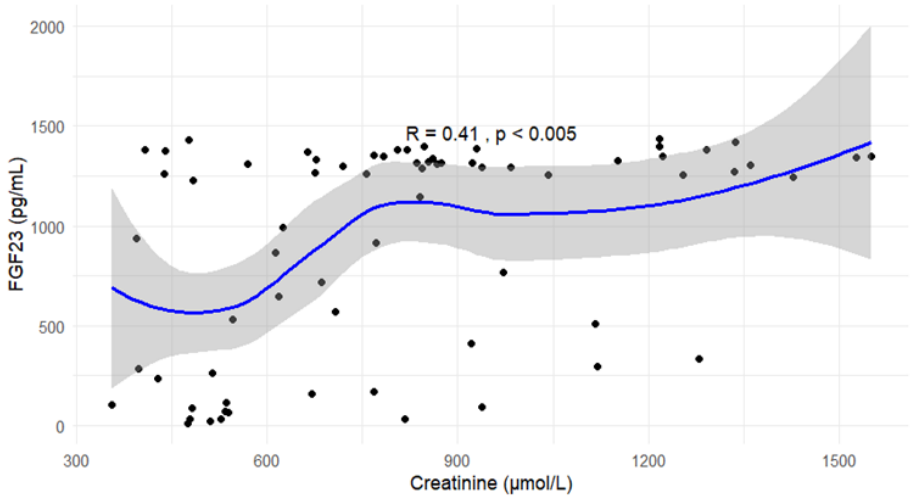

Fig. 1. Correlation between FGF23 and creatinine levels.

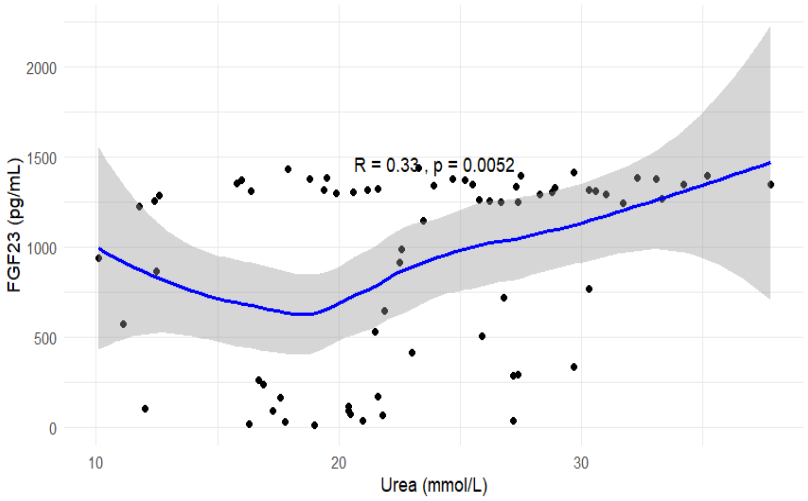

Fig. 2. Correlation between FGF23 and urea levels.

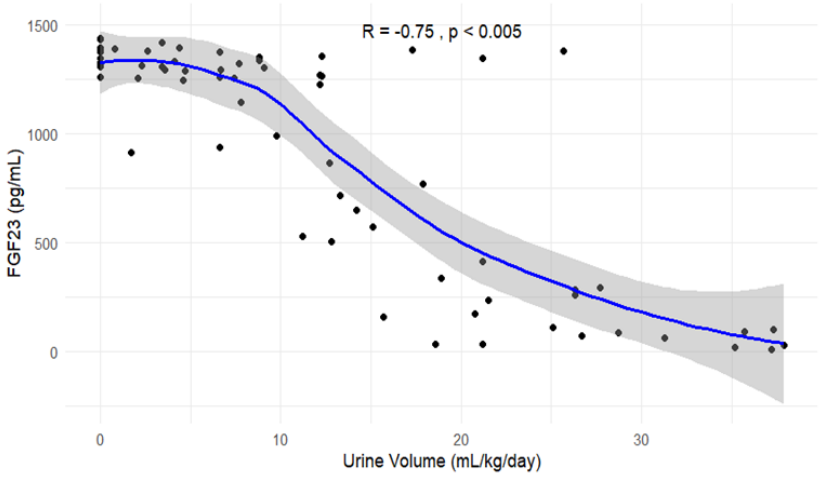

Fig. 3. Correlation between FGF23 and urine volume levels

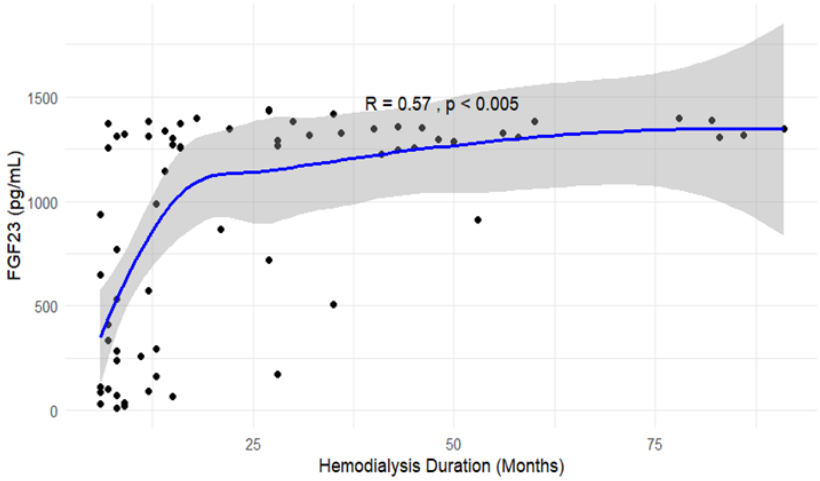

Fig. 4. Correlation between FGF23 levels and hemodialysis treatment duration.

Subsequently, a backward stepwise multiple linear regression analysis was conducted to develop a model predicting urine output (mL/kg/day) using initial candidate predictors: FGF23, creatinine, urea, and hemodialysis duration. The final model, after the removal of non-significant predictors (creatinine, p=0.7991; urea, p=0.9156), included FGF23 and hemodialysis duration (Table 2).

Table 2

Final Model from Backward Stepwise Regression Predicting Urine Output

Variable	Estimate	Standard Error	t-value	P-value
(Intercept)	29.306665	1.436711	20.398	<0.0001
FGF23	-0.015690	0.001562	-10.044	<0.0001
Dialysis Duration	-0.101998	0.036838	-2.769	0.0073

R2=0.7369, indicating that the model can predict 73.7% of urine output variations. F-statistic: 92.45 tests the overall significance of the model. The p-value of the F-statistic is <0.0001, indicating that the model is statistically significant. Residual standard error: 5.843. Residual degrees of freedom: 66. From these data, we can draw the following conclusions:

1. The model is statistically significant for predicting urine output.
2. FGF23 has a significant negative impact on urine output.
3. The amount of time on dialysis has a significant impact on urine output.
4. Creatinine and urea do not have a significant impact on urine output, so they were excluded from the model.
5. The model explains 73.7% of the variance in urine output. Thus, we can conclude that the model is adequate for explaining the influence of FGF23 and dialysis duration on urine output (Fig. 5, 6).

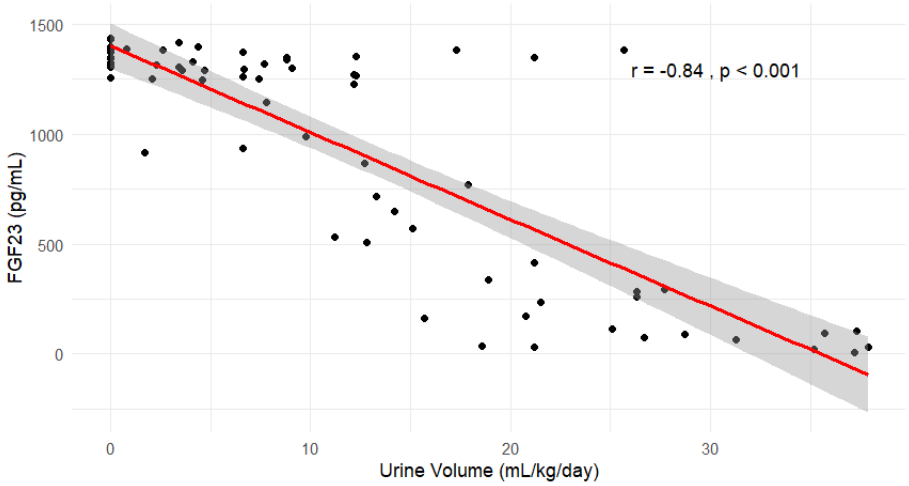

Fig. 5. Effect of FGF23 on urine output.

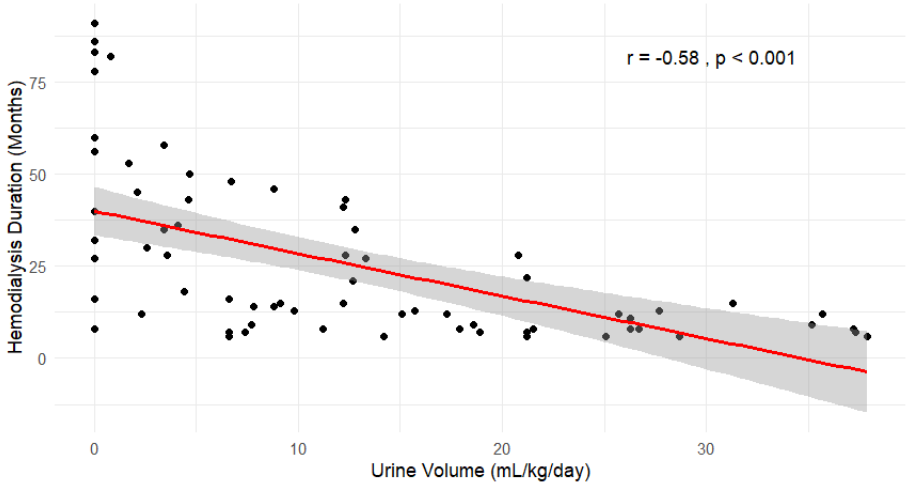

Fig. 6. Effect of hemodialysis duration on urine output.

Discussion. Fibroblast growth factor 23 is a key hormone in the regulation of phosphate homeostasis. Over the past decades, FGF23 has been the subject of intensive research in nephrology and cardiology. The uniqueness of FGF23 lies in its early appearance at the preclinical stages of CKD. In a study by Fauconnier C., the authors report high sensitivity and specificity of the FGF23 assay for the diagnosis of mild to moderate CKD, compared to the use of eGFR and creatinine [10]. Some scientific papers show that FGF23 can be a marker of hypophosphatemia [8], while others point to its potential as an early marker of calcium-phosphorus disturbances in the early stages of CKD [9]. In a Chinese study, the authors show that FGF23 can be a marker of vascular calcification, especially in stage 5 renal failure. They state that eGFR, serum creatinine, and FGF23 are independent risk factors for heart valve calcification in patients with CKD [11].

Our findings in patients undergoing maintenance hemodialysis, however, revealed no significant associations between FGF23 and phosphorus or parathyroid hormone, and only very weak significant associations with total and ionized calcium. These results suggest that FGF23 may not be a reliable diagnostic marker for assessing ongoing mineral metabolism disturbances in this specific patient population. Interpreting FGF23 levels is further complicated by its dual role reflecting not only mineral metabolism but also the degree of residual renal function (RRF). This dual role should be considered.

While literature supports FGF23's utility in early CKD or for vascular calcification risk assessment, our results do not confirm its diagnostic effectiveness for routine CPM monitoring in maintenance hemodialysis patients. Conversely, consistent with studies highlighting FGF23's potential for assessing renal function [12, 13], possibly with advantages over eGFR in certain contexts, our study found strong associations related to RRF.

We observed a strong, statistically significant negative correlation between FGF23 concentration and urine output (an indicator of RRF). This underscores the potential value of FGF23 as a biomarker reflecting RRF in patients with advanced kidney disease.

The interpretation of this negative association (higher FGF23, lower urine output) involves complex physiology. Potential mechanisms contributing to lower urine output with higher FGF23 could include direct hormonal effects, such as suggested activation of the

renin-angiotensin-aldosterone system (RAAS) [17] or upregulation of the Na-Cl cotransporter (NCC) [18], both promoting sodium and water retention. Alternatively, and perhaps more likely in this population, the markedly elevated FGF23 levels primarily serve as a sensitive marker of the severity of underlying renal dysfunction and loss of nephron mass, which is the principal determinant of reduced urine output. The phosphaturic effect of FGF23 [15], while physiologically important, is unlikely to be the dominant factor driving the negative correlation with overall urine volume observed in our stage 5 CKD cohort.

Further complicating the picture are these potential non-phosphaturic effects of FGF23. Evidence suggests possible activation of the RAAS [17] and regulation of NCC expression in the distal tubules [18], both of which typically lead to sodium and water retention (an antidiuretic effect) and increased blood pressure. While seemingly counterintuitive given FGF23's primary phosphaturic role [19], these fluid-retaining mechanisms [17] could potentially contribute to, rather than contrast with, the observed overall negative association between markedly elevated FGF23 levels and reduced urine output in our cohort with severe renal failure. This highlights the complex, multifactorial influence of FGF23 on water and salt balance in CKD, where its role extends beyond phosphate regulation and likely reflects the profound loss of renal function.

We also observed increased FGF23 levels with a longer duration of dialysis treatment, although this correlation was less significant for predicting urine output. This aligns with literature data indicating a progressive decline in residual renal function in patients on chronic dialysis [19]. Decreased RRF leads to impaired phosphate excretion and the development of hyperphosphatemia, which is a potent stimulus for compensatory FGF23 hypersecretion by osteocytes [20]. Therefore, high FGF23 levels in patients with a long history of dialysis primarily reflect the loss of RRF and the body's attempt to maintain phosphate homeostasis [20]. This explains why FGF23 level, rather than dialysis duration, correlates more closely with urine output as an indicator of preserved renal function.

The results of the regression analysis demonstrate that FGF23 is a significant predictor of changes in urine output in patients on hemodialysis, surpassing traditional markers such as creatinine and urea in its predictive value. Backward stepwise regression analysis revealed a statistically significant negative correlation between FGF23 levels and urine output, confirming the hypothesis of its effect on renal excretory function. The model, which includes FGF23 and hemodialysis duration, explains 73.7% of the variation in urine output, indicating its high predictive ability. This underscores the clinical significance of FGF23 as a biomarker for assessing residual renal function in dialysis patients. When interpreting the regression analysis model, it is necessary to note factors that were not included in the model but could potentially influence its results. These factors include: the etiology CKD, patient age, the presence and severity of comorbidities (especially cardiovascular disease), dialysis therapy parameters other than duration (specifically, modality [HD/HDF], and the ultrafiltration strategy and rate, which affect intradialytic hemodynamics), individual fluid and sodium intake, nutritional status, the degree of systemic inflammation, as well as the use of certain medications, particularly diuretics or renin-angiotensin system inhibitors. Potentially accounting for these factors could enhance the model's predictive performance.

We found a negative correlation between hemodialysis duration and urine output, which is consistent with known data on the progressive decline in residual renal function in patients on long-term dialysis [14]. The lack of significant correlation between urine output and creatinine and urea may be due to limitations of these markers in assessing residual renal function, especially in dialysis patients, but this issue requires further study. A deeper understanding of the role of FGF23 in the pathogenesis of CKD and calcium-phosphorus disorders should expand the clinical indications for its use as a diagnostic and prognostic marker and identify new therapeutic targets for the treatment of CKD and heart failure.

Limitations. Firstly, the cross-sectional design precludes establishing causality or tracking changes over time. Secondly, the sample size provided insufficient statistical power to definitively assess correlations between FGF23 and phosphorus/PTH, limiting conclusions about FGF23's diagnostic role for specific CPM parameters in this group, despite non-significant findings. Thirdly, focusing on stage 5 CKD patients on maintenance hemodialysis limits the generalizability of findings, particularly regarding FGF23 and RRF assessment, to patients with earlier CKD stages or on different RRT modalities. Fourthly, the regression model did not include several potential confounders, which could influence the observed associations.

Conclusions:

1. FGF23 is unsuitable as a diagnostic marker for calcium-phosphorus disorders in patients undergoing programmed hemodialysis, not only because of its weak correlations with calcium-phosphorus metabolism parameters but also due to its strong negative correlation with residual kidney function.
2. FGF23 is a significant predictor of urine output in hemodialysis patients according to the results of the regression analysis.
3. The model, which includes FGF23 and hemodialysis duration, explains 73.7% of the variation in urine output, indicating its high predictive ability. This underscores the clinical significance of FGF23 as a biomarker for assessing residual renal function in dialysis patients. A model integrating these parameters shows promise as a tool for identifying patients with lower residual urine output, reflecting reduced RRF among those on maintenance hemodialysis.

Future research should focus on elucidating the mechanisms behind the strong negative association between FGF23 and residual diuresis in hemodialysis patients. Investigating the impact of different therapeutic interventions on FGF23 levels and subsequent preservation of RRF is warranted. Additionally, developing standardized FGF23 assays remains crucial for facilitating its broader clinical application.

Ethical declaration: The research protocol was reviewed and approved at the meeting of the Commission on Ethics of Scientific Research, Experimental Development, and Scientific Works of Danylo Halytsky Lviv National Medical University on March 18, 2024, protocol number 3. Written informed consent was provided to patients for participation in the study.

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Author contributions

Bardash V.O.: conceptualization and design, data collection, statistical analysis.

Maksymets T.A.: writing and interpretation

Skliarov E.Ya.: review and editing.

Data Availability: The data analyzed in the study can be provided upon reasonable request.

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The current role of pentafecta outcomes in open and laparoscopic partial nephrectomy for localized renal tumors

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Abstract. *Background: Partial nephrectomy (PN) is the standard treatment for managing clinical T1 renal masses. The "trifecta" and "pentafecta" metrics are commonly used to assess the complexity and success of PN procedures. The present study aimed to identify predictive factors associated with the achievement of pentafecta outcomes following PN.*
Methods. A prospective randomized study was conducted between May 2022 and May 2024, involving 70 patients with clinical T1–T2a N0M0 renal tumors suitable for partial nephrectomy. Participants were randomly assigned into two groups: Group A (n = 38) underwent open partial nephrectomy (OPN), and Group B (n = 32) underwent laparoscopic partial nephrectomy (LPN). Preoperative assessment included lab tests and imaging. All surgeries were performed via a transperitoneal approach under general anesthesia. OPN and LPN techniques followed standardized protocols, each performed by an experienced surgeon. Postoperative follow-up included clinical, laboratory, and imaging assessments at set intervals. Primary outcomes focused on predictors of pentafecta achievement; secondary outcomes included blood loss, operative time, hospital stay, pain scores, complications, recurrence, and renal function. Statistical analysis was performed using SPSS v26.0, with significance set at $p < 0.05$. The study was registered at ClinicalTrials.gov (Identifier: NCT06960135).
Results. Both surgical approaches yielded comparable oncological outcomes. However, patients in the LPN group experienced significantly lower intraoperative blood loss, shorter operative times, reduced opioid requirements, and lower postoperative pain scores ($p < 0.05$) compared to the OPN group. Additionally, the length of hospital stay was significantly shorter in the LPN group ($p < 0.0001$). A significant positive association was observed between glomerular filtration rate and the use of tumor enucleation ($p = 0.0073$), as well as between PADUA score and body mass index ($p = 0.0004$).
Conclusions. While LPN is associated with longer ischemia time, it offers significant benefits over OPN, including reduced blood loss, lower analgesia requirements, and shorter hospital stays.

Keywords: *laparoscopic, partial nephrectomy, trifecta, pentafecta achievement, renal tumors.*

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Сучасна роль показників "пентафекти" як критеріїв ефективності відкритої та лапароскопічної часткової нефректомії у пацієнтів з локалізованими пухлинами нирок

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Резюме. *Часткова нефректомія (ЧН) є стандартом лікування пацієнтів із клінічними T1-пухлинами нирок. Показники «трифекта» та «пентафекта» широко застосовуються для оцінки складності та ефективності проведення ЧН. Метою цього дослідження було визначення прогностичних факторів досягнення пентафекти після часткової нефректомії.*

Методи. У період з травня 2022 до травня 2024 року проведено проспективне рандомізоване дослідження за участю 70 пацієнтів із пухлинами нирки стадії T1–T2a N0M0, придатними для часткової нефректомії. Пацієнтів випадковим чином розподілено на дві групи: група A (n = 38) — відкрита часткова нефректомія (ОЧН), група B (n = 32) — лапароскопічна часткова нефректомія (ЛЧН). Передопераційне обстеження включало лабораторні аналізи та візуалізацію. Усі операції виконувалися трансперитонеальним доступом під загальним наркозом за стандартизованими методиками. Первинна кінцева точка — предиктори досягнення пентафекти; вторинні — крововтрата, тривалість операції, госпіталізація, больовий синдром, ускладнення, рецидиви та функція нирок. Статистичний аналіз виконано в SPSS v26.0; рівень значущості — p < 0,05. Дослідження зареєстровано в ClinicalTrials.gov (ідентифікатор: NCT06960135).

Результати. Обидва хірургічні підходи забезпечили порівнянні онкологічні результати. Однак пацієнти групи ЛЧН мали достовірно меншу інтраопераційну крововтрату, коротший операційний час, нижчу потребу в опіоїдних анальгетиках та нижчі показники післяопераційного болю (p < 0,05) у порівнянні з групою ВЧН. Тривалість госпіталізації також була значно коротшою в групі ЛЧН (p < 0,0001). Було встановлено достовірно позитивну асоціацію між швидкістю клубочкової фільтрації та застосуванням енуклеації пухлини (p = 0,0073), а також між балом PADUA і індексом маси тіла (p = 0,0004).

Висновки. Попри довший час ішемії ЛЧН, цей метод має переваги над відкритою ЧН, зокрема меншу крововтрату, нижчі анальгетичні потреби та скорочену тривалість перебування в стаціонарі.

Ключові слова: *лапароскопія, часткова нефректомія, трифекта, пентафекта, пухлини нирки.*

Introduction. PN is widely considered the preferred treatment for clinical T1 (cT1) renal masses, primarily due to its superior preservation of kidney function compared to radical nephrectomy [1].

The trifecta metric includes three components: warm ischemia time (WIT) of ≤ 25 minutes or cold ischemia time (CIT) of ≤ 60 minutes, negative surgical margins, and the absence of perioperative major complications. The pentafecta expands upon these criteria by adding two additional parameters: preservation of ≥ 90% of the estimated glomerular filtration rate (eGFR) and no progression in the chronic kidney disease (CKD) stage within 12 months postoperatively [2].

Over the past decade, advances in surgical techniques and technology have positioned LPN as a viable alternative to OPN demonstrating comparable outcomes in terms of perioperative complications, oncological efficacy, and kidney function preservation [3].

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A critical determinant of postoperative kidney function is the extent of preserved parenchymal mass, assuming that ischemia duration is minimized [4]. In this context, tumor enucleation (TE) — a surgical technique involving blunt dissection along the tumor pseudocapsule — has gained attention. TE is thought to preserve more healthy renal parenchyma than conventional sharp dissection techniques, which typically remove tissue within a 2–5 mm margin around the tumor [5].

This study aims to evaluate pentafecta outcomes in patients undergoing OPN and LPN for localized renal tumors. Additionally, it seeks to identify clinical, surgical, and pathological factors that predict the likelihood of achieving pentafecta status.

Methods. A prospective, randomized study was conducted between May 2022 and May 2024, involving 70 patients of both sexes diagnosed with clinical T1 or T2a N0M0 renal tumors who were eligible candidates for partial nephrectomy. The study was approved by the Local Ethics Committee of South Valley University, Egypt (Ethical Approval Code: SVU-MED-URO016-2-22-5-400). Written informed consent was obtained from all participants or, when applicable, from their parent, legal guardian, or next of kin, for both participation in the study and publication of medi-

cal data and related images. The study was registered at ClinicalTrials.gov (Identifier: NCT06960135). Inclusion criteria required patients to have localized renal tumors suitable for nephron-sparing surgery. Exclusion criteria included: severe and irreversible coagulopathy, anatomically unfavorable tumor location, extensive encasement of the renal pedicle, diffuse invasion of the renal vein or central collecting system, adjacent organ invasion consistent with stage cT4 disease, regional lymphadenopathy (stage cTxN1), or anticipated preservation of less than 20% of total nephron mass.

Randomization. Random allocation of the cases into two groups was performed using a block randomization method in Stata, version 13.1, Stata Corp, for Microsoft Windows: group A (OPN) and group B (LPN group) (Fig. 1).

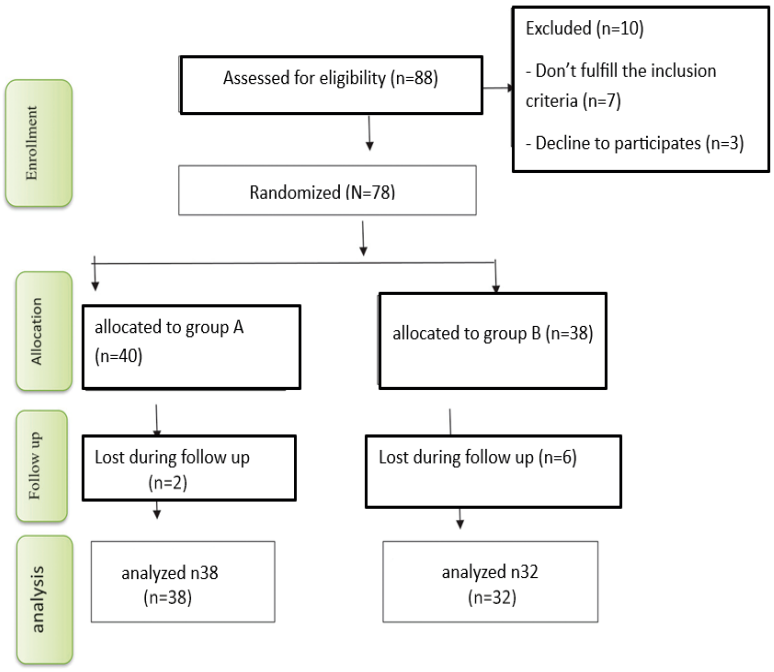

Fig. 1. CONSORT diagram for patient flow throughout the research

Participants were randomly assigned into two groups using block randomization: Group A (n = 38) underwent OPN, while Group B (n = 32) underwent LPN, with all procedures performed under general anesthesia. A transperitoneal approach was used in all cases.

Preoperative assessment. Physical examination, medical history, and laboratory investigations. Laboratory tests included complete blood count (CBC), serum creatinine, glomerular filtration rate (GFR), liver function tests, random blood glucose, blood grouping, coagulation profile, and urinalysis. Radiological evaluation included ultrasonography (US), computed tomography (CT), chest X-ray, and magnetic resonance imaging (MRI).

Surgical techniques. Group A – Open Partial Nephrectomy (OPN). An extraperitoneal flank incision was made over the 11th or 12th rib. In some patients, a transperitoneal Chevron incision was performed. The renal hilum was exposed, and the renal artery was occluded using a bulldog clamp. Tumor excision was then performed using cold scissors in a nearly bloodless field, followed by renal parenchymal repair using interrupted horizontal mattress sutures (0-polyglactin). The bulldog clamp was removed following repair, and warm ischemia time was recorded in minutes. Operative time was calculated from skin incision to skin closure.

Surgical techniques. Group B – Laparoscopic Partial Nephrectomy (LPN). Patients were positioned in a 45-degree modified lateral decubitus position with mild table flexion. A Veress needle was inserted periumbilically to establish pneumoperitoneum with carbon dioxide. After removal of the needle, a 12-mm trocar was inserted at the same site, and intra-abdominal pressure was maintained at 12–15 mmHg. A 30-degree endoscopic camera was used. Under direct vision, two additional trocars were placed: a 12-mm trocar in the ipsilateral midclavicular line (one finger breadth caudal to the camera port) and a 5-mm trocar (one finger breadth cephalic). Operative time was calculated from port entry to port exit.

The colon was mobilized along Toldt's fascia to fully expose the retroperitoneum. Dissection proceeded until the psoas muscle was visualized, revealing the ureter dorsal to the gonadal vein. The lower pole of the kidney was elevated and mobilized. The renal hilum was identified, and the arteries were meticulously dissected. The kidney was mobilized within Gerota's fascia and

defatted, preserving peritumoral fat. Tumor excision or enucleation was performed using cold scissors. Renal parenchymal repair was completed with V-Loc sutures, and warm ischemia time was recorded.

To minimize the variability in surgical expertise and enhance the reliability of the findings, all OPNs were performed by a single surgeon, and all LPNs were performed by another single surgeon. Each surgeon had independently performed over 100 procedures using their respective approach prior to the start of this study. This standardization aimed to control for operator-dependent variability and reduce potential bias related to differences in surgical experience.

Follow-up. This included physical examination and laboratory testing, along with radiological imaging at 3, 6, and 12 months postoperatively, and annually for three years. Imaging included abdominal CT or MRI, and chest X-ray annually, with chest CT performed when clinically indicated. These imaging procedures were mainly performed to assess renal tumor recurrence or local metastases.

Outcome measures. The primary outcomes include predictors of pentafecta achievement among variables. The secondary outcomes included blood loss, surgery duration, length of hospital stay, intraoperative complications, VAS, analgesic requirements, complications following surgery, local recurrence, kidney function, distant metastases, contralateral kidney recurrence, and cardiovascular events, all of which have been documented, with monitoring based on the Clavien-Dindo grading system [6, 7].

Statistical analysis. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Quantitative variables were presented as means and standard deviations (SD) and compared between the two groups using the unpaired Student's t-test. Categorical variables were summarized as frequencies and percentages and analyzed using the Chi-square test or Fisher's exact test, depending on the expected cell counts. Receiver operating characteristic (ROC) curve analysis was employed to evaluate diagnostic performance, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A two-tailed p-value < 0.05 was considered statistically significant.

Results. *The patients' characteristics*. A total of 70 patients were enrolled between May 2022 and May 2024, with 38 undergoing OPN and 32 undergoing LPN. The two groups were comparable across preoperative demographic, clinical, and tumor-related variables.

As shown in Table 1, there were no statistically significant differences between groups in terms of age, sex distribution, smoking index, body mass index (BMI), Eastern Cooperative Oncology Group (ECOG) performance status, comorbidities (hypertension [HTN], diabetes mellitus [DM], chronic kidney disease [CKD], ischemic heart disease [IHD]), or baseline laboratory values. Tumor characteristics, including laterality, location, size, PADUA score, and clinical stage (T1a, T1b, T2a), were also not significantly different between the two groups.

Table 1

Preoperative demographic, disease history and laboratory data of the studied patients

		Open surgery (n =38)	Lap. approach (n =32)	P
Age (years)		61.63 ± 10.96	58.28 ± 16.03	0.098
Sex	Male	28 (73.68%)	18 (56.25%)	0.2928
	Female	10 (26.32%)	14 (43.75%)	
Smoking index		0.47 ± 0.5	0.48 ± 0.56	0.94
BMI		28.99 ± 3.98	30.91 ± 4.39	0.2036
ECOG score		0.21 ± 0.69	1 (6.25%)	0.6575
Disease history data				
CKD		8 (21.05%)	2 (6.25%)	0.2243
IHD		0	2 (6.25%)	0.2824
HTN		12 (31.58%)	12 (37.5%)	0.7229
DM		6 (15.79%)	16 (50.0%)	0.1586
Laboratory data				
Hb (g/dl)		12.84±1.37	13.39 ± 1.66	0.7274
Creatinine		1.15 ± 0.28	1.07 ± 0.42	0.1104
GFR (CKD-EPI)		67.69 ± 13.61	77.2 ± 33.57	0.3328

Continuation of Table 1

		Open surgery (n =38)	Lap. approach (n =32)	P
Tumor data				
Side	Left	20 (52.63%)	16 (50.0%)	0.8811
	Right	18 (47.37%)	16 (50.0%)	0.8811
Site	Lower polar	20 (52.63%)	10 (31.25%)	0.2143
	Mid polar	8 (21.05%)	14 (43.75%)	0.1586
	Upper polar	10 (26.32%)	8 (25.0%)	0.9319
Size (mm)		44.05 ± 23.13	38.38 ± 14.67	0.7525
PADUA score		7.47 ± 1.09	7.25 ± 1.09	0.5605
Clinical staging data				
t1a		22 (57.89%)	24 (75.0%)	0.2882
t1b		12 (31.58%)	6 (18.75%)	0.387
t2a		4 (10.52%)	2 (6.25%)	0.9039

Intraoperative outcomes and histopathological findings. Intraoperative parameters are summarized in Table 2.

Table 2

Intraoperative evaluation and histopathology data of the studied patients

		Open surgery (n = 38)	Lap. approach (n = 32)	P
Operative data				
Tumor Resection	Enucleation	0(0.0%)	28(87.5%)	0.0001
	Excision	38(100.0%)	4(12.5%)	0.0001
Ischemia time (min.)		8.17±7.05	16.94±3.8	0.0005
Pelvicalyceal injury		2(5.26%)	4(12.5%)	0.4609
Pleural injury		6(15.79%)	0(0.0%)	0.102
Blood loss (cc)		365.79±167.06	171.88±72.82	0.0001
Operative time (min.)		153.89±39.12	126.88±20.22	0.0179
Histopathology data				
Clear Cell RCC		24(63.16%)	32(100.0%)	0.0056
Chromophobe		6(15.79%)	0(0.0%)	0.102
Papillary RCC		8(21.05%)	0(0.0%)	0.0531
Surgical margin free		38(100%)	32(100%)	

Enucleation was performed significantly more often in the LPN group (87.5%) compared to none in the OPN group ($P < 0.0001$). Conversely, tumor excision was performed in all OPN cases and only 12.5% of LPN cases. Mean ischemia time was significantly longer in the LPN group (16.94 ± 3.8 minutes) compared to the OPN group (8.17 ± 7.05 minutes; $P = 0.0005$). The LPN group also had significantly lower estimated blood loss (171.88 ± 72.82 mL vs. 365.79 ± 167.06 mL, $P < 0.0001$) and shorter operative time (126.88 ± 20.22 minutes vs. 153.89 ± 39.12 minutes, $P = 0.0179$). Rates of pelvicalyceal and pleural injuries were not significantly different.

Histopathological evaluation showed clear cell renal cell carcinoma (CCRCC) as the most common subtype, with significantly more cases in the LPN group (100%) than in the OPN group (63.16%, $P = 0.0056$). All patients in both groups had negative surgical margins.

Postoperative outcomes. Postoperative clinical and laboratory outcomes are detailed in Table 3.

Table 3

Postoperative evaluation and lab. Investigations of the studied patients

Postoperative data				
Analgesia required	**Paracetamol**	38(100.0%)	32(100.0%)	–
	NSAID	38(100.0%)	0(0.0%)	–
	Opioid	24(63.16%)	0(0.0%)	0.0001
Clavien Dindo **1** **2**		1.05±0.22 36(94.74%) 2(5.26%)	32(100.0%) 32(100.0%) 0(0.0%)	0.3896 0.3666 –
VAS		8.16±0.67	3.94±0.83	0.0001
Hospital stays (day)		7.47±2.37	1.94±0.66	0.0001
Post operative investigations				
Post operative Hb (g/dl)		10.92±0.56	12.59±1.69	0.0272
Post. Week1 Creatinine		1.17 0.35	1.13 0.35	0.64
Post. Week1 GFR		71.1 16.3	70.3 17.5	0.85
Post. 3 months Creatinine		1.19 0.29	1.17 0.3	0.78
Post. 3 months GFR		68.9 12.5	69.1 13.2	0.95
Post. Year 1 Creatinine		1.22±0.3	1.22±0.44	0.7776
Post. Year 1 GFR		63.57±13.98	67.56±16.21	0.2732
GFR deficit>10%		13 (34.21%)	18(56.25%)	0.06
Pentafecta achievement		28(73.68%)	16(50.0%)	0.1575

Pain scores (VAS) and opioid analgesia requirements were significantly lower in the LPN group compared to the OPN group (VAS: 3.94 ± 0.83 vs. 8.16 ± 0.67; P < 0.0001; opioids: 0% vs. 63.16%; P < 0.0001). The length of hospital stay was significantly shorter in the LPN group (1.94 ± 0.66 days vs. 7.47 ± 2.37 days; P < 0.0001).

No statistically significant differences were observed in postoperative creatinine levels or GFR at one week, three months, or one year. The rate of GFR decline >10% was higher in the LPN group (56.25%) than in the OPN group (34.21%), but this difference did not reach statistical significance (P = 0.06). Pentafecta achievement rates were 73.68% in the OPN group and 50.0% in the LPN group (P = 0.1575).

Predictors of pentafecta achievement. As presented in Table 4, comparisons between patients who achieved pentafecta (n = 44) and those who did not (n = 26) revealed that patients in the failure group had significantly higher BMI (31.64 ± 3.42 vs. 28.82 ± 4.39; P = 0.0496), smoking index (P = 0.04), and higher prevalence of HTN (76.92% vs. 9.09%; P = 0.0004) and DM (69.23% vs. 9.09%; P = 0.0002). Postoperative kidney function measures (GFR at one week, three months, and one year; creatinine levels at three months and one year; and GFR deficit >10%) were significantly worse in the pentafecta failure group (Fig. 2, 3).

Operative approach, ischemia time, intraoperative blood loss, complications, and hospital stay were not significantly associated with pentafecta achievement.

Table 4

Analysis of variables among success and failure of pentafecta achievement groups

		Pentafecta achievement (n = 44)	Pentafecta failure (n = 26)	P
Age (years)		54.32±15.33	61.23±11.66	0.1555
Sex	**Male**	30(68.18%)	16(61.54%)	0.6995
	Female	14(31.82%)	10(38.46%)	0.6995
Smoking index		0.39 ±0.69	0.52 ±0.1	0.04
BMI		28.82±4.39	31.64±3.42	0.0496
Disease history data				
CKD		6(13.64%)	4(15.38%)	0.8905
IHD		0(0.0%)	2(7.69%)	0.1977
HTN		4(9.09%)	20(76.92%)	0.0004

Continuation of Table 3

		Pentafecta achievement (n = 44)	Pentafecta failure (n = 26)	P
DM		4(9.09%)	18(69.23%)	0.0002
PADUA score		7.36±1.07	7.38±1.15	0.9859
Operative data				
Tumor Resection	**Enucleation**	14(31.82%)	14(53.85%)	0.21
	Excision	30(68.18%)	12(46.15%)	0.21
Ischemia time (min.)		11.1±6.86	14.23±7.39	0.1926
Intraoperative complication		2(4.55%)	4(15.38%)	0.3029
Blood loss (cc)		304.55±189.44	230.77±91.02	0.2259
Operative time (min.)		136.82±37.06	158±27.88	0.0501
Histopathology data				
CCRCC		32(72.73%)	24(92.31%)	0.1713
Chromophobe		4(9.09%)	2(7.69%)	0.8905
Papillary RCC		8(18.18%)	0(0.0%)	0.1083
Post operative data				
Analgesia required				–
Paracetamol		44(100.0%)	26(100.0%)	–
NSAID		28(63.64%)	10(38.46%)	0.1575
Opioid		18(40.91%)	6(23.08%)	0.2967
Clavien Dindo	**1**	44(100.0%)	24(92.31%)	0.1977
	2	0(0.0%)	2(7.69%)	--
VAS		6.68±2.18	5.46±2.1	0.1131
Post operative Hb (g/dl)		11.48±1.15	12.03±1.85	0.5838
Post. Week1 Creatinine		1.08±0.3	1.19±0.5	0.25
Post. Week1 GFR		74.8±22.7	59.15±11.8	0.001
Post. 3 months Creatinine		1.09 ±0.3	1.34±0.6	0.02
Post. 3 months GFR		74.9 ±22.8	56.6±11.5	<0.0001
Post. Year 1 Creatinine		1.1±0.3	1.42±0.4	0.001
Post. Year 1 GFR		75.1±22.82	54.15±12.11	0.0012
GFR deficit>10%		0.45±1.88	24(92.31%)	<0.0001
Hospital stays (day)		5.36±3.13	4.23±3.45	0.2261

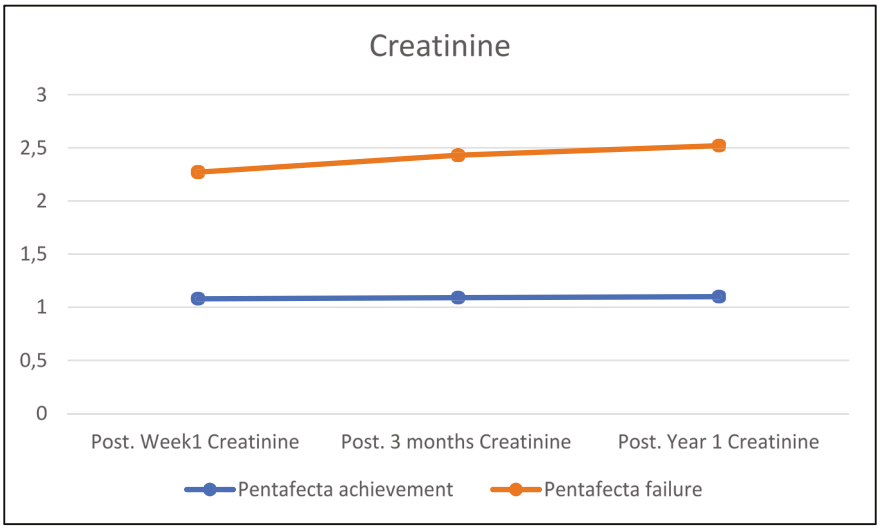

Fig. 2. Creatinine level during the follow-up period.

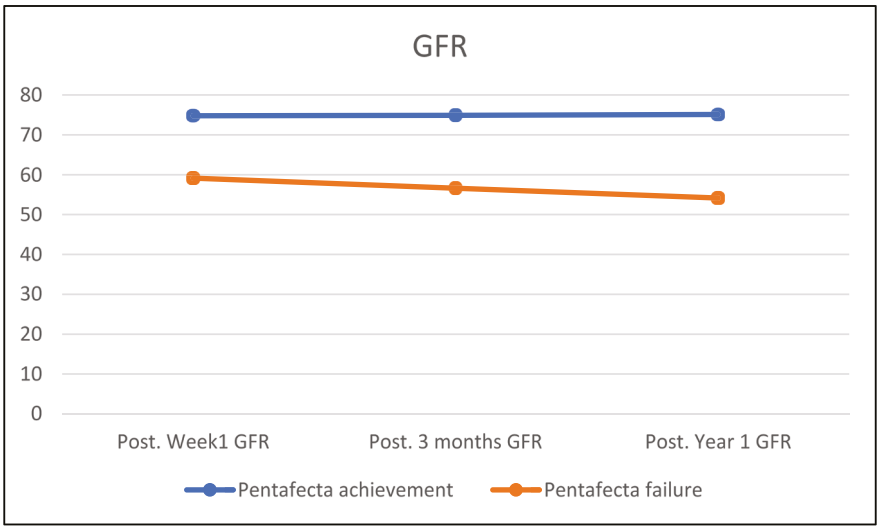

Fig. 3. eGFR changes during the follow-up period.

There was a significant negative correlation between pentafecta achievement and HTN and DM ($P<0.05$). There was a significant positive correlation between GFR and the performance of enucleation ($P = 0.0073$), indicating that enucleation preserves kidney function. There was a significant positive correlation between Padua score and BMI ($P = 0.0004$); however, non-significant associations were found with other parameters as seen in Table 5.

Table 5

Association between BMI, HTN, DM, enucleation, GFR, padua score and comorbidities with pentafecta achievement

	Unstandardized Coefficients		OR	T	P	95.0% CI for B	
	B	Std. Error				Lower bound	Upper bound
BMI	-0.01681	0.012565	0.983331	-1.3378	0.1907	-0.0424	0.00881
HTN	-0.50474	0.122878	0.603664	-4.1076	**0.0003**	-0.7553	-0.25413
DM	-0.40863	0.124896	0.664562	-3.2717	**0.0026**	-0.6633	-0.1539
GFR	0.01004	0.003162	1.010091	3.175	**0.0073**	0.00320	0.01687
pentafecta achieved	-0.3602	0.178398	0.697537	-2.0190	0.0646	-0.7456	0.02520
HTN	0.355945	0.319216	1.427529	1.11506	0.2737	-0.2959	1.00787
DM	0.000769	0.3155	1.000769	0.00243	0.9981	-0.6435	0.64510
BMI	0.1884	0.047038	1.207317	4.00529	**0.0004**	0.09233	0.28446

Results of all follow-up imaging procedures revealed no local recurrence or metastases.

Discussion. Renal tumors are among the most common neoplasms of the urinary system, second only to bladder cancer in prevalence. Due to their relatively low biological aggressiveness, these tumors typically demonstrate a subdued malignant potential [8].

In the present study, the preoperative characteristics – including age, sex, smoking index, BMI, ECOG score, comorbidities, and laboratory parameters – did not differ significantly between OPN and LPN groups. These findings align with Kang et al. [9]. who examined outcomes following robot-assisted partial nephrectomy (RAPN) for localized renal cell carcinoma (RCC). Their cohort had a median age of 50 years, a male predominance (70.7%), and notable rates of hypertension (29.3%) and diabetes mellitus (13.5%), with a median BMI of 25 kg/m^2. Similarly, Deka et al. [2] compared trifecta and pentafecta outcomes across OPN, LPN, and RAPN and reported comparable baseline characteristics.

While LPN is associated with a longer ischemia time, it is important to note that the mean ischemia duration in our study remained below the commonly accepted threshold of 30 minutes [1], which is used in defining favorable trifecta outcomes. Therefore, the prolonged ischemia observed in the LPN group may not have had a significant impact on Pentafecta outcomes. Nevertheless, a more detailed analysis with a larger sample size or a multi-institutional cohort would provide greater clarity on the potential influence of ischemia time on long-term functional outcomes and overall Pentafecta achievement.

Tumor-related variables, including tumor side, site, and size, also showed no statistically significant differences between the surgical groups or between cases achieving versus failing to achieve pentafecta outcomes. This is consistent with Porpiglia et al. [10] who reported no significant differences in tumor location or diameter between OPN and LPN groups.

Evaluation of disease complexity using the PADUA score similarly demonstrated no significant differences between the groups or between pentafecta achievers and non-achievers. Although Abdelhafez et al. [11]. found PADUA score differences to be significant across their study groups, our findings may reflect the inclusion of a broader clinical spectrum (cT1 and T2a tumors) with low complexity scores.

Clinical staging analysis revealed a nonsignificant increase in T1a and T1b tumors in the LPN group compared to the OPN group. T2a tumors were evenly distributed between groups. These results are consistent with Chang et al. [12] who observed no significant differences in clinical staging across surgical approaches.

In agreement with our result about operative data, Mehra et al. [13], reviewed fifty-five PN procedures: 28 OPN, 14 LPN, and 13 RAPN. OPN, LPN, and RAPN had similar median tumor size, nephrometry score, and preoperative creatinine. Blood loss was higher for OPNs than for LPNs. Our study disagrees with Soisrithong, Sirisreetreerux, et al. [14], which reported that the operative time was significantly shorter in the OPN group compared to the LPN and RPN groups.

Postoperative outcomes further supported the advantages of LPN. Patients undergoing LPN required significantly less analgesia – especially opioids and NSAIDs – and reported lower visual analog scale scores and decreased blood loss. No significant differences were observed between groups in terms of blood transfusion requirements, Clavien-Dindo complications, postoperative creatinine or GFR levels, or GFR deficit >10%.

Among patients failing to achieve pentafecta, significantly higher postoperative creatinine, lower GFR, and increased GFR deficit were observed. These findings are consistent with those of Ghavimi et al. [15] who reported similar eGFR trends across surgical modalities, with >10% GFR reductions observed in 59% of LPN and OPN cases and 52% of RAPN cases.

Histopathological comparisons revealed no significant differences in tumor subtype or margin status between surgical groups or pentafecta achievement groups. These results are in line with Mehra et al. [13] who found no significant difference in histologic subtypes (e.g., papillary RCC) across surgical modalities. It is worth noting that all cases included in the study were consecutively enrolled and randomized without any selection bias. The observed difference in CCRCC rates (100% in LPN vs. 63.16% in OPN) likely reflects random variation due to the small sample size, rather than a systematic bias in patient selection or treatment assignment. Histopathological outcomes were determined independently and postoperatively, thus not influencing surgical decision-making.

Hospital stay duration was significantly shorter in the LPN group, a finding supported by Mehra et al. [13] who reported median postoperative durations of 5, 6.5, and 10 days for OPN, LPN, and RAPN, respectively. However, pentafecta achievement rates did not differ significantly between groups, consistent with results from both Mehra et al. [13] and Deka et al. [2] who reported pentafecta rates of 71.6% for OPN, 82.6% for LPN, and 62.6% for RAPN.

In our study, there was a significant negative association between pentafecta achievement and HTN and DM. Also, there was a significant positive association between GFR and the performance of enucleation, indicating that enucleation preserves kidney function. Our study can be supported by Xu et al. [16] who found that TE was not only less traumatizing and beneficial for recovery but also better for kidney function protection.

Strengths and limitations of the study. This study provides valuable insights into the comparative performance of OPN and LPN regarding pentafecta outcomes. However, several limitations should be noted. While our analysis revealed a numerical trend favoring OPN descriptively, these differences did not reach statistical significance, due mainly to the limited sample size, which may have limited the ability to detect small but potentially meaningful differences between groups. Accordingly, future studies with larger sample sizes may help clarify whether the trend observed reflects a true clinical benefit of OPN. Specifically, based on a post-hoc power analysis with a medium effect size, a sample size of 51 in each group is needed to potentially detect the existing effect. Additionally, the follow-up period was limited, which necessitates making it longer in future research. Moreover, variations in surgeon expertise and technique could have influenced both operative outcomes and the achievement of pentafecta.

Conclusions. Both approaches show similar rates of pentafecta achievement, BMI and comorbidities such as HTN and DM negatively impact pentafecta outcomes, while the enucleation technique appears beneficial for preserving kidney function. These findings highlight the need for tailored surgical approaches to optimize outcomes in PN for localized renal tumors.

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Author contributions.

Aly M Abdel-Karim: Conceptualization and methodology;

Gamal Alsagheer: Validation and formal analysis;

Omar A.Bakeet: Writing – original draft preparation;

Mostafa AbdelRazek: Writing – review, editing and supervision.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

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The impact of cord blood stem cell transplantation on the functional status of living-donor kidney allografts

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Abstract. *Standard immunosuppressive therapy (SIST) remains the cornerstone in preventing early rejection episodes in kidney transplantation. However, long-term graft survival rates have seen limited improvement over the past three decades. Recent research suggests that stem cells possess immunomodulatory properties, contribute to the reduction of ischemia-reperfusion injury, and enhance graft function.*

The present study aimed to evaluate the effect of cord blood stem cell (CBSC)-enhanced induction immunosuppression on the functional outcomes of kidney allografts during the first post-transplantation year.

Methods. This prospective study included 45 recipients of living-donor kidney transplants, who were divided into two groups: Group I (n = 20) received CBSC in combination with SIST, while Group II (n = 25) received SIST alone. Graft function was assessed over three post-transplant periods: Period I (days 1–7), Period II (days 8–89), and Period III (days 90–360).

Parameters assessed included serum creatinine levels, estimated glomerular filtration rate (eGFR), daily urine output, urinary neutrophil gelatinase-associated lipocalin (NGAL) levels, and protocol biopsies at 3 and 12 months post-transplantation.

Results. CBSC administration in Group I was well tolerated without adverse events. During the first week, daily urine output in Group I was significantly higher (9,400 ± 950 mL) compared to Group II (7,545 ± 750 mL; $p < 0.01$). Normalization of serum creatinine occurred within 2–3 days in Group I, whereas in Group II it occurred by days 5–7. At 12 months, the mean eGFR was significantly better in Group I (98.2 mL/min/1.73 m^2) compared to Group II (61.4 mL/min/1.73 m^2; $p < 0.01$). Urinary NGAL levels on days 1 and 3 post-transplant were substantially lower in Group I (79.4 and 28.4 pg/mL, respectively) versus Group II (375 and 121 pg/mL; $p < 0.01$), indicating reduced tubular injury. No opportunistic infections were recorded in Group I during the 12-month follow-up. In contrast, two cases of cytomegalovirus infection were documented in Group II.

Protocol biopsies at 3 and 12 months showed no pathological findings in Group I. In Group II, 10% of biopsies revealed glomerulitis and peritubular capillaritis with C4d deposition, and one patient exhibited lymphocytic tubulitis.

Conclusions. The addition of CBSC to SIST appears to mitigate ischemia-reperfusion injury and accelerates early recovery of graft function, with improved eGFR and reduced tubular injury markers observed within the first year post-transplant. These findings support the potential of CBSC in enhancing transplant outcomes. Further research is warranted to define optimal dosing strategies, administration frequency, and long-term immunological impacts.

Key words: *kidney transplantation, standard immunosuppressive therapy, cord blood stem cells, induction immunosuppressive therapy, glomerular filtration rate.*

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О.С. Вороняк

Вплив трансплантації стовбурових клітин кордової крові на функцію трансплантованої нирки від родинного донора.

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Резюме. *Стандартна імуносупресивна терапія (СІСТ) забезпечує профілактику раннього відторгнення ниркового трансплантата. Проте віддалені результати трансплантації нирки (ТН) не зазнали суттєвих змін протягом останніх 30 років. Декілька досліджень впливу застосування стовбурових клітин продемонстрували їх імуносупресивний вплив, зменшення ішемічно-реперфузійного пошкодження та покращення функціонального стану трансплантата. Метою цієї роботи було оцінити влив індукційної імуносупресії (ІІС) поєднаної з застосуванням стовбурових клітин кордової крові (СККК) на функціональний стан ниркових трансплантатів протягом першого року посттрансплантаційного періоду.*

Методи. Проведено проспективне порівняння результатів впливу СІСТ терапії поєднаної з застосуванням СККК у реципієнтів ниркових трансплантатів (І група, n = 20) та СІСТ (ІІ група, n = 25) протягом першого року посттрансплантаційного періоду (І період 1-7днів; ІІ період 8-89днів; ІІІ період 90-360 днів), за даними концентрації креатиніну сироватки крові, швидкості клубочкової фільтрації (ШКФ), об'єму добового діурезу, показників NGAL сечі та протокольних біопсій, виконаних на 3-ій та 12-ий місяць після ТН.

Результати. Всі реципієнти першої групи перенесли введення СККК без ускладнень. Діурез у реципієнтів І групи протягом першого періоду спостереження складав 9400±950 мл на добу, а у хворих ІІ групи - 7545±750 мл на добу (p < 0,01). У реципієнтів І групи відмічали швидшу (через 2-3 доби) нормалізацію креатинінемії порівняно з групою реципієнтів ІІ групи, де нормалізація концентрації рівня креатиніну в крові мала місце на 5-7 добу після ТН. Через 1 рік спостереження ШКФ у реципієнтів І групи складала 98.2мл/хв/1,73 м^2, а ІІ групи - 61.4 мл/хв/1,73 м^2 (p < 0,01).

Концентрація NGAL в сечі на І та ІІІ добу у І групі складав 79.4 та 28.4пг/мл, у ІІ групі - 375 та 121 пг/мл відповідно (p < 0,01). У хворих першої групи не спостерігали опуртуністичних інфекцій пацієнтів у термін спостереження до 12 місяців спостереження, тоді як у ІІ групі у 2 пацієнтів було діагностивно цитомегаловірусну інфекцію.

Протокольні біопсії на третій та 12 місяць після ТН у хворих І групи не виявили патологічних змін, у пацієнтів ІІ групи гломеруліт та перитубулярний капілярит з експресією С4d спостерігалася у 10% випадків, а запальна лімфоцитарна інфільтрація канальців у одного пацієнта.

Висновки. Поєднання СІСТ з введенням СККК реалізується через зменшення ішемічно-реперфузійних пошкоджень структур трансплантату, відновлюючи ШКФ протягом 2-3 доби після ТН, порівняно з 5-7 добою у хворих без застосування СККК. Для подальшого вивчення механізмів впливу СККК та створення методики їх застосування (доза, частота, об'єм моніторингу) необхідним є проведення подальшого вивчення впливу СІСТ поєднаної з застосуванням СККК на функціональний стан ТН.

Ключові слова: *трансплантація нирки, стандартна імуносупресивна терапія, стовбурові клітини кордової крові, індукційна імуносупресивна теарпія, швидкість клубочкової фільтрації.*

Вступ. Кращим методом лікування хворих на хронічну хворобу нирок Vст. (ХХН Vст.) є трансплантація нирки [1]. Стандартна імуносупресивна терапія (СІСТ) запобігає ранньому та сповільнює виникнення та прогресування хронічного відторгнення (ХРВТ) ниркового трансплантата (НТ) [2, 3], яке є основною причиною втрати його функції у пізньому періоді [4].

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Побічними ефектами постійного застосування СІСТ у реципієнтів НТ є виникнення опуртуністичних інфекцій, ендокринних порушень, онкологічних, серцево-судинних захворювань, які можуть навіть закінчуватися фатально за наявності функціонуючого трансплантата [5-7].

Трансплантологам вдалося досягнути значних успіхів щодо профілактики раннього відторнення, проте Coemans et. al [8] продемонстрували, що за останні 30 років віддалені результати ТН суттєво не покращилися. Отже необхідним є пошук нових методів запобігання прогрусування ХРВТ, та частоти пов'язаних з нею ускладнень [3].

Hafeez et al. [9] показали переваги застосування АТГ для індукційої імуносупресивної терапії

(ІІТ) у разі ТН від померлого донора, повторної ТН, особливо, у разі раннього відторгнення, використанні нирок від маргінальних донорів, тривалому часі холодової ішемії НТ [10].

Доведено, що без застосування ІІС результати ТН є значно гіршими [11].

Отже, удосконалення ІІС є нагальною необхідністю сьогодення.

В попередніх дослідженнях нами були продемонстровані обнадійливі результати застосування СККК поєднаних з ІІС.

Опубліковані роботи показали їх безпеку в експериментах [12] та ефективність в проведених клінічних дослідженнях [13, 14]. Дана робота є продовженням наших попередніх досліджень.

Мета роботи – оцінити влив ІІС поєднаної з застосуванням СККК на функціональний стан ниркових трансплантатів протягом першого року посттрансплантаційного періоду.

Матеріали та методи. До цього проспективного дослідження було включено 45 пацієнтів з ХХН Vст., яким у 2020 – 2023 рр. в Національному науковому центрі хірургії та трансплантології ім. О.О. Шалімова НАМН України була виконана перша алотрансплантація нирки від живого родинного донора. Всі пацієнти були поділені на 2 групи: група I – дослідна (n = 20) та група II – порівняння (n = 25). Обидві групи отримували ІСТ АТГ в дозі 3мг/кг маси та інгібітор кальциневрину (такролімус або циклоспорин), препарат мікофенолової кислоти та метилпреднізолон, яку розпочинали за 5-7 днів до планованої операції. Крім того, I група реципієнтів за 3-4 години до трансплантації нирки, після премедикації дексаметазоном в дозі 8 мг отримувала додатково внутрішньовенне введення фракції ядровмісних клітин пуповинної крові людини (ФЯКПКЛ) в дозі 2-3х106/кг (табл. 1).

Моніторинг лабораторних показників здійснювали у три періоди: I період включав 1-7день; II період – 8-89день; III період – 90-360день, концентрації інгібіторів кальциневрину (С0), клініколабораторних показників крові та сечі здійснювали тричі на тиждень протягом першого місяця, двічі на місяць протягом 2-3 місяців та 1 раз на місяць до кінця року. Визначення NGAL сечі здійснювали у всіх реципієнтів в I періоді на першу та третю добу після трансплантації нирки.

Таблиця 1

Характеристики реципієнтів ниркового трансплантата

Параметри	I група (n=20)	II група (n=25)
вік, роки, M±m	25,50±4,55	31,58±3,55
стать: – чоловіки, n (%) – жінки, n (%)	 11 (55%) 9 (45%)	 14 (56%) 11 (44%)
ІМТ (індекс маси тіла)	20,4 ± 0,55	20,8 ± 0,64
Індукційна ІСТ: – АТГ	 20 (100%)	 25 (100%)
Введення ФЯКПКЛ	20 (100%)	0
Підтримуюча ІСТ:		
Cyclosporin, n (%)	4 (20 %)	5 (20%)
Tacrolimus, n (%)	16 (80%)	20 (80%)
Mycophenolate, n (%)	20 (100%)	25 (100%)
Methylprednisolone	20 (100%)	25 (100%)
Cross-match негативний	20 (100%)	25 (100%)
ЦМВ статус реципієнта – позитивний – негативний	 15 (75%) 5 (25%)	 19 (76%) 6 (24%)

Для профілактики розвитку ЦМВ інфекції всі пацієнти отримували валганцикловір 450 мг/добу, для профілактики пневмоцистої інфекції – котримоксазол 480 мг/добу терміном до 3-5 місяців.

Пункційна біопсія ТН проводилась всім пацієнтам згідно протоколу через 3 та 12 місяців після трансплантації, морфологічні зміни визначали згідно Banff-класифікації [15].

Статистичну обробку результатів досліджень проводили за допомогою статистичного пакету StatSoft (2010) STATISTICA 9.1 for Windows StatSoft Inc, Tusla. Проводився дескриптивний аналіз кожної вибірки з розрахунком середнього значення (M) та стандартного відхилення (SD). Порівняння двох незалежних сукупностей проводилось за непараметричним тестом – U-тест Манна–Уітні (U–

testMann–Whitney). Розбіжності між сукупностями вважались статистично значимими при значеннях коефіцієнта достовірності p < 0,05. Характер зв'язку між змінними показниками оцінювався за рівнем коефіцієнта кореляції Pearson (rp) при значеннях коефіцієнта достовірності p < 0,05.

Результати. Самопочуття пацієнтів оцінювалося за 10-ти бальною шкалою на основі суб'єктивних відчуттів (загальна слабкість, задишка), рівень артеріального тиску, частоти пульсу, температури тіла та інших скарг. Відповідно до операції цей показник складав 6-7 балів у хворих обох груп, а вже через два тижні після ТН він відповідав 10 балам у всіх пацієнтів I групи, та 9,8-9,9 у пацієнтів групи порівняння.

Дані об'єму добового діурезу у різні періоди після ТН (табл.2):

Таблиця 2

Динаміка діурезу, мл

Термін / Показник	Групи реципієнтів	При поступленні	I період (1-7день)		II пероід (8-90 день)		III пероід (91-360 день)		
			1 день	7 днів	14 днів	30 днів	90 днів	180 днів	360 днів
Діурез, (мл)	I	800± 370	9400± 950	3750± 350	2850± 100	2440± 121	2452± 69	2350± 90	2341± 50
	II	740± 430	7545± 750	3130± 455	2978± 225	2543± 109	2243± 70	2450± 70	2437± 40

I період: у групі дослідження відмічався достовірно більший рівень поліурії на 1-у добу (9400±950 мл) та на 7-му добу (3750±350 мл), порівняно з II групою реципієнтів, де добовий діурез на 1-у та 7-му добу складав 7545±750 мл та 3130±455 мл відповідно .

II пероід, III період – достовірної різниці за рівнем діурезу не спостерагалося.

Динаміка показників концентрації сироваткового креатиніну та ШКФ у I періоді свідчила про швидке відновлення функції НТ в обох групах пацієнтів. Причому в першій групі ми спостерігали нормалізацію рівня креатинінемії через 48-72 години після операції, тоді як в II групі нормалізація рівня креатиніну сироватки крові спостерагалося лише на 5-7 день після операції. (рис. 1, 2). В II періоді, на 14 післяопераційний день, концентарція креатиніну крові хворих I групи була також достовірно нижчою, ніж у пацієнтів II групи.

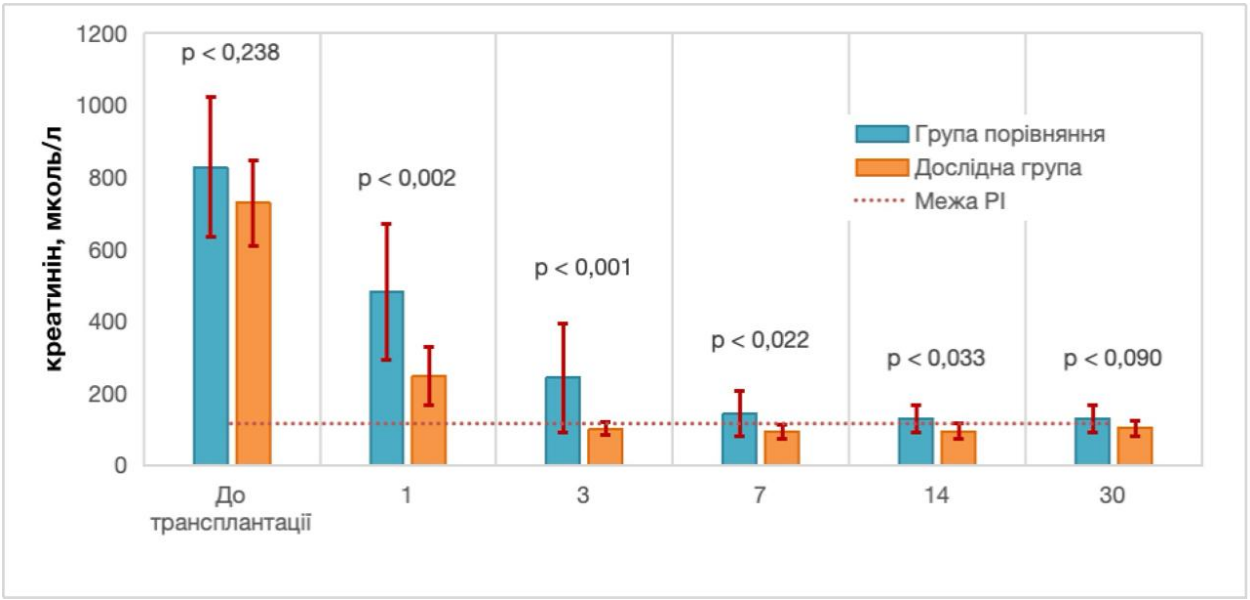

Рис. 1. Динаміка рівня концентрації сироваткового креатиніну у пацієнтів після ТН, мкмоль/л.

Розрахункову ШКФ (CKD-EPI, мл/хв/1,73 м^2) визначали в III посттрансплантаційному періоді (рис. 2). З 90 по 360 день у I групі ШКФ була достовірно вищою, порівняно з II групою.

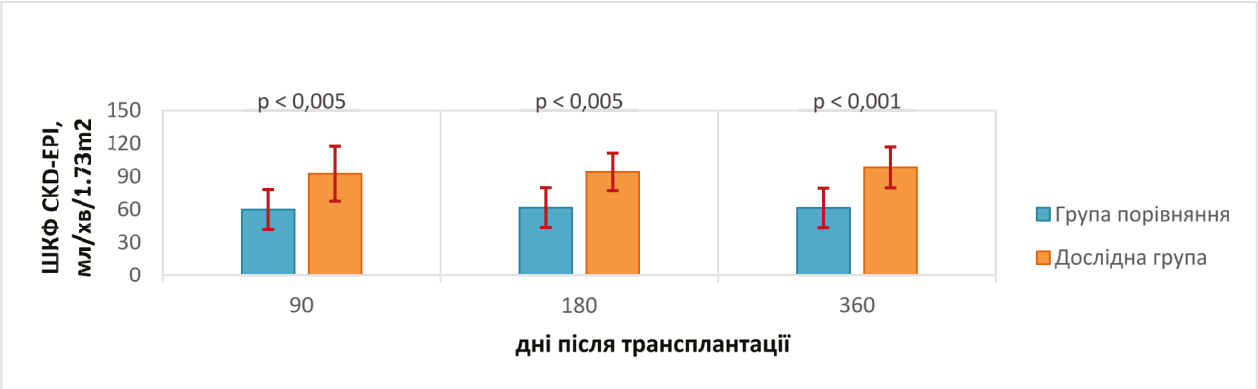

Рис. 2. Швидкость клубочкової фільтрації у пацієнтів після ТН, CKD-EPI, мл/хв/1,73 м^2 в III посттрансплантаційному періоді.

Досліджували концентрацію NGAL сечі в першому пероді після трансплантації нирки (рис. 3).

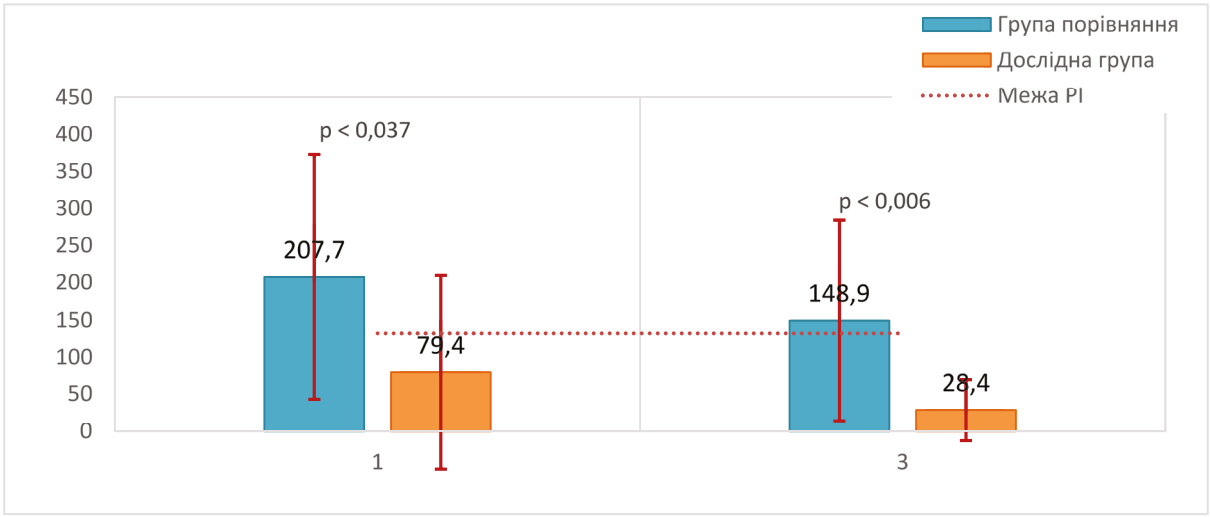

Рис. 3. Динаміка концентрації NGAL сечі у пацієнтів після ТН на 1-ий та 3-ій день після трансплантації нирки, пг/мл.

Концентрація NGAL сечі у реципієнтів I групи на 1-ий та 3-ій день після ТН складала 79.4 та 28.4 пг/мл та була достовірно нижчою, порівняно з реципієнтами II групи – 375 та 121 пг/мл на 1-ий та 3-ій день відповідно ($p < 0,05$).

Біопсії всім пацієнтам обох груп виконувалися через 3 місяці після трансплантації. У I групі клубочки були без ознак склерозування, без сегментоядерних нейтрофілів у просвіті петель капілярів клубочків, без ознак подвоєння базальної мембрани та розширення мезангіального матриксу (g0, mm0, cg0). Канальці мали збережений епітелій, у 20% пацієнтів були мінімальні ділянки тубулярної атрофії (ct1), інтраепітеліальних лімфоцитів виявлено не було (t0). У просвіті канальців патологічного вмісту виявлено не було, у інтерстиції ознак фіброзу, запальної інфільтрації виявлено не було (ci0, i0, i-IFTA0, у перитубулярних капілярах нейтрофілів виявлено не було (ptc0), стінки артерій без патологічних змін (v0, cv0, ah0), ознаки запалення були відсутні (ti0) (рис. 4-7).

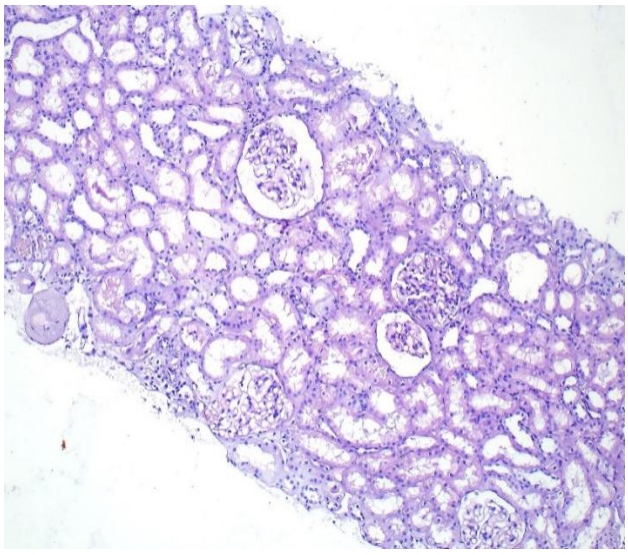

Рис. 4. Матеріал біопсії ниркового трансплантату без істотних морфологічних змін, забарвлення гем-еозин, збільшення x100.

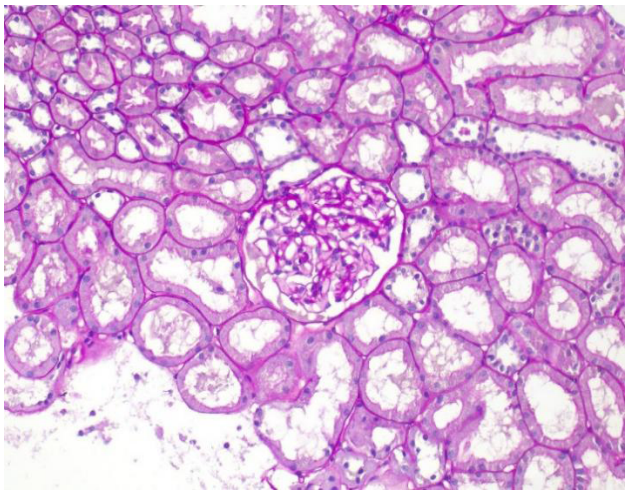

Рис. 5. Забарвлення ШИК-реакція, збільшення х200. Відсутні істотні морфологічні зміни у корковому шарі ниркового трансплантату.

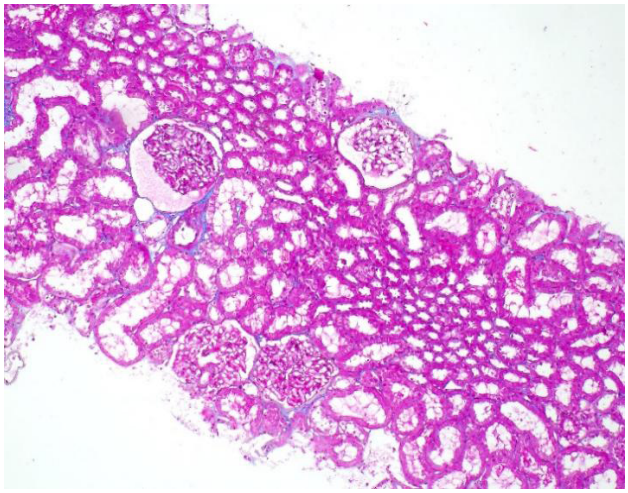

Рис. 6. Забарвлення Масон-Трихром, збільшення х100. Відсутні істотні ознаки склерозування інтерстицію.

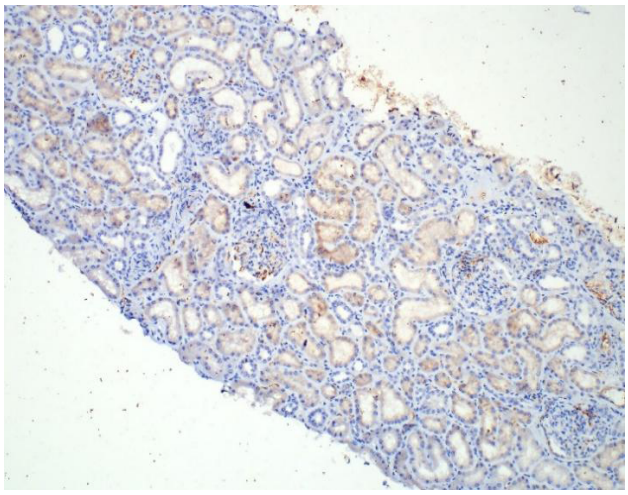

Рис. 7. Забарвлення гем-еозин, збільшення х100. Відсутнє лінійна експресія C4d у перитубулярних капілярах.

Результати імуногістохімічного дослідження реципієнтів I групи: CD3 (Polyclonal) – позитивна реакція в Т-лімфоцитах, C4d позитивна реакція в перитубулярних капілярах та клубочкових капілярах у 5% хворих .

Banff score: g0, mm0, cg0, ct1, ci0, t0, ptc0, i-IFTA0, i0, ti0, v0, cv0, ah0, c4d1 (5%).

У II групі встановлено ознаки активного антитіло-опосередкованого відторгнення (гломеруліт та перитубулярний капіляріт із наявністю лінійної експресії C4d у капілярах) у 8% хворих (рис. 8).

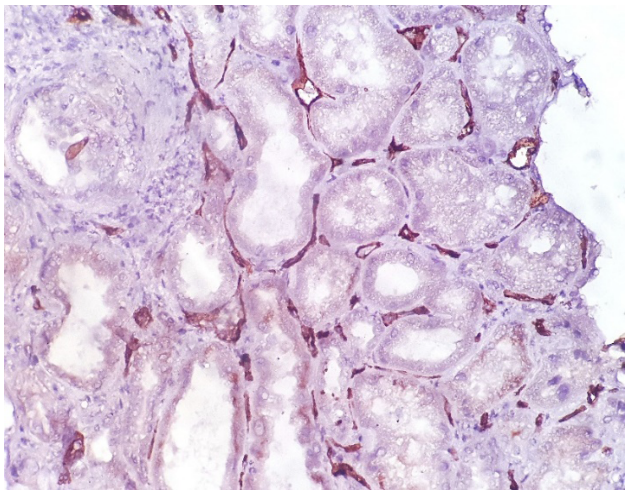

Рис. 8. Імуногістохімія біоптата нирки, збільшення х400. Лінійна експресія C4d у перитубулярних капілярах.

Гломеруліт характеризувався наявністю сегментоядерних нейтрофілів у просвіті петель капілярів частини клубочків на рівні g1-2. Перитубулярний капіляріт проявлявся наявністю клітин запалення у просвіті капілярів інтерстицію на рівні ptc1-2. У одному з випадків виявлено розширення перитубулярних капілярів. У всіх випадках активного антитіло-опосередкованого відторгнення спостерігалися явища гострого канальцевого некрозу. Не було виявлено випадків з ураженням артеріальних судин.

У одного пацієнта II групи було виявлено ознаки гострого Т-клітинного відторгнення з тубулітом та запальною лімфоцитарною інфільтрацією у несклерозованому інтерстиції.

Імуногістохімічне дослідження продемонтрувало експресію CD3 лімфоцитів (рис. 9). Тубуліт характеризувався наявнісю лімфоцитів у епітелії канальців на рівні t2.

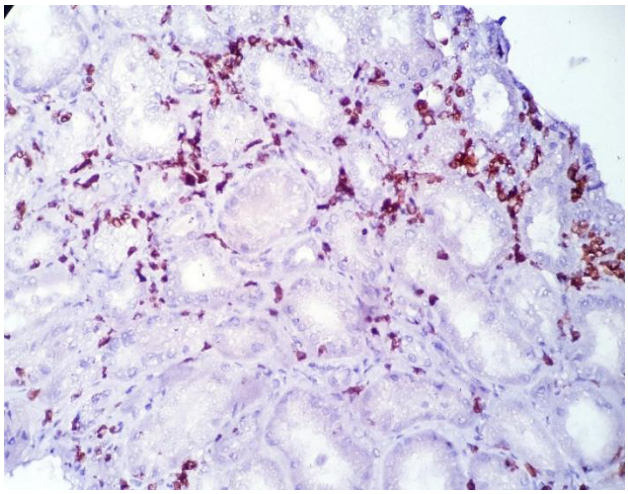

Рис. 9. Імуногістохімія, збільшення х400
Експресія CD3 у Т-лімфоцитах у інтерстиції та в епітелії окремих канальців.

Обговорення. За даними літератури, введення гемопоетичних стовбурових клітин може супроводжуватися різними ускладненнями, включаючи гостре пошкодження нирок, нефротичний синдром, хронічне захворювання нирок, інфекційні ускладнення [16]. Проте в нашому дослідженні під час та після введення СККК ускладнень не спостерігали, за винятком незначного підвищення артеріального тиску в окремих пацієнтів, що може бути пов'язано з премедикацією дексаметазоном, а також емоційними переживаннями, оскільки їх введення проводилося в умовах відділення інтенсивної терапії. Анафілактичних чи гіпертермічних реакцій на введення препарату ФЯКПКЛ не було. Відстроченої функції НАТ також не було у жодному випадку.

Зменшення креатинінемії у пацієнтів I групи виникало раніше (через 48-72 години), на відміну від реципієнтів II групи, де нормалізація рівня креатиніну наступала на 5-7-ий день, що може бути пов'язано з достовірно більшою поліурією в I періоді у першій групі реципієнтів та можливими ішемічно-реперфузійними пошкодженнями НТ, що підтверджено нижчим рівнем NGAL в сечі у реципієнтів I групи на I та III добу після трансплантації нирки ($p < 0,05$).

Середній час перебування пацієнтів в стаціонарі реципієнтів I групи був 10 днів, II групи – 14 днів. Неспроможності чи стриктур сечових анастомозів у групі дослідження і порівняння не відмічалося. Загоєння післяопераційних ран у пацієнтів першої групи було без ускладнень. У двох пацієнтів II групи з надлишковою масою тіла виникли сероми післяопераційних ран, а також у 4 із 30 пацієнтів – епізоди пієлонефритів НАТ, що потребувало дещо більшого часу перебування пацієнтів в стаціонарі.

Ми не спостерігали розвитку опуртуністичних інфекцій у пацієнтів I групи, на відміну від II групи, у 1 пацієнта була ЦМВ інфекція та COVID-19 у 1 пацієнта.

Патоморфологіні дослідження біопсій графтів у I групі не виявлили ознак суттєвих патологічних змін.

Загалом морфологічна картина біоптатів була без значних патологічних змін, що відповідало нормальним показникам функції всіх НТ. Лише експресія C4d у перитубулярних капілярах без клінічних та морфологічних проявів антитіло-опоседкованого відторгнення, яка спостерігалася у 2 реципієнтів, не мала достовірного діагностичного значення.

У II групі виявлено 2 випадки антитіло-опосередкованого відторгнення (10%) з гломерулітом та перитубулярним капілярітом та лінійною експресією C4d у капілярах, а також ознаки гострого канальцевого некрозу та один випадок гострого Т-клітинного відторгнення, що були успішно консервативно проліковані з застосуванням пульс терапії метилпреднізолону, сеансів плазмаферезу, введенням внутришньовенного імуноглобуліну, ритуксимабу та підвищенням концентрації інгібітора кальціневрину.

Zhao L. et al. [17] стверджували, що індукційна терапія з використанням СККК є безпечною у реципієнтів НТ, а в віддаленому посттрансплантаційному періоді мала нижчий рівень інфекційних захворювань, порівняно з пацієнтами, які отримували СІСТ (RR = 0,65, 95% ДІ: 0,46–0,9, P = 0,01), що також відповідає результатам проведного нами дослідження.

Висновки. Поєднання СІСТ з введенням СККК реалізується через зменшення ішемічно-реперфузійних пошкоджень структур трансплантату, відновлюючи ШКФ протягом 2-3 доби після ТН, порівняно з 5-7 добою у хворих без застосування СККК. Для подальшого вивчення механізмів впливу СККК та створення методики їх застосування (доза, частота, об'єм моніторингу) необхідним є проведення подальшого вивчення впливу СІСТ поєднаної з застосуванням СККК на функціональний стан ТН.

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Конфлікт інтересів. Автор заявляє про відсутність конфлікту інтересів.

Внесок автора.

Вороняк О.С.: збирання та обробка клінічного матеріалу, аналіз отриманих результатів, написання тексту рукопису.

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Research article

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Burden of anemia in kidney transplant patients: Epidemiology, pathophysiology, and management

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Abstract. *Anemia following kidney transplantation (KTx) is a prevalent complication that adversely affects allograft function, graft survival, and patient survival. Its etiology is multifactorial, encompassing general causes of anemia and KTx-specific factors, such as immunosuppression and reduced erythropoietin production. Management primarily involves iron supplementation and erythropoiesis-stimulating agents (ESAs); however, specific guidelines for post-KTx anemia are lacking, and the optimal methods for treating iron deficiency in KTx recipients remain undefined. Emerging evidence suggests that sodium-glucose cotransporter-2 inhibitors may improve hemoglobin and hematocrit levels in patients with chronic kidney disease and KTx recipients.*

To review recent advances in the pathogenesis, epidemiology, treatment, and outcomes of post-KTx anemia, we conducted a literature search using PubMed, Google Scholar, and Google, with keywords including "anemia in kidney transplantation," "anemia etiology in KTx recipients," "iron deficiency in renal transplantation," and "short- and long-term effects of anemia in KTx recipients."

This review synthesizes evidence indicating that effective management of post-KTx anemia, through ESAs and supplementation of erythropoiesis essentials (iron, folate, vitamin B12), is safe and may confer renoprotective benefits. Targeted anemia correction enhances quality of life, reduces mortality, improves transplanted kidney function, and lowers the risk of graft rejection, underscoring the need for standardized treatment protocols.

Keyword: *anemia, kidney transplantation, erythropoietin, iron deficiencies, sodium-glucose transporter 2 inhibitors, graft rejection, graft survival, quality of life.*

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Анемія у реципієнтів ниркового трансплантату: епідеміологія, патофізіологія та підходи до лікування

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Резюме. *Анемія у реципієнтів ниркового тнсплантата (НТ) негативно впливає на функцію алотрансплантата, виживаність трансплантата та пацієнтів. Її етіологія є мультифакторною, охоплюючи загальні причини анемії і специфічні для НТ фактори, такі як імуносупресія та зниження продукування еритропоетину. Лікування переважно включає застосування засобів заліза та еритропоез-стимулюючі агенти (ЕСА); однак специфічні рекомендації щодо менеджменту пост-трансплантаційної анемії відсутні, а оптимальні методи лікування дефіциту заліза у реципієнтів НТ залишаються невизначеними. Нові дані свідчать, що інгібітори котранспортера натрію-глюкози 2 можуть покращувати рівні гемоглобіну та гематокриту у реципієнтів НТ з хронічною хворобою нирок.*

Для огляду останніх досягнень у вивченні патогенезу, епідеміології, лікування та наслідків пост-трансплантаційної анемії ми провели пошук літератури за допомогою PubMed, Google Scholar і Google, використовуючи ключові слова, зокрема «анемія за трансплантації нирки», «етіологія анемії у реципієнтів НТ», «дефіцит заліза у реципієнтів НТ» та «коротко- і довгострокові ефекти анемії у реципієнтів НТ».

Цей огляд узагальнює докази, які вказують на те, що ефективний менеджмент пост-трансплантаційної анемії за допомогою ЕСА та необхідних для еритропоезу компонентів (залізо, фолати, вітамін В12) є безпечним і може забезпечувати нефропротекторні переваги. Цілеспрямована корекція анемії покращує якість життя, знижує смертність, сприяє кращій функції трансплантованої нирки та зменшує ризик відторгнення трансплантата, підкреслюючи необхідність розробки стандартизованих протоколів лікування.

Ключові слова: *анемія, трансплантація нирки, еритропоетин, дефіцит заліза, інгібітори котранспортера натрію-глюкози 2, відторгнення трансплантата, виживаність трансплантата, якість життя.*

Introduction. Kidney failure is a major public health problem, and its impact is expected to escalate significantly because of the aging population and the increasing rates of diabetes (DM) and hypertension (HTN) [1]. The commonest cause of renal failure that requires regular dialysis and kidney transplantation (KTx) is chronic kidney disease (CKD). CKD's overall prevalence is almost 14% [2]. Globally, DM and HTN are the prevalent etiologies of CKD [3, 4]. Although dialysis is widely used globally for kidney failure, research has shown that KTx is more cost-effective.

KTx is linked with improved quality of life, greater survival rates, and higher economic productivity [5]. The framework established by the International Society of Nephrology (ISN) prioritizes KTx as the preferred method of kidney replacement treatment (RRT) [6]. However, the use of KTx is limited by many reasons, such as patient eligibility, the availability of donors, cultural prejudice against organs from dead donors, local or regional proficiency, and the expenses associated with KTx surgery and immunosuppressive drugs [7]. While it is probable that low-income nations have more challenges in accessing KTx, there is a lack of recorded data on the availability, accessibility, and quality of KTx care compared to countries with good economic levels.

A survey was conducted in 182 nations, with 155 responding to inquiries related to KTx. Approximately 74% reported the availability of KTx, with a median occurrence rate of 14/million and a median prevalence rate of 255/million. The accessibility of knowledge about KTx has shown significant disparities, with even high-income nations demonstrating significantly worse accessibility for ethnic minorities. Around 31% of individuals had access to universal health coverage for all KTx treatment expenses, whereas 57% maintained kidney transplant registries [8], affecting the rate of KTx.

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The American Society of Transplantation and the World Health Organization (WHO) define anemia as Hb levels < 12 g/dL for females and < 13 g/dL for males [9, 10]. Normocytic normochromic anemia is a common CKD anemia linked to many negative clinical consequences [11]. A successful KTx can rectify anemia. Nevertheless, a significant proportion of KTx individuals, ranging between 20% and 51%, have anemia at different stages after KTx [12, 13]. Post-KTx (PKTx) anemia is often subdivided into early and late. Early anemia usually develops within 6 months of PKTx, affecting around 50% of patients, while Late anemia occurs in 23%-35% of KTx individuals after 6 months [13, 14].

PKTx anemia is connected with decreased physical ability, persistent tiredness, cognitive deterioration, and worsening life quality [15]. Furthermore, increasing evidence suggests that anemia might have an adverse correlation with long-term clinical results after KTX, including graft failure, death, and progression of kidney dysfunction. Therefore, addressing anemia in KTx recipients (KTRs) is justifiable, preferably initiating treatment promptly after transplantation. However, the guidelines need more appropriate or precise recommendations for managing anemia in KTxRs [16]. The KDIGO recommendations for KTRs and the position statement from the European Renal Best Practice Group both recommend managing anemia in KTxRs by adhering to the treatment guidelines for anemia in CKD. However, these recommendations have some drawbacks, as will be discussed later.

Epidemiology of post-kidney transplantation anemia. PKTx anemia prevalence was reported as 20–51% at different points in time after transplantation [12, 13]. The prevalence of early anemia in PKTxRs was about 50% in various studies [17, 18], and the prevalence decreased to approximately 23–35% during the eight-year follow-up [17, 18]. The prevalence rates of PKTx anemia at 1, 3, 6 months, and 1 year were 84.3%, 39.5%, 26.2%, and 21.6%, respectively [19]. Subsequent research indicates that the prevalence of PKTx anemia varies between 25% and 41.4% [20, 21], with a 2-year prevalence of 36.6%. The anemia incidence at 3, 5, and 10 years PKTx has been reported as 41.5%, 35.3%, and 93.2%, respectively [22]. Another study reported that anemia rates vary by definition and transplantation time. Research indicates that 50% of KTxRs are anemic at 6 months, 40% at 1 year, and 30% at 5 years of PKTx, with 12%-15% experiencing severe PKTx anemia (Hb <11 g/dL) [23]. PKTx anemia has a high prevalence, and its severity increases the risk of graft failure [24], mortality rate [12, 13], left ventricular hypertrophy [25], congestive heart failure [26], and the estimated glomerular filtration rate (eGFR) reduction [17]. The variation in PKTx anemia prevalence and anemia-related death rate may be due to heterogeneity in anemia definitions, patient characteristics, graft function, immunosuppression, inclusion criteria, and study methodologies. Standardized reporting and longer follow-ups could improve comparability.

Post-kidney transplantation anemia etiology and pathophysiology. Anemia incidence and severity are linked to kidney transplant graft dysfunction [27]. KTx causes folate, iron, and vitamin B12 deficits, causing anemia [28]. The graft secretes erythropoietin (EPO), which rises to 100% by 16 weeks PKTx, improving erythropoiesis and anemia. Second plasma EPO peaks depend on renal function recovery [29]. Serum EPO levels may vary with renal function in KTxRs [30]. As the transplanted kidney's glomerular filtration rate (GFR) drops, EPO production diminishes [30]. Acute rejection can rapidly lower serum EPO [31]. PKTx anemia patients may have high serum EPO or insufficiency and may develop intolerance to high EPO levels. Chronic allograft rejection, inflammation, iron insufficiency, hyperparathyroidism, infections, and immunosuppressive medication misuse increase resistance to the EPO effect [30].

Iron deficiency PKTx can cause early or late-stage anemia. Increased iron consumption due to allograft-induced serum EPO generation, hemorrhage during or after surgery, frequent sampling, anticoagulant usage, commencement of heavy menstrual cycles, and malignancies can cause an absolute iron deficiency [32]. Inflammation and mTOR inhibitors raise hepcidin levels, lowering blood iron and affecting erythropoiesis [32].

Chronic bacterial or viral infections impair erythropoiesis by directly impacting the bone marrow, increasing the risk of PKTx anemia [33]. In the early months after transplantation, immunosuppressive medication increases the risk of infectious diseases [34]. Due to bone marrow suppression by parvovirus B19, reticulocyte counts might drop below 1% within 2 weeks of kidney transplantation, causing anemia [37].

Anemia caused by medication may be investigated when no other causes are found [20]. Calcineurin inhibitors, anti-thymocyte globulin, mTOR inhibitors, and antimetabolites can cause anemia. Anemia can be caused by toxic antivirals such as ganciclovir and valganciclovir. Antibacterials such as trimethoprim-sulfamethoxazole can impact bone marrow, and immune-mediated hemolysis can cause anemia [14, 35]. Azathioprine and mycophenolate mofetil can cause hemolytic or megaloblastic anemia in kidney transplant recipients [36, 37]. Long-term proton pump inhibitor use may impede intestinal iron absorption, causing iron-deficiency anemia [38]. PKTx anemia is linked to RAAS inhibitors [27]. The RAAS increases serum angiotensin II, which boosts erythropoiesis by increasing EPO synthesis in response to hypoxia and decreasing renal blood flow [16]. It also decreases hepcidin and stimulates red blood cell precursor production. The effect of RAAS on erythropoiesis may be minor in the general population, but blocking RAAS receptors decreases hematocrit in immunosuppressed individuals. RAAS inhibitors in PKTx may affect erythropoiesis, causing anemia [20, 39]. Delayed graft function delays EPO synthesis by

the transplanted kidney, causing PKTx anemia. Interstitial fibrosis and tubular atrophy are independently related to 12-month post-PKTx anemia [40].

Pathophysiologically, PKTx anemia has many causes. Iron deficiency, sometimes caused by preexisting deficits, causes anemia in PKTxRs. Vitamin B12 and folate deficits, albeit less common than iron deficiency, can cause anemia and impair erythropoiesis since they are essential for erythropoiesis. Rejection of transplanted kidneys, especially in situations of chronic injury, can cause interstitial fibrosis and tubular atrophy, limiting kidney function and erythropoietin synthesis.

Early kidney impairment often coincides with donor kidney health. Late dysfunction can arise from cumulative damage or rejection, affecting EPO production over time. Hepcidin, ferritin, and HIF dysregulation affect iron availability and EPO response. Hepcidin, an iron-regulatory hormone, is raised during inflammation, especially in transplant recipients, limiting iron availability. Erythropoietin synthesis and iron metabolism depend on hypoxia-inducible factors [41]. The primary causes and pathogenesis of PKTx anemia are shown in Table 1 and Fig. 1.

Table 1

Etiologies of post-kidney transplant early and late anemia

Early Anemia Etiology	Late Anemia Etiology
Iron deficiency	Allograft Dysfunction
Infections – Viruses causing aplastic anemia (parvovirus B19, Epstein-Barr virus, cytomegalovirus, adenovirus, BK virus, herpesviruses, and varicella-zoster virus) – Indolent infections (bacterial, fungal, viral, and parasitic)	Infections: Late-viral (cytomegalovirus, hepatitis B virus, and hepatitis C virus) Community-acquired pathogens
Allograft Quality	Immunosuppressive Treatment
Immunosuppression induction	Iron Deficiency
Extended donor criteria	Vitamin B12 and Folic acid deficiency
Acute Rejection	Acute rejection
Delayed graft function	Antimicrobials
Ischemia time	Drugs Renin-angiotensin system inhibitors Proton-pump inhibitors
Aggressive Hydration (dilutionary effect)	
Transplanted kidney	Transplanted kidney

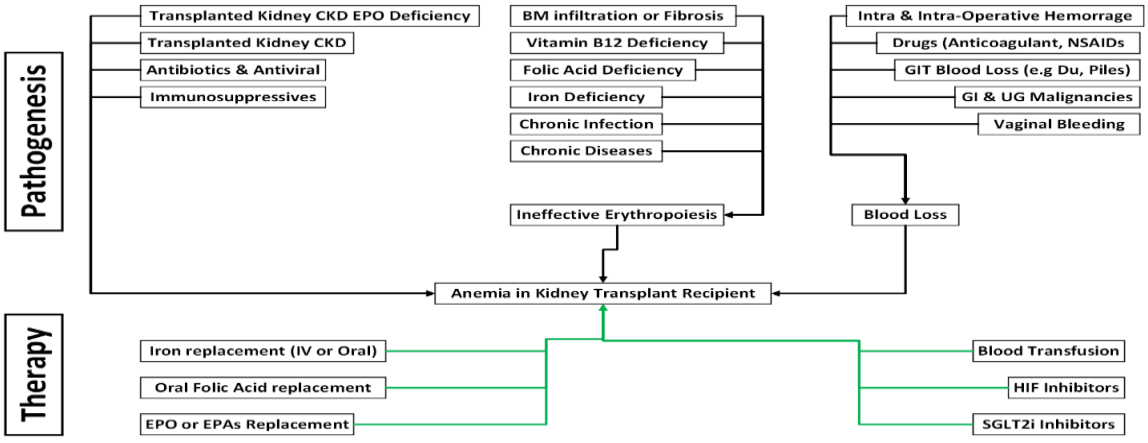

Fig. 1. Pathogenesis, etiology, and treatment of Post-kidney Transplantation Anemia.

Predictors of post-kidney transplant anemia. Multiple studies have sought to identify factors that might predict the occurrence of PKTx anemia. In retrospective research, 266 KTxRs between 2008 and 2011 were included [17]. Female sex, lower eGFR, and presence of hypochromic red blood cells were identified as variables linked with early PKTx anemia in a multivariate analysis. Early PKTx anemia and receiv-

ing a live donor kidney were identified as important factors related to late PKTx anemia occurring 2 years after transplantation. The latter factor, having a living donor as the source of transplantation, was shown to have a protective effect for late PKTx anemia [17]. The Transplant European Survey on Anemia Management (TRESAM) gathered figures from 72 transplant facilities in 16 countries, which included 4,263 KTxRs [20]. An evident correlation was seen between graft performance and Hb levels. In 904 KTx individuals with serum creatinine > 176.8 mmol/dL, 60.1% were anemic, compared to 29.0% of serum creatinine < 176.8 mmol/dL individuals, with a significant difference, $P < 0.01$. High serum creatinine levels (over 176.8 mmol/dL) were related to anemia occurrence in PKTx. Additional variables linked to PKTx anemia included donor age (especially over 60 years), the administration of ACE inhibitors, immunosuppressives such as mycophenolate mofetil and azathioprine, angiotensin receptor blockers, and infections.

In a study involving 626 patients who received transplantation at a single center in Pennsylvania, several factors were significantly linked to anemia 12 months after PKTx. These factors include anemia occurring 3 months after PKTx, the donor's age, creatinine levels at 3 months, and being female [42]. It has been concluded that after 12 months of PKTx, the occurrence of PKTx anemia has led to a higher risk of patient mortality. The presence of anemia 3 months after the transplantation is a significant factor in determining the occurrence of 12-month PKTx anemia [42]. Furthermore, a separate study conducted in Michigan, which included 192 kidney transplant patients, found that being female and having higher creatinine levels at the 3-month mark are indicators of late post-transplant allograft dysfunction [43]. The finding that anemia shortly after PKTx indicates that anemia will occur later [17] implies that improved measures to avoid and manage anemia in the early stages of PKTx might avert late PKTx anemia and graft failure, especially in severe and moderate anemia [12].

Approach to post-kidney transplant anemia. The diagnosis and the investigatory scheme of PKTx anemia include a good history and a physical examination. The important parts of the history are the history of bleeding, stool color, urine color, drug history, gastrointestinal chronic diseases, and previous surgical history. A history of anticoagulation and the type of immunosuppressive medication used are essential. Moreover, the history of when the anemia started after KTx is important to recognize the type of PKTx anemia and the history of anemia pre-KTx. Furthermore, careful, thorough clinical examination for signs of bleeding, anemia, and chronic kidney, liver, and cardiac diseases. A thorough general and systematic clinical examination is necessary to look for any causes of anemia. Complete laboratory workup, including complete blood count, differential count, urine examination, serum vitamin B12, folate, iron status (serum iron, ferritin, total iron binding capacity), serum thyroid, and parathyroid hormone levels, is all needed. In some instances, invasive investigations, such as endoscopies, bone marrow aspiration, and biopsy, might be required.

Post-kidney transplantation anemia and graft failure. Previous studies reported that KTx is linked to the combined occurrence of death from any cause and loss of the transplanted organ. Nevertheless, whereas most research has shown a noteworthy link with graft failure, findings addressing the connection between anemia and death are inconclusive. An investigation of 4,217 French PKTx individuals revealed that both death and graft failure were linked to PKTx anemia at 12 months [21]. Research conducted in Hungary followed 938 KTx patients and found that anemia was linked to increased death and graft failure after 4 years [44] and 8 years of follow-up [45]. An Austrian study on 2,031 KTx individuals revealed a substantial correlation between anemia and graft failure and mortality over a median period of 6 years [46]. A study conducted in Pennsylvania, including 626 KTx recipients, found that anemia at the 12-month mark had a higher mortality risk [42]. A study of 1,023 KTx recipients found that anemia after 3 months of PKTx was linked to increased graft failure and mortality [47]. A study of 170 KTxRs concluded that PKTx anemia during the first 30 days indicates poor graft outcome [48]. Another research, including 266 transplant patients, found that anemia occurring after PKTx at 2 years was also linked to higher death rates [44] and even during 8 years of follow-up [45]. An Austrian study of 2,031 KTx individuals revealed a substantial correlation between anemia and graft failure and mortality over a median period of 6 years [46]. A study of 1,023 KTx recipients found that anemia after 3 months of PKTx was linked to increased graft failure and mortality [47]. A study of 170 KTxRs concluded that PKTx anemia during the first 30 days indicates poor graft outcome [48]. Another research, including 266 transplant patients, found that PKTx anemia is significantly related to late mortality, graft dysfunction, and a rise in graft failure rate [12]. Another trial, including 1,139 patients, showed that anemia occurring between 6 and 18 months after PKTx is common and linked to graft failure and mortality [13]. A Japanese [51] and a Slovakian [52] study had comparable findings. A recent systematic review concluded that therapy with RAAS inhibitors can decrease death and graft loss in PKTx [53], although they may precipitate PKTx anemia [16]. However, further well-designed prospective trials are mandatory to validate these conclusions. A retrospective study that included 145 KTx recipients recently concluded that PKTx anemia significantly enhances graft failure, loss, and mortality [45].

On the contrary, subsequent research has shown contradictory findings concerning long-term fatality. In a retrospective analysis of 825 KTxRs in Europe during a follow-up period of 8 years, anemia was not shown to be correlated with all-cause mortality [49]. According to a study conducted on 2,102 Danish KTxRs, there

was no observed correlation between Hb levels and CV morbidity or death over 5 to 6 years [50]. China's retrospective research, examining 887 PKTx individuals, concluded that 12 months of PKTx anemia was not significantly linked to death [19]. On the contrary, subsequent research has shown contradictory findings concerning long-term fatality. Anemia was not shown to be correlated with all-cause mortality in a retrospective analysis of 825 KTxRs in Europe during a follow-up period of 8 years [49]. According to a study conducted on 2,102 Danish KTxRs, there was no observed correlation between Hb levels and CV morbidity or death over 5 to 6 years [50]. China's retrospective research examining 887 PKTx individuals concluded that 12 months of PKTx anemia was not linked significantly to death [19].

Regarding graft survival, anemia was consistently shown to be substantially correlated with graft loss in different studies. However, other studies did not find a connection with overall mortality, but a substantial link was noted between anemia and graft loss [13, 51]. However, other studies did not find a connection with overall mortality, but a substantial link was noted between anemia and graft loss [13, 51, 52]. Regarding graft survival, anemia was consistently shown to be substantially correlated with graft loss in different studies. However, other studies did not find a connection with overall mortality, but a substantial link was noted between anemia and graft loss [13, 51].

A study reported an established correlation between anemia and mortality, which was detected to depend on the degree of severity [13]. The presence of severe anemia (Hb < 11 g/dL) was continuously linked to a higher death risk. However, moderate anemia has not been reported to correlate significantly with mortality. Moreover, iron deficiency triggers the activation and simultaneous splitting of fibroblast growth factor (FGF) 23 [53]. High levels of FGF23 have been observed as an independent predictor for graft loss, cardiovascular disease (CVD), and death in KTxRs. This is likely due to the unintended effects of FGF23 in non-target sites [32]. It is yet unknown if FGF23 might serve as a link between iron deficiency and negative consequences in KTx anemia cases, necessitating further research.

Research indicates that high serum ferritin in the absence of infection is linked to improved graft performance and survival [54]. In contrast, the study conducted on 438 KTx recipients did not find any connection between graft failure and the proportion of hypochromic anemia [49]. Nevertheless, one study found a significant correlation between the proportion of hypochromic anemia and overall mortality [49]. Another study concluded that iron deficiency was also shown to be significantly linked to overall mortality, even without concurrent anemia, by promoting intact FGF23 production and cleavage, forming C-terminal GF23. A high level of C-terminal GF23 is significantly prospectively related to patient survival [55]. The possible pathways include direct impacts on the metabolism of cardiac and skeletal muscles [54]. In contrast, the study conducted on 438 KTx recipients did not find any connection between graft failure and the proportion of hypochromic anemia [49].

Nevertheless, one study found a significant correlation between the proportion of hypochromic anemia and overall mortality [49]. The possible pathways include direct impacts on the metabolism of cardiac and skeletal muscles [55]. Iron deficiency may weaken the functioning of cardiac and skeletal muscle cells by reducing the amount of oxygen available inside the cells and hindering the Krebs cycle [32]. These findings may explain the inconsistency in research that demonstrated a correlation between death and anemia compared to those that did not report a relationship. Hence, further research is required to clarify this subject.

Post-kidney transplantation anemia effcts on the cardiovascular system. Canadian retrospective research of 473 KTxRs revealed that anemia is a recognized independent factor for LVH, which was detectable in electrocardiography for 1 to 5 years [56]. The presence of LVH and anemia was shown to be strongly correlated with a substantial risk of mortality. LVH before KTx is linked to a higher incidence of CVD among KTx patients [57]. A study involving 1063 individuals who underwent pretransplant echocardiography revealed a significant occurrence of LVH and increased wall thickness. In a multivariable survival regression analysis, these were statistically significant (P=0.02 and 0.04) and were independent factors associated with CVD events. This association remained significant even after accounting for common pretransplant CVD risk factors [58]. KTx results in regression of LVH, as shown by research that demonstrated a substantial reduction in LV mass index (P<0.001), together with a significant improvement in ejection fraction (P = 0.009) over a 24-month follow-up period [59]. Paoletti et al. demonstrated that enhancing LVH improved post-transplant outcomes [60]. A retrospective analysis was undertaken at two Canadian institutions, including 638 consecutive KTx recipients who were clear of cardiac illness one year after the transplantation. Moreover, they reported that anemia was an independent risk factor for congestive heart failure (CHF) in anemic KTxRs. The presence of LVH and anemia was shown to be strongly correlated with a substantial risk of mortality. LVH before KTx is linked to a higher incidence of CVD among KTx patients [57].

KTx generally leads to an increase in ejection fraction over time for most recipients. Following KTx, there is a significant occurrence of new-onset CHF, ranging from 10% to 18% at 12 and 36 months, which is linked to more graft function loss and death rates [61,62]. While KTx leads to improvements in LV volumes during systole and diastole, as well as a reduction in LV muscular masses, it is important to note that the dysfunction of the cardiorenal axis before and after the KTx can still contribute to ongoing LV dilation, myocardial infarction, CHF, and arrhythmia in recipients [62]. The exact prevalence of CHF with preserved ejec-

tion fraction (HFpEF) in KTxRs has yet to be fully understood [61, 62]. However, data obtained from echocardiographic strain measurements indicate that even individuals with normal LVEF may exhibit subtle abnormalities in global longitudinal strain, a highly sensitive measure of LV function. These abnormalities were observed in KTx recipients during a mean follow-up period of 11 months. A decrease in global longitudinal strain during the peri-transplant period may also be linked to an increased likelihood of CV disease events or mortality after kidney transplant [63]. Although it appears that KTx improves most, if not all, CKD CVS complications after KTx, there are some controversies regarding the reported evidence. Hence, further larger studies are required to clarify these controversies.

Post-kidney transplantation anemia effect on glomerular filtration rate. A study reported a decline of eGFR with time in patients with anemia and vice versa. There was more reduction of the eGFR at 2 years compared to 6 months in anemic recipients, while the eGFR improved in nonanemic recipients [17]. In the Correction of Anemia and Progression of Renal Insufficiency in Transplant Patients (CAPRIT) trial, normalization of Hb (13–15 g/dL), PKTx reduced the eGFR, progression to ESRD, improved creatinine clearance, and death-censored graft survival rates [64]. Another prospective study suggested that anemia correction (12.5-13.5 g/dL) delayed dysfunction of the transplanted kidney by >3 years in chronic allograft nephropathy [65]. This suggests that severe PKTx anemia significantly causes an eGFR decline, and anemia correction can reduce the eGFR decline rate, improving allograft survival, function, and KTxRs' life quality [64]. Another prospective study suggested that full anemia correction (12.5-13.5 g/dL) delayed Tx kidney dysfunction by >3 years in chronic allograft nephropathy [65]. This suggests that severe PKTx anemia significantly causes an eGFR decline, and anemia correction can minimize the eGFR decline rate, improving allograft survival, function, and KTxRs' life quality. Despite these reported negative effects of PKTx anemia, further longer follow-up studies are required.

Post-kidney transplant anemia and mortality. Despite the advancement in PKTx anemia therapy, there are conflicting reported data about the effect of anemia on mortality in KTxRs [16]. Studies on PKTx anemia and death showed inconsistent findings. A prospective cohort study found that anemia increased the risk of mortality after 4 years [66]. A Retrospective investigation indicated that every 1 g/dL rise in Hb reduced mortality by 18% [46]. Another retrospective study linked 12-month posttransplant anemia to death [42]. Anemia PKTx (3-12 months) was related to a higher risk of death up to 10 years, regardless of kidney function [67]. Early and late PKTx increased 4-year mortality [12]. However, other research has found no link between PKTx and death. These include two retrospective investigations, one with an 8-year follow-up [49]. Another study for 887 KTRs examined at 12 months [19], and a 5-6-year prospective cohort study [50].

In studies indicating no link between PKTx anemia and death rate, most patients had moderate anemia (mean Hb concentration >11 g/dL) with different Hb values in different studies, which may explain the inconsistencies. Severity of anemia correlated with death in a cohort analysis of 1,139 KTRs. Anemia (Hb concentration <11 g/dL) was closely linked to death [13]. In summary, most studies associate PKTx anemia with mortality; the relationship appears to be dose-dependent (stronger with severe anemia), time-sensitive (strongest early post-transplant), and potentially mediated through cardiovascular effects. Table 2 summarizes the effects of PKTx anemia on mortality.

Table 2

Summary of the effects of post-kidney transplant anemia on mortality

Study Population	Study Design	Follow-up	Key Findings	Reference
4,217 PKTxRs	Retrospective	12 months	Anemia associated with mortality & graft failure	[21]
938 PKTxRs	Retrospective	4-8 years	Anemia linked to mortality & graft failure	[45, 46]
2,031 PKTxRs	Retrospective	6 years (median)	Significant mortality/graft failure correlation	[46]
626 PKTxRs	Retrospective	12 months	12-month anemia mortality risk	[42]
1,023 PKTxRs	Retrospective	3 months	Anemia linked to graft failure & mortality	[47]
170 PKTxRs	Retrospective	30 days	Early anemia predicted poor outcomes	[49, 48]
266 PKTxRs	Retrospective	2-8 years	Late mortality association	[12, 44, 45]

Continuation of Table 2

Study Population	Study Design	Follow-up	Key Findings	Reference
1,139 PKTxRs	Retrospective	6-18 months	Graft failure & mortality link	[13]
145 PKTxRs	Retrospective	-	Graft failure & mortality	[45]
825 PKTxRs	Retrospective	8 years	No mortality association	[49]
2,102 PKTxRs	Retrospective	5-6 years	No Hb-CV mortality correlation	[50]
887 PKTxRs	Retrospective	12 months	No mortality link	[19]

Legend: PKTxRs (post-kidney transplant recipients).

The summary of the table and the texts above are: 1. Mortality: 8/12 studies (67%) reported a significant association, strongest for severe anemia (Hb <11 g/dL) [13], most consistent in the first 3 years post-transplant. 2. Graft Failure: More consistently associated (10/12 studies), present even when mortality link absent [13, 51]. 3. Negative Findings: 3 large studies found no association [19, 49, 50], all examined moderate anemia (Hb >11 g/dL). 4. Cardiac Impact: Anemia+LVH ↑ mortality risk [56-58], may explain some mortality associations.

Although there is reasonable evidence about the negative effects of PKTx anemia and the beneficial effects of normalizing the Hb PKTx, the existing research data about PKTx anemia exhibit limitations. Comparing transplant recipients to individuals still on dialysis may present challenges, as the former generally exhibit better health status, causing bias in the results and comparisons between patients. The other limitation is heterogeneity. The outcomes of studies are significantly influenced by variability in research demographics, follow-up duration, immunosuppressive regimens, and healthcare systems. Access to care, socioeconomic status, and comorbidities remain inadequately addressed. Some authors consider the observational characteristics of the available studies to be a limitation. Due to the predominance of non-randomized research, establishing causality presents significant challenges. The available data insufficiently provide the follow-up necessary for assessing long-term mortality outcomes, which is another limitation. Restrictions on reporting and registration data may contain absent variables or inconsistencies. Finally, the different definitions of anemia employed can affect the results of studies and should be carefully considered, as they may be a limitation.

Post-kidney transplant anemia therapy. PKTx anemia is frequent in KTxRs. Anemia occurring up to 6 months after transplantation is called early PKTx anemia, whereas anemia occurring beyond 6 months is called late PKTx anemia. Early PKTx anemia predicts late PKTx anemia. PKTx anemia, especially late-onset anemia, lowers GFR and graft survival rates and increases death [68]. The severity and cause of anemia affect mortality significantly. Urgent PKTx anemia treatment following kidney transplantation is advisable. Renal transplant patients with anemia should aim for 12.5-13 g/dL, which is greater than the recommended value for CKD patients. ESA and iron therapies are needed to reach this goal

Iron therapy. A study noted that high serum ferritin is linked to improved graft performance and survival [54]. In contrast, other studies conducted for KTxRs found no connection between graft failure and the proportion of hypochromic anemia [16, 69]. Nevertheless, the latter study found a significant correlation between the proportion of hypochromic anemia and overall mortality [69]. Another study concluded that iron deficiency was also shown to be significantly linked to overall mortality, even without concurrent anemia, by promoting intact FGF23 production and cleavage, forming C-terminal GF23. A high level of C-terminal GF23 is significantly and prospectively related to mortality [55]. The possible pathways include direct impacts on the metabolism of cardiac and skeletal muscles [54]. Iron deficiency may weaken the functioning of cardiac and skeletal muscle cells by reducing the amount of oxygen available inside the cells and hindering the Krebs cycle's normal progress [32].

Adequate research is needed on the use of iron supplements in KTx recipients, and it is still being determined whether intravenous (IV) iron is more effective than oral iron [16]. Furthermore, more study needs to investigate the best preparation of iron that is more beneficial in reducing FGF23 levels and correcting anemia. Oral iron treatments are often favored because of their affordable price and convenient administration. Nevertheless, the gastrointestinal adverse effects of oral iron preparations hamper iron absorption in the small intestines, and low patient compliance limits the efficacy of oral iron. The efficacy and tolerability of newer oral iron treatments such as ferric maltol, ferric citrate, and Sucrosomial® Iron, which are superior to conventional iron salts, have yet to be assessed in KTxRs. Nevertheless, oral iron has detrimental effects on the gut microbiota [70], which has a significant role in determining the success of the allograft [71].

Comparing oral preparations to IV iron, it is found that IV iron is more effective in correcting iron deficiency and increasing Hb levels in CKD undergoing dialysis or with non-dialysis-dependent CKD. Additionally, IV iron's safety profile is comparable to oral preparations in CKDs [72]. Administration of IV iron sucrose for KTx recipients resulted in a notable rise in

Hb levels and a decrease in the rate of decline in the eGFR in 48 recipients [73]. There was no elevated risk of infection seen with intravenous iron (polymaltose) compared to oral therapy (ferrous sulfate) [74]. Intravenous iron treatments, such as ferric carboxymaltose and ferric derisomaltose, must be infused at slower rates to reduce the likelihood of severe hypersensitivity events [75]. IV ferric carboxymaltose has excellent tolerability and safety [76].

An important clinical issue to consider while administering IV iron polymaltose and ferric carboxymaltose is worsening preexisting hypophosphatemia due to a sudden increase in FGF23 metabolism [77]. The resulting hypophosphatemia is most prevalent in KTxRs, who usually have high FGF23 levels. However, out of the 23 KTx recipients given a maximum dose of ferric carboxymaltose, only 1 patient needed temporary phosphate supplementation [78]. Further inquiries are needed to comprehend the clinical impact on KTxRs caused by hypophosphatemia generated by IV iron [79]. Individualized Hb level goals were advisable in PKTx anemia. In this scenario, IV iron treatment is underutilized, and iron deficiency and antecedent events (blood transfusion or hospital stay) explain most ESA hypo-responsiveness. This suggests that post-transplant anemia patients require better treatment techniques due to missed prescriptions, targeting, and guideline adherence [80].

Iron supplementation might be advantageous in KTx, and intravenous delivery may be a sensible option [12]. Nevertheless, several matters remain unsettled. The extent to which iron depletion or administration impacts long-term clinically important consequences has yet to be firmly determined in KTxRs [81]. Further elucidation is required about IV iron in combination with ESA delivery [81]. Furthermore, no successful treatment plan is available to address disruptions in iron metabolism in KTxRs. Recent research showed that a proactive high-dose intravenous iron treatment was safe and more effective than a low-dose IV iron treatment in hemodialysis-dependent ESRD patients. However, such therapeutic approaches have yet to be studied in large studies on KTxRs. Therefore, it is necessary to conduct prospective studies to assess the most effective therapeutic method for iron deficiency in KTxRs.

Erythropoietin or erythropoietin-stimulating agents therapy. ESA treatment stimulates the production of erythrocytes. It has significantly transformed the treatment of anemia in CKD [11]. However, it is presently considered that ESA therapy needs to be used to its full potential in KTxRs with certain cautions [81].

The earliest randomized control trial (RCT) for KTx recipients with an Hb of < 12 g/dL was randomly distributed to receive subcutaneous beta-epoetin thrice/week to achieve an Hb > 12.5 g/dL, and a control group received a placebo. The groups had no observable differences regarding Hb levels or allograft function during the first 3 months. However, the group receiving ESAs achieved the goal of Hb level more quickly and needed fewer blood transfusions [82]. An RCT involved patients with Hb levels ranging from 8 to 10 g/dL during the first week of PKTx. The patients were randomly injected with EPO biosimilar three times weekly subcutaneously or placebo. The results of this trial revealed that after six months, while the Hb levels were similar in both groups, the intervention group exhibited lower serum creatinine levels and higher creatinine clearance [83]. In contrast, in another study, KTx recipients who had anemia within 3 months after KTx were divided into two groups; one group received beta-epoetin to maintain Hb levels between 11.5 and 13.5 g/dL, while the placebo group received no medication [84]. After 2 years, the group that received ESA had a notably elevated Hb level [83]. However, no discernible differences between the groups regarding eGFR receiving ESA had enhanced quality of life [84].

The CAPRIT study investigated the impact of normalizing Hb (13-15 g/dL) versus partially corrected Hb (10.5-11.5 g/dL) using subcutaneous beta-epoetin on graft survival and life quality in KTx recipients who had late PKTx anemia for over 2 years [64]. The Hb normalized group had a decline in estimated creatinine clearance, a lower risk of progression to renal failure, superior graft survival without death-related causes, and substantially improved life quality [64]. Tsujita et al. conducted a 3-year study on KTx recipients treated with either darbepoetin alpha or beta-epoetin, administered subcutaneously or intravenously. Hb correction (12.5-13.5 g/dL) versus low Hb levels (10.5-11.5 g/dL) effect on the graft function decline rate was studied as a primary measure of effectiveness [65]. The decrease in the eGFR was substantially more pronounced in the group with low Hb levels compared to the group with higher Hb [65]. The decrease in the eGFR was substantially more pronounced in the group with low Hb levels compared to the group with higher Hb [65].

Another study investigated a total of 153 KTx recipients who were randomly allocated to two groups: one with a high goal level of Hb (≥12.5 g/dL) and the other with a low target level (<10.5 g/dL). Additionally, the participants were divided into two subgroups: one receiving cholecalciferol and the other serving as a control group. All participants received subcutaneous beta-epoetin [85]. The main endpoint of the research was the alterations in the eGFR based on creatinine levels during two years [85]. Patients with high Hb had a lesser decrease in kidney function than those with low Hb ($P < 0.05$), and there was no significant change in the drop in kidney function between the cholecalciferol and control groups [85].

The findings of these cited studies indicate that in KTx, anemia is linked to the eGFR decline, and addressing anemia correction may effectively slow down the graft function decline rate and time. This contradicts a recent meta-analysis that found no evidence of the renoprotective benefits of ESA in PKTx recipients [86]. Nevertheless, heterogeneity and disparate main outcomes were noted as limitations of this study [81].

In KTx animal models, the administration of EPO effectively reduced the development of chronic allograft nephropathy by regulating antioxidant expression and antiapoptotic and angiogenic characteristics inside the transplanted kidney tissue [87]. Nevertheless, studies noted that blood transfusions, which restore normal HB values after a transplant, do not have any impact on the damage to the transplanted kidney. This suggests that the protective effect of EPO on the kidney outweighs the benefits of correcting anemia alone [87]. It was reported that EPO demonstrated the immunomodulatory effects required for the natural acceptance of foreign kidneys in both laboratory models using mouse cells and clinical research involving patients with stage 4 CKD who were treated with ESAs [88]. Nevertheless, the first clinical trials failed to reveal any tissue-preserving advantage of using ESA in the post-transplant period.[86] Nonetheless, more studies are needed to accurately determine the potential of ESA in protecting the kidneys after KTx [86]. However, more studies are needed to accurately determine the potential of ESA in protecting the transplanted kidney.

ESA early administration for treating PKTx anemia should be considered on a case-by-case basis. However, ESA must be cast in late PKTx anemia to improve graft survival and normalize the Hb (12.5-13.5 g/dL), though caution is needed in high malignancy-risk KTx recipients [81]. EPO is prescribed for cancer- and chemotherapy-associated anemia. Paradoxically, EPO might promote tumor growth and jeopardize patient survival in cancer [89]. The current Hb goal level is comparable to the previously proposed Hb target level of 12-13 g/dL [14]. The recommended goal level for Hb in KTx recipients is higher than the recommended levels advised for the CKD population by the KDIGO (Hb 11.5 g/dL) and KDOQI (Hb 11 g/dL) recommendations [90]. ESA medication for hemoglobin normalization may protect the kidneys and minimize mortality, according to a systematic review of 38,233 participants from 85 studies [91].

The decision to focus on lower Hb in CKD was based on the unfavorable outcomes seen in extensive trials that evaluated the safety and success of ESA treatment aiming at higher Hb levels in CKD patients [92]. Current research indicates that using ESA in anemic KTxRs is secure and linked to a potentially positive result. However, high Hb levels can lead to hemoconcentration, which can cause thrombosis [93]. Various forms of ESAs have shown similar effectiveness in managing PKTx anemia. Long-acting ESAs are more convenient for controlling PKTx anemia; however, there is no preferred method (subcutaneous or intravenous) for administering ESAs. Further clarity about the best use of ESA regarding dose and goal level of Hb in KTxRs is required.

Blood transfusion. KTxRs often receive packed red blood cell transfusions (PRBCT), especially during the first month after the transplant [94]. A study that included 12,000 KTxRs found a link between early transfusion and transplant failure (defined as graft loss or death with a functioning graft) [94]. Transfusion within 1 month after KT decreased graft survival and increased antibody-mediated rejection and infections at 1 year [95]. Transfusion may also cause venous thromboembolism [96]. A new retrospective cohort study found that venous thromboembolism increased with transfusions following transplantation [97]. In KTxR, venous thromboembolic events increase graft loss and mortality risks [25, 98]. Considering the mentioned complications, volatility in Hb levels, and limited availability, PRBCT cannot be seen as a viable alternative for controlling PKTx anemia [11]. Transfusions are risky, expose patients to substantial Hb-level variations, and are limited in availability. Thus, they cannot be used to treat posttransplant anemia [11]. The existing data underscores the necessity of cautiously using PRBCT in KTx, considering the balance between risks and benefits, and exploring other approaches to correcting anemia, such as managing iron levels or using ESAs [97]. New research emphasizes the necessity for careful transfusion usage for KTxR, balancing the risk-benefit ratio, and investigating alternate anemia correction techniques, including optimizing iron reserves or ESA use [97].

Hypoxia-inducible factor-prolyl hydroxylase inhibitors. Recently, there have been oral medications called hypoxia-inducible factor-prolyl hydroxylase (HIF-PH) inhibitors that may be used to treat CKD-induced anemia. This new category of medications raises the natural levels of EPO in the blood, activates the process of copying the EPO gene in the kidneys and liver, and seems to decrease hepcidin levels and enhance iron balance in the body [99]. Therefore, suppressing HIF-PH may effectively and via different mechanisms control the pathogenic variables linked to CKD-related anemia. Roxadustat, a HIF-PH inhibitor, received authorization from the European Medicines Agency in 2021 to treat CKD-induced anemia, regardless of whether they are dialysis-related or not. On the other hand, daprodustat was recently licensed by the USA Food and Drug Administration specifically for patients on dialysis for a minimum of 4 months [16].

Data on the use of HIF-PH inhibitors in KTx recipients is scarce. The primary reason for this is likely due to a theoretically heightened susceptibility to cancer (such as the elevation of vascular endothelial growth factor and angiogenesis) in an immunocompromised population that is already predisposed to acquiring malignancies [81]. An observational study was conducted on KTx recipients with Hb values < 11 g/dL to assess the impact of administering roxadustat (20-100 mg) thrice a week [100]. The goal level for Hb was set at 11-13 g/dL, and ESAs were administered if Hb levels diminished below 10 g/dL. Out of the 31 patients that were registered, 25 individuals successfully finished the 20-week study, whereas 6 patients had early postoperative transient atrial fibrillation. The average Hb levels gradually rose from 9.8 g/dL at the beginning, reaching a stable value of 12.4

g/dL after 20 weeks. Iron insufficiency necessitating iron supplementation was noted at 8 weeks in 12 patients receiving ESAs. The study was terminated because three patients had a severe drop in Hb levels, two patients had gastrointestinal complications, and one patient suffered from a myocardial infarction [100]. Li et al. conducted a study on 21 KTx recipients, 6 of whom were treated with ESA. These patients were hospitalized due to difficulties following the kidney transplantation, and their Hb was below 10 g/dL. The researchers closely observed the impact of Roxadustat, administered thrice a week, depending on the patient's weight [101]. Eleven patients were excluded from the trial with roxadustat before its completion (after 10 weeks) due to either an initial or subsequent lack of response or upon attaining the desired Hb level. Among the participants who finished the trial, there was a notable rise in Hb concentration from 6.9 to 10.4 g/dL.

Additionally, the treatment response rate was observed in 71.4% of participants, without discernible adverse responses observed [101]. Among 5 KTx recipients with late PKTx anemia who transitioned from using epoetin beta l to roxadustat thrice a week for 9 months, all patients had a rise in their Hb after just 1 month. Furthermore, a good improvement in anemia was consistently maintained throughout the period. Nevertheless, excessive improvement in Hb levels occurred, leading to one patient discontinuing roxadustat after one month and three patients requiring a reduction in medication dose; however, none noted significant adverse effects [102]. Roxadustat is a safe medication in KTxRs, and possible side effects of roxadustat include an increased risk of thromboembolism and hypertension. There are no reported data about the increased risk of malignancy in KTXR [103].

Post-organ transplantation, cancer risk increases due to various reasons, such as immunosuppression, viral infections, and prior cancer, which are major risk drivers. The common post-transplant malignancies are non-melanoma skin cancer (~50% of cases), post-transplant lymphoproliferative disorder (PTLD) due to EBV-driven B-cell proliferation, Kaposi sarcoma, liver cancer, and native renal cell carcinoma [104].

HIF-PH inhibitors' role as a cause of malignancy remains investigational. In KTxRs, the use of HIF-PH inhibitors data is limited [16]. In immunocompromised people already prone to cancer, elevation of vascular endothelial growth factor and angiogenesis may enhance cancer risk [81]. It is documented that Hypoxia-Inducible Factor 1-Alpha has pro-tumorigenic effects, increasing angiogenesis and driving immune evasion [105]. Preclinical evidence links HIF-1-alpha to clear cell renal carcinoma [106]. However, limited safety data in KTxRs. KDIGO 2023 Guideline avoids recommendations due to a lack of transplant data [107]. Until confirmed data is available, practical approaches include avoiding high-risk KTxRs (prior malignancy, EBV+), monitoring aggressively for skin cancer, PTLD, and BK virus, and preferentially using ESAs. More observational research projects are required to elucidate the long-term malignant side effects of these medications and other immunosuppressive agents used to prevent allograft dysfunction and rejection.

Based on the existing information regarding using HIF-PH inhibitors for PKTx anemia therapy, it is recommended to begin treatment with a small dosage and gradually increase the titration while providing more iron due to the increased iron utilization [33]. Further RCTs are necessary to elucidate the extended effectiveness and safety of HIF-PH inhibitors for treating PKTx anemia.

Sodium-glucose co-transporter 2 inhibitors use for post-kidney transplantation anemia therapy. Diabetes prevalence is predicted to rise to 10.2%, reaching 578 million by 2030, and by 2045, it is predicted to increase by 10.9%, reaching 700 million people [108]. Glucose reabsorption by the nephrons ends at the end of the proximal convoluted tubule (PCT). PCT is divided into three segments. Both segments one and two reabsorb almost 90% of the filtered glucose mediated by SGLT2 receptors. In contrast, SGLT1, located in the straight segment of the proximal tubule (segment 3), accounts for roughly a tenth of total glucose reabsorption [109]. Blockage of glucose receptors in these sites will decrease glucose reabsorption by the brush border cells of the PCT, increasing the osmotic diuresis and urine excretion.

Seven SGLT2 inhibitors are currently in clinical use: empagliflozin, dapagliflozin, ipragliflozin, tofogliflozin, luseogliflozin, and canagliflozin. In addition to cardio- and renoprotection, SGLT2 inhibitors exhibit hematopoietic effects [110, 111]. Although SGLT2i shows unforeseen renal-protective properties in diabetes mellitus type 2 (DM2) and those without DM2 with and without CKD, doubts about their usage in KTx remain owing to worries about an elevated risk of genital mycotic and urinary tract infections. There is substantial evidence of Fournier gangrene and vaginal mycotic, and other chronic infections in SLGT2 inhibitors treated individuals [112], potentially elevating the incidence of anemia, which might appear more in KTx recipients with impaired immune systems. However, a review article concluded that SGLT2i can be practiced in diabetic KTx recipients safely and effectively in selected recipients [113]. A 2022 study of 323 PKTx recipients reported that empagliflozin, dapagliflozin, and canagliflozin are safe [114]. Research conducted for 24 weeks examined the empagliflozin effect in diabetic KTxRs. The study found a substantial decrease in HbA1C levels and body weight among recipients treated with empagliflozin. However, no significant change was seen in the eGFR [115]. Another study noted no change in the eGFR in diabetic KTxRs on SGLT2 inhibitors [113]. The lack of considerable improvement in the eGFR was attributed to the vasoconstriction of the afferent arterioles caused by SGLT2 inhibitors. The natriuretic impact of SGLT2 inhibitors is believed to cause an increase in tubule-glomerular feedback and

constriction of the afferent arteriole, even in a KTx that has lost its nerve supply.

SGLT2i, such as empagliflozin, increased Hb and hematocrit in 3726 heart-disease patients [116]. Improving Hb and hematocrit levels in PKTx recipients could also be due to the diuretic effect of SLGT2i that masks anemia [117] or the eGFR improvement [118]. Although SLGT2 inhibitor usage appears promising, further larger studies in diabetic and non-diabetic KTxRs are required to assess this thought further. It was reported that SGLT2 inhibitors improve PKTx anemia, possibly by improving the effectiveness of EPAs [119]. Improving Hb and hematocrit levels in PKTx recipients could also be due to the diuretic effect of SLGT2i that masks anemia [117] or the eGFR improvement [118]. Although SLGT2 inhibitor usage appears promising, larger studies in diabetics and diabetic KTxRs are required to assess this hypothesis further.

SGLT2i boosts EPO synthesis, decreasing kidney hypoxia. However, the precise process is unknown [120,121]. SGLT2 inhibitors improved Hb and increased hematocrit in T2D and CKD patients [122]. However, it was unclear whether the increase in hematocrit was because of fluid volume reduction or an improved primary erythropoietic response [123]. Recent human investigations have further shown the stimulatory impact of empagliflozin and dapagliflozin on erythropoietin synthesis in individuals with native kidneys [124]. Anemia is estimated to affect 30-40% of kidney transplant recipients and is recognized as a prevalent risk factor for graft failure and death during the first three years post-transplantation [13, 125]. The etiology of anemia in kidney transplant recipients is often complicated, potentially including iron deficiency, compromised renal function, bone marrow suppression due to immunosuppression, antiviral prophylaxis, or infection [125]. SGLT2 inhibitors may mitigate anemia in kidney transplant recipients and enhance allograft outcomes [123].

Two post hoc analyses of the CREDENCE (Canagliflozin and Renal Events in Diabetes With Established Nephropathy Clinical Evaluation) and DAPA-CKD trials suggest additional anemia-related benefits from canagliflozin and dapagliflozin treatment [126]. Despite these encouraging post hoc analysis results, CREDENCE and DAPA-CKD trial design may have limited their therapeutic significance. A cohort study comparing SGLT2i and SGLP receptor antagonists concluded that SGLT2i could be deemed an adjunct treatment to diminish the anemia rate by 19% in 13,799 CKD-DM2 patients [121]. A study showed a significant difference in Hb concentrations between DM2 patients with coronary artery disease treated with 10 mg empagliflozin and placebo at six months: 13.9 (g/dl) in the placebo group and 14.6 in the empagliflozin group [124]. Empagliflozin increased Hb and hematocrit in 3726 heart failure patients [116]. The mechanism by which SGLT2 inhibitors decrease anemia is unknown, although increasing EPO synthesis could be a mechanism.

Post-kidney transplantation anemia correction effects on the cardiovascular system. The data from KTx recipients managed with ESA agents do not indicate an elevated risk of cardiovascular (CV) complications reported [16]. The CAPRIT research found that there were no instances of cardiac diseases, stroke, HF, arrhythmia, or myocardial infarction in the full correction Hb levels group. However, certain CV events occurred in a lower Hb level group [64]. No CV events were noted in the trial conducted by Tsujita et al. [65], and research conducted by Obi et al. [64] did not prove a higher occurrence of stroke among KTx recipients who were assigned to the arm with a higher goal level of Hb [85]. Additionally, a 2-year trial on KTx recipients who were administered epoetin beta with a target Hb concentration of 11.5-13.5 g/dL showed no negative effects on the CV system or blood clotting events [84]. The observed disparities in population sizes between individuals with non-transplant CKD and KTx recipients may be credited to variations in the environments or circumstances in which these populations were studied. Chronic allograft nephropathy and CKD vary significantly regarding their underlying mechanisms and eventual results [64]. Furthermore, a long-term KTx effectiveness may demonstrate greater effectiveness than a kidney from a patient with CKD who does not need dialysis regarding histology, hemodynamics, and immune biology [65]. However, further studies are required to explore these issues in KTxRs.

Guidelines: Challenges in post-kidney transplant anemia. Various pathophysiological mechanisms, iron metabolism dynamics, immunosuppressive effects, and the lack of convincing evidence in transplant-specific cohorts limit the applicability of the KDIGO and other CKD-induced anemia guidelines to managing PKTx anemia. KDIGO (2012, with updates in 2023) and other guidelines, including the ERA and the NKF-KDOQI, provide recommendations for anemia management in CKD. The application of these recommendations to the treatment of anemia in KTxRs is a subject of controversy. Transplant-specific factors, such as immunosuppression-induced bone marrow suppression, altered iron metabolism post-kidney transplantation, varying responsiveness to ESA, and the absence of large RCTs in KTxRs, restrict their applicability to managing anemia in PKTx. Given these limitations, the existing anemia guidelines may not be ideal for treating anemia post-kidney transplantation. Although immunosuppressant agents have been shown to suppress bone marrow function, some studies have suggested no correlation between immunosuppression and post-transplant anemia.

Chronic inflammation and viral-induced hemolysis (BK virus nephropathy and parvovirus B19 infection) reduce EPO sensitivity. Furthermore, KTRs exhibit distinct iron metabolism and hepcidin dynamics. ESO agents' hyporesponsiveness has also been documented in KTRs [127]. KDIGO recommends ESA therapy for hemoglobin levels below 10 g/dL in CKD

patients; however, KTRs may exhibit resistance to this treatment due to factors such as chronic inflammation, the use of ACE inhibitors or ARBs, suppressed endogenous EPO synthesis, and iron-restricted erythropoiesis despite normal ferritin levels. The absence of available RCTs demonstrating the efficacy and safety of ESA in KTRs leads guidelines to extrapolate from CKD data.

Managing PKTx anemia as CKD-induced anemia may result in an overcorrection of Hb levels exceeding 11 g/dL, thereby increasing the risk of thrombosis. The KDIGO guideline advises against allowing Hb levels to exceed 11.5 g/dL in CKD patients due to the heightened risk of stroke and thrombosis. Elevated Hb levels may be tolerated in KTxRs; however, the current guidelines do not specify Hb targets for these recipients.

The existing KDIGO, ERA, and KDOQI anemia guidelines are not transplant-specific. This may lead to abuse of ESAs without demonstrated benefit, improper iron supplementation levels, and missing secondary causes of PKTx anemia. Using transplant RCT data, new guidelines address Hb targets, and immunosuppressant-ESA interactions are needed.

Conclusions and prospects for further research. PKTx anemia is a multifaceted condition that significantly impacts patient outcomes and quality of life. PKTx anemia is mainly caused by iron deficiency. Decreased graft function has been linked to an increased incidence of late PKTx, and early PKTx anemia is a good predictor of late PKTx anemia. Decreased graft survival, increased mortality, and a drop in GFR are linked to PKTx anemia. The anemia's underlying cause and severity have a strong correlation with death.

Kidney transplantation should generally start as soon as feasible. The ideal goal Hb level for KTX patients with anemia is likely closer to 12.5–13 g/dL. A suitable course of therapy with iron and erythropoiesis-stimulating drugs is recommended to reach this goal, manifesting in better life quality, graft survival, and reduced mortality rates. Therapy with SGLT2I for anemic KTxRs showed promising potential. However, further research in different aspects of PKTx anemia effects and treatment, and setting a target Hb level for PKTx is required. Large-scale controlled RCTs for SGLT2i and standardized protocols for iron therapy are needed. Finally, there are no guidelines for anemia definition and targeted hemoglobin levels for KTxRs in CKD. Therefore, guidelines are urgently required based on large-scale studies for kidney transplanted patients.

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Authors contribution.

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Diagnostic and therapeutic potential of interleukin-37 in kidney diseases: A mini-review

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Abstract. *Interleukin-37 (IL-37) is a newly discovered anti-inflammatory cytokine from the IL-1 family that plays a key role in regulating both innate and adaptive immune responses. It is secreted in healthy tissues, reflecting a homeostatic function. Intracellularly, IL-37 suppresses diverse inflammatory signals in various cells, including dendritic cells, macrophages, epithelial cells, and endothelial cells. Although it has been studied in many conditions, such as autoimmune disorders, cancer, and cardiovascular disease, its specific role in kidney diseases remains relatively understudied.*

In this mini-review, we summarize current evidence on the biology of IL-37, with a focus on its relevance to kidney disease. We explore its molecular structure, patterns of expression, and the immunomodulatory mechanisms that may influence kidney diseases, including acute kidney injury, diabetic nephropathy, and autosomal dominant polycystic kidney disease. We also discuss the challenges of translating IL-37-based therapies into clinical practice and highlight key areas for future research aimed at unlocking its potential in the treatment of kidney diseases.

Key words: *interleukin-37, kidney diseases, acute kidney injury, diabetic nephropathy, autosomal dominant polycystic kidney disease, immunomodulatory mechanisms, interleukin-based therapy.*

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Потенціал інтерлейкіну-37 в діагностиці та лікуванні захворювань нирок: міні-огляд

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Анотація. *Інтерлейкін-37 (ІЛ-37) — це відносно нещодавно відкритий протизапальний цитокін із сімейства ІЛ-1, який відіграє ключову роль у регуляції як вродженої, так і адаптивної імунної відповіді. ІЛ-37 секретується в здорових тканинах, що відображає його гомеостатичну функцію. Внутрішньоклітинно, ІЛ-37 пригнічує запальні сигнали в різних клітинах, включаючи дендритні клітини, макрофаги, епітеліальні клітини та ендотеліальні клітини. Хоча ІЛ-37 ретельно досліджувався при аутоімунних розладах, онкологічних і серцево-судинних захворюваннях, його специфічна роль у патології нирок залишається недостатньо вивченою.*

У цьому міні-огляді ми узагальнюємо сучасні дані щодо біології ІЛ-37, зосереджуючись на його значенні для захворювань нирок. Огляд розглядає його молекулярну структуру, закономірності експресії та імуномодуляторні механізми, які можуть впливати на захворювання нирок, зокрема гостре ураження нирок, діабетичну нефропатію та аутосомно-домінантну полікістозну хворобу нирок. Робота також обговорює виклики впровадження терапії на основі ІЛ-37 у клінічну практику та виділяє ключові напрями для майбутніх досліджень, спрямованих на розкриття потенціалу ІЛ-37 в лікуванні захворювань нирок.

Ключові слова: *інтерлейкін-37, захворювання нирок, гостре ураження нирок, діабетична нефропатія, аутосомно-домінантна полікістозна хвороба нирок, імуномодуляторні механізми, терапія на основі інтерлейкіну.*

Introduction. Kidney diseases, encompassing both acute kidney injury (AKI) and chronic kidney disease (CKD), represent a growing global health burden with significant morbidity, mortality, and socioeconomic impact [1]. Despite advances in understanding the molecular mechanisms underpinning renal pathology, therapeutic options remain largely supportive, and effective biomarkers for early diagnosis and progression monitoring are still limited [2, 3]. In recent years, the role of inflammation in the initiation and progression of kidney diseases has gained increasing attention, opening new avenues for targeted interventions [4, 5]. For example, inflammatory cytokines play a central role in mediating the interplay between metabolic disturbances, such as dyslipidemia, and vascular injury in systemic diseases like diabetes mellitus. A recent study demonstrated that structural changes in the vascular wall and alterations in lipid metabolism are closely linked in diabetic patients, underscoring the importance of inflammatory regulation in cardiorenal pathophysiology [6, 7].

Interleukin-37 (IL-37), a relatively novel member of the IL-1 cytokine family, has emerged as a potent anti-inflammatory and immunomodulatory factor. Unlike classical pro-inflammatory interleukins, IL-37 functions as a natural suppressor of innate and adaptive immune responses [8, 9]. Its expression is elevated in various inflammatory and autoimmune diseases, and preclinical studies suggest a protective role in tissue injury, including renal damage [10, 11]. Moreover, IL-37's dual function, as a secreted cytokine and an intracellular regulator, highlights its unique therapeutic and diagnostic promise [8].

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This review aims to synthesize current knowledge on the biology of IL-37, its regulation, and its mechanistic involvement in kidney disease pathophysiology. We explore the potential of IL-37 as a biomarker for disease activity and progression and evaluate emerging evidence supporting its use as a therapeutic agent.

Interleukin-37: structure and function. IL-37 is a unique cytokine encoded by the IL1F7 gene on chromosome 2. It is produced in five isoforms (a–e) through alternative splicing, with IL-37b being the most active and extensively investigated in both healthy and disease states [8, 10]. IL-37 is expressed in a variety of tissues and cell types, including the lymph nodes, thymus, lung, colon, uterus, and bone marrow, as well as in monocytes, epithelial cells, breast carcinoma cells, and endothelial cells. Expression patterns of IL-37 isoforms are tissue-specific and linked to distinct exon usage and protein lengths are presented in Table 1.

Table 1

Tissue distribution, exon composition, and amino acid lengths of IL-37 isoforms

Isoform	Tissue Distribution	Exons Included	Length (Amino Acids)
IL-37a	Brain	3, 4, 5, 6	192
IL-37b	Kidney, bone marrow, blood, skin, respiratory and urogenital tract	1, 2, 4, 5, 6	218
IL-37c	Heart	1, 2, 5, 6	197
IL-37d	Bone marrow	1, 4, 5, 6	197
IL-37e	Testis	1, 5, 6	157

Abbreviations: IL, interleukin.

Genetic studies indicate that common variants of the IL-37 gene are maintained through balanced selection, though the functional significance of this variability remains to be fully elucidated [**11, 12**]. Notably, some of these variants influence IL-37 protein stability and, consequently, its immune-inhibitory potency [**13, 14**]. These findings suggest a potential link between IL37 genetic variation and susceptibility to various inflammatory or autoimmune conditions.

Structurally, IL-37 adopts the typical β-trefoil fold seen in the IL-1 family but is distinct in its potent anti-inflammatory properties. It functions in both extracellular and intracellular compartments [**10, 12**]. On the cell surface, IL-37 binds to IL-18 receptor α (IL-18Rα) and recruits the co-receptor SIGIRR (also known as IL-1R8), an inhibitor of pro-inflammatory signaling cascades. Intracellularly, IL-37 forms a complex with Smad3, enabling its translocation to the nucleus where it modulates transcriptional programs that attenuate immune responses [**10, 12, 13**].

Functionally, IL-37 downregulates a wide range of pro-inflammatory cytokines, including IL-1β, IL-6, TNF-α, and IFN-γ, while enhancing regulatory T cell activity and promoting immune tolerance [**8, 10, 13**]. These properties have been demonstrated across numerous models of inflammation, including gastrointestinal, cardiovascular, autoimmune, and kidney disease [**8, 13**].

IL-37 in acute kidney injury (AKI). IL-37 has shown significant promise as a protective, anti-inflammatory cytokine in the context of AKI, particularly in models of renal ischemia-reperfusion injury (IRI). It reduces the production of key pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6 in response to renal injury [**13, 14**]. These effects are mediated through IL-37's interaction with the interleukin-18 receptor alpha (IL-18Rα) and the co-receptor single immunoglobulin IL-1 receptor-related molecule (SIGIRR/IL-1R8), which collectively inhibit downstream inflammatory signaling [**13, 14**]. Furthermore, IL-37 exerts intracellular effects by binding to Smad3, facilitating its translocation into the nucleus where it modulates transcriptional responses, leading to the suppression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) pathways [**15**].

In mouse models of renal IRI, overexpression of IL-37 or administration of recombinant IL-37 results in significant reductions in oxidative stress, as evidenced by decreased levels of malondialdehyde (MDA), and in apoptotic signaling, reflected by lower caspase-3 activity [**14**]. These molecular changes correspond with improved histological preservation of renal tissue and enhanced renal function, as indicated by reduced serum creatinine and blood urea nitrogen levels [**14**].

Beyond its role in modulating inflammation and oxidative stress, IL-37 also influences the phenotype of immune cells in the renal microenvironment. It promotes the polarization of macrophages toward the anti-inflammatory M2 phenotype and enhances the activity of tolerogenic dendritic cells, further supporting an immunosuppressive milieu. Additionally, innovative delivery systems such as neutrophil-derived nanovesicles carrying IL-37 have demonstrated the ability to target injured renal endothelial cells [**15**]. This targeted approach reduces leukocyte adhesion and transmigration, promotes angiogenesis, and helps preserve microvascular integrity during IRI.

Transgenic mouse models expressing human IL-37, as well as studies utilizing exogenous IL-37 administration, consistently report improved renal outcomes following AKI. These include diminished inflammatory cytokine levels, reduced cellular injury, and better preservation of renal architecture and function [**14, 16, 17**]. While these findings are currently limited to preclinical studies, they collectively underscore the therapeutic potential of IL-37 in managing AKI, particularly in conditions driven by immune activation and cytokine-mediated damage. A summary of IL-37's effects in AKI models is provided in Table 2.

Table 2

Summary of IL-37's actions in experimental models of AKI [13,15,16]

Mechanism	Effect in AKI models
Suppresses pro-inflammatory cytokines	↓ TNF-α, IL-1β, IL-6, HMGB1
Reduces oxidative stress	↓ Malondialdehyde
Limits apoptosis	↓ Caspase-3 activity
Modulates immune cell phenotypes	↑ M2 macrophages, ↑ tolerogenic dendritic cells
Protects renal endothelium	↓ Leukocyte infiltration, ↑ angiogenesis
Improves renal function	↓ Serum creatinine and BUN; improved tubular structure and histological integrity

Abbreviations: BUN, blood urea nitrogen; IL, interleukin; HMGB1, high mobility group box 1; TNF-α, tumor necrosis factor alpha.

In conclusion, IL-37 serves as a critical modulator of the inflammatory and immune responses implicated in AKI. Its multifaceted mechanisms, including suppression of pro-inflammatory signaling, reduction of oxidative stress, modulation of immune cell behavior, and support of vascular integrity, position it as a promising therapeutic candidate.

IL-37 in diabetic kidney disease (DKD). Recent evidence indicates that IL-37 plays a protective role in DKD through multiple molecular and cellular mechanisms. Its expression is markedly reduced in the serum and kidney tissues of patients with DKD [**16, 18**]. This downregulation correlates strongly with markers of disease severity, including increased proteinuria, elevated serum creatinine, reduced estimated glomerular filtration rate (eGFR), and more extensive interstitial fibrosis [**16, 18**]. One of the key protective mechanisms of IL-37 in DKD involves the inhibition of renal fibrosis. In diabetic mouse models, both transgenic overexpression and treatment with recombinant IL-37 significantly attenuate fibrotic remodeling [**16**]. IL-37 achieves this by restoring fatty acid oxidation (FAO) in tubular epithelial cells, primarily through upregulation of carnitine palmitoyl-transferase 1A (CPT1A), an essential enzyme in FAO. Improved FAO prevents lipid accumulation in renal tissues, thereby reducing the activation of fibrotic pathways [16]. Transgenic DKD mice expressing human IL-37 exhibit lower levels of proteinuria, serum creatinine, and blood urea nitrogen, alongside reduced expression of tubular injury markers such as kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) [**16**].

Furthermore, IL-37 exerts potent anti-inflammatory and anti-oxidative effects in the diabetic kidney. It downregulates the expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, while also suppressing MDA [**18**]. Simultaneously, it enhances the activity of protective molecules including superoxide dismutase (SOD) and Bcl-2, thereby limiting apoptosis in podocytes and tubular epithelial cells [**18**]. In addition to these effects, IL-37 has been shown to inhibit the STAT3–cyclophilin A (CypA) signaling pathway in podocytes, which is activated under hyperglycemic conditions and contributes to inflammatory and oxidative damage [**18**]. These multifaceted actions of IL-37 in DKD are summarized in Table 3.

Table 3

IL-37 mechanisms of action in diabetic kidney disease

Mechanism	Effect in DKD
Reduced expression in DKD	Correlates with ↑ fibrosis, ↑ serum creatinine, ↑ proteinuria, ↓ eGFR
Anti-fibrotic activity	Restores FAO via ↑ CPT1A; ↓ lipid accumulation and fibrotic signaling
Improvement of renal function	↓ Proteinuria, ↓ serum creatinine, ↓ BUN, ↓ KIM-1, ↓ NGAL
Anti-inflammatory and antioxidant roles	↓ TNF-α, IL-1β, IL-6, MDA; ↑ SOD, Bcl-2; ↓ apoptosis in renal cells
Inhibition of STAT3–CypA signaling	Prevents high glucose-induced podocyte injury

Abbreviations: Bcl-2 – B-cell lymphoma; BUN, blood urea nitrogen; CPT1A, carnitine palmitoyltransferase 1A; CypA, cyclophilin A; DKD, diabetic kidney disease; eGFR, estimated glomerular filtration rate; FAO, fatty acid oxidation; IL, interleukin; KIM-1, kidney injury molecule-1; MDA, malondialdehyde; NGAL, neutrophil gelatinase-associated lipocalin; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TNF-α, tumor necrosis factor alpha.

In summary, IL-37 is significantly downregulated in DKD, and its restoration confers protection against inflammation, oxidative stress, fibrosis, and functional deterioration.

IL-37 in autosomal dominant polycystic kidney disease (ADPKD). Experimental models of ADPKD have demonstrated that transgenic expression of human IL-37b significantly suppresses cyst development in the collecting ducts [19]. This protective effect is observed in both early-onset and adult-onset forms of the disease. Furthermore, exogenous administration of recombinant IL-37b also reduces cyst burden in early-stage models, suggesting that IL-37 may be effective both prophylactically and therapeutically. Mechanistically, IL-37b mediates its effects through the modulation of innate immune signaling pathways. Specifically, IL-37b enhances type I interferon responses in kidney-resident macrophages [19]. Activation of this pathway leads to suppression of cyst initiation and limits cyst expansion. Importantly, the therapeutic effect of IL-37b is abrogated when type I interferon signaling is blocked, confirming the central role of this pathway in mediating IL-37's action. Interestingly, the reduction in cyst formation is not associated with a decrease in the total number of macrophages within the kidney. Instead, IL-37b appears to reprogram macrophage function toward a protective, anti-cytogenic phenotype, rather than reducing macrophage infiltration or presence. This functional modulation underscores the precision of IL-37's immunoregulatory effects in the renal microenvironment. These findings suggest that IL-37 represents a promising immunotherapeutic candidate for ADPKD. The main mechanisms by which IL-37 exerts its effects in ADPKD are summarized in Table 4

Table 4

IL-37 mechanisms of action in polycystic kidney disease

Mechanism	Effect in ADPKD models
Suppression of cyst initiation	↓ Cyst burden in collecting ducts (early- and adult-onset disease)
Activation of type I interferon signaling	↑ IFN response in renal macrophages; essential for anti-cyst effect
Functional reprogramming of macrophages	No change in macrophage numbers; shift toward anti-cystogenic phenotype
Therapeutic impact	↓ Cyst growth with transgenic or recombinant IL-37b; potential therapeutic use

Abbreviations: IFN, interferon; IL, interleukin.

Therapeutic applications and delivery strategies. Although IL-37's contribution has been well-documented in preclinical models of kidney disease, the translation of these findings into viable therapeutic interventions requires the development of effective delivery strategies and rigorous validation in clinical settings.

Recombinant IL-37 protein therapy. As discussed above, the administration of recombinant human IL-37 (rhIL-37) has demonstrated therapeutic benefits in experimental nephropathies. For example, in diabetic kidney disease (DKD) models, rhIL-37 reduces inflammatory cytokines (such as TNF-α, IL-6, and IL-1β), mitigates oxidative stress, and improves renal function, including reductions in proteinuria and renal fibrosis [16]. However, protein-based therapies face challenges including short half-life, limited tissue penetration, potential immunogenicity, and the need for repeated administration [20]. Optimization of protein stability and formulation will be key for clinical viability.

Gene therapy approaches. Gene delivery methods, such as plasmid or viral vector-mediated transfection, enable sustained expression of IL-37 in target tissues. Transgenic mouse models expressing human IL-37 have been instrumental in demonstrating long-term protective effects, particularly in models of AKI, CKD, and DKD [16, 19]. AAV (adeno-associated virus)-based vectors targeting renal parenchyma represent a promising avenue for organ-specific gene therapy, although safety, dose control, and regulatory barriers remain significant hurdles [8].

Cell-based and nanocarrier delivery systems. Emerging strategies leverage cell-derived vesicles and nanoparticles for targeted IL-37 delivery. Neutrophil-derived nanovesicles loaded with IL-37 have shown efficacy in localizing treatment to injured renal endothelial cells, reducing inflammation and preserving microvascular integrity during ischemia-reperfusion injury [17]. Similarly, liposome or polymer-based nanoparticles may enhance delivery specificity and protect IL-37 from degradation in circulation [21].

Combination therapies. IL-37's broad anti-inflammatory effects suggest potential synergy with existing therapies. Co-administration with immunosuppressants (corticosteroids, calcineurin inhibitors), antifibrotic agents (pirfenidone, ACE inhibitors), or metabolic modulators (SGLT2 inhibitors) may enhance therapeutic outcomes in multifactorial kidney diseases such as DKD or lupus nephritis [10, 15, 22]. However, such approaches warrant systematic evaluation in controlled studies.

Challenges and clinical translation. Key translational obstacles include defining optimal therapeutic windows, establishing dosing regimens, and monitoring potential off-target effects, particularly given IL-37's broad immunosuppressive capabilities [8, 10]. Importantly, isoform-specific functions and tissue distribu-

tion may influence treatment outcomes and need to be considered in therapy design.

Diagnostic and biomarker potential of IL-37 in kidney disease. IL-37 has emerged not only as a cytokine with therapeutic relevance but also as a potential biomarker reflecting disease severity and inflammatory activity in various kidney disorders. Its expression patterns in both circulating blood and renal tissue correlate with pathological features of kidney injury, offering potential diagnostic and prognostic utility. In AKI, elevated IL-37 levels correspond with reduced inflammation and oxidative stress [14, 17]. Similarly, in DKD, serum and renal IL-37 levels are significantly reduced, with lower expression strongly associated with increased proteinuria, elevated serum creatinine, reduced eGFR, and greater interstitial fibrosis [16]. In contrast, clinical studies focusing on broader CKD populations have reported a different pattern. Li et al investigated plasma IL-37 levels in patients with CKD and nephrotic syndrome (NS) compared to healthy controls [23]. The researchers enrolled 57 CKD patients, 13 NS patients, and 22 healthy individuals, further stratifying the CKD group by disease stage (stages 1, 3, and 5). Using ELISA, they found that plasma IL-37 levels were significantly higher in both CKD and NS patients than in healthy controls. Interestingly, IL-37 levels did not differ significantly among the different CKD stages, suggesting that its elevation is a general feature of CKD rather than stage-specific. After treatment, IL-37 levels decreased in both CKD and NS groups, and in CKD patients, IL-37 levels positively correlated with white blood cell and lymphocyte counts [23]. In line with these findings, another observational study found that serum IL-37 levels were significantly higher in patients undergoing hemodialysis (HD), especially in those with subclinical or overt hypothyroidism, compared to healthy controls [24]. In addition, IL-37 levels have been shown to be significantly elevated in patients with systemic lupus erythematosus compared to healthy controls and were correlated with high disease activity, mucocutaneous involvement, and renal involvement [22].

These findings suggest that elevated plasma IL-37 may serve as an auxiliary diagnostic marker for CKD, potentially reflecting an ongoing inflammatory or immune response. Because IL-37 levels inversely correlate with pro-inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β), particularly in cardiovascular diseases [25], measuring IL-37 alongside traditional markers may improve diagnostic accuracy. Specifically, a combined cytokine profile (high IL-6/IL-1β and low IL-37) may better distinguish inflammatory from non-inflammatory conditions, addressing the limitations of current markers in clinical practice [26, 27].

Limitations and future directions. Despite compelling preclinical evidence supporting the anti-inflammatory and tissue-protective functions of IL-37 in kidney diseases, several important limitations must be addressed before translating these findings into clinical applications. One of the fundamental biological challenges lies in the species-specific nature of IL-37. Mice, commonly used in preclinical research, lack an endogenous IL-37 gene, necessitating the use of human IL-37 transgenic models or recombinant protein administration [16, 19]. While informative, these approaches do not fully recapitulate the endogenous regulation, isoform dynamics, or receptor interactions that might occur in humans. Furthermore, the distinct roles of IL-37 isoforms (a–e), including their tissue-specific expression and functional relevance in renal physiology and pathology, remain poorly understood [12, 13].

From a therapeutic development perspective, optimal dosing strategies, routes of administration, and pharmacokinetic profiles for IL-37-based therapies are not yet established. Most available data stem from short-term animal studies, and there is limited insight into the long-term safety of exogenous IL-37, particularly regarding its immunosuppressive capacity and potential to interfere with host defense mechanisms [10, 12]. The development of targeted delivery platforms, such as nanoparticle systems or gene therapy vectors, offers promise but also introduces additional regulatory and safety considerations.

Emerging applications of IL-37 in clinical nephrology offer exciting but as yet untested possibilities. For instance, chronic inflammation is a key contributor to adverse outcomes in patients undergoing peritoneal dialysis (PD) or HD. Peritoneal inflammation and fibrosis are major complications in PD, often driven by recurrent peritonitis and exposure to bioincompatible dialysis fluids, leading to increased levels of inflammatory cytokines such as IL-1β and IL-6, and activation of profibrotic pathways such as TGF-β1 [28, 29]. Given IL-37's broad anti-inflammatory and anti-fibrotic properties, suppression of pro-inflammatory cytokines, inhibition of immune cell activation, and attenuation of fibrosis in various tissues-it is plausible that IL-37 could counteract the inflammatory and fibrotic processes in the peritoneum. Although direct studies in PD are lacking, the mechanistic rationale is strong, and IL-37's efficacy in other models of tissue fibrosis and inflammation supports this hypothesis. However, the findings that IL-37 is already elevated in the HD population complicate the rationale for further enhancing its activity [24]. The increased IL-37 may represent the body's attempt to counteract ongoing inflammation, but it may be insufficient to fully control the inflammatory burden, or it may indicate a state of cytokine resistance or immune dysregulation. Rather than simply increasing IL-37 levels, strategies may need to focus on enhancing its functional activity or overcoming possible resistance mechanisms. Additionally, the immunosuppressive effects of IL-37 raise concerns about infection risk in an already immunocompromised population.

To advance IL-37 toward clinical utility, several key research priorities should be addressed. First, early-phase clinical trials are needed to evaluate safety, tolerability, and pharmacodynamics in both acute and chronic kidney disease populations. Second, the devel-

opment of isoform-specific recombinant proteins or gene constructs will facilitate more precise therapeutic strategies. Third, IL-37 should be explored not only as a therapeutic agent but also as a biomarker for inflammation, fibrosis, and disease progression. Finally, combination approaches integrating IL-37 with existing immunosuppressants, antifibrotics, or metabolic agents may offer synergistic benefits and should be explored in preclinical models and trial settings.

Conclusions. The review provides an updated perspective on the immunobiological functions and potential clinical applications of IL-37, with a particular focus on its relevance to kidney disease. IL-37 is recognized for its broad immunomodulatory roles, including suppression of inflammatory responses, anti-tumor activity, and enhancement of antimicrobial defenses. These properties have positioned IL-37 as a promising candidate in emerging cytokine-based immunotherapeutic strategies. Although its functions have been explored across a wide spectrum of pathological conditions, growing evidence highlights its relevance in renal pathophysiology, including AKI, CKD, DKD, and ADPKD. The pleiotropic actions of IL-37, mediated through its interaction with IL-18 receptor α and SIGIRR/IL-1R8, suggest its potential utility not only in modulating kidney inflammation and fibrosis but also as a target for immune gene therapy. Thus, IL-37 represents a compelling immunotherapeutic molecule with promising diagnostic and therapeutic applications in a range of kidney diseases.

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Immunological determinants of long-term kidney graft survival as therapeutic targets

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Abstract. *This article analyzes current research on the mechanisms underlying acute and chronic rejection of kidney transplants (KT) to identify key immunological determinants of long-term graft survival.*

According to contemporary understanding, both forms of allograft rejection are mediated by effector responses of the innate and adaptive immune systems. Immune-mediated damage to the graft remains the leading cause of transplant loss, regardless of the post-transplantation period.

Advancements in methodology, including the use of novel biomarkers, allow for earlier diagnosis of rejection mechanisms, while artificial intelligence and genome/proteome-based monitoring provide tools for predicting the progression of alloimmune responses.

Several immunological determinants influencing kidney graft longevity have been identified as potential therapeutic targets to enhance transplant survival.

Key words: *kidney transplantation, graft survival, immune response, donor-specific antibodies, graft rejection, acute rejection, chronic rejection, complement system proteins, biomarkers, artificial intelligence.*

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Імунологічні детермінанти тривалого функціонування трансплантованої нирки як терапевтичні мішені

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Резюме. *У статті проаналізовані дослідження, присвячені механізмам гострого або хронічного відторгнення трансплантованої нирки (ТН), для встановлення імунологічних детермінант тривалості її виживання.*

Відповідно до теперішнього розуміння цієї проблеми, обидва варіанти аллогенного відторгнення реалізуються через ефекторні реакції вродженого та адаптивного імунітету.

Саме імуноопосередковані пошкодження ТН є провідними в структурі причин її відторгнення незалежно від тривалості післяопераційного періоду.

Діагностувати причини відторгнення на новому методологічному рівні дозволяє використання відповідних біомаркерів, і прогнозувати подальший перебіг алоімунного конфлікту, - застосування штучного інтелекту та ганомно-протеомного моніторингу.

Визначені імунологічні детермінанти тривалості виживання ТН, які можуть бути терапевтичними мішенями для його подовження.

Ключові слова: *трансплантація нирки, виживання трансплантата, імунна відповідь, антитіла донор-специфічні, відторгнення трансплантата, гостре відторгнення, хронічне відторгнення, система комплемента, біомаркери, штучний інтелект.*

Вступ. Хронічна хвороба нирок (ХХН) є значною медико-соціальною проблемою сьогодення [1]. Особливої актуальності ця проблема набуває з огляду на стабільне щорічне 2,3% збільшення кількості хворих на ХХН 5 ст., які потребують лікування методами ниркової замісної терапії (НЗТ) [**1, 2**].

На сьогоднішній день кращим методом лікування цієї категорії пацієнтів є трансплантація нирки [**3**]. За даними European Renal Association (ERA) Registry 2022 року питама вага трансплантації нирки (ТН) в структурі НЗТ складала 39%. Решта 56% пацієнтів лікувались методом гемодіалізу (ГД), а ще 5% пацієнтів – методом перитонеального діалізу. Частота ТН у 2022 році в країнах ЄС складала 40,0 на 1 мільйон населення, переважна більшість яких (66%) – трансплантації від посмертного донора [**4**].

Частка ТН у структурі НЗТ значно коливається залежно від економічного розвитку конкретної країни (за високого рівня ВВП - вона відповідно вища).У країнах з низьким та середнім рівнем ВВП методи діалізної ниркової замісної терапії (ДНЗТ) залишаються превалюючими модальностями лікування хворих на ХХН 5 ст. [**5**].

Протягом останніх десятиліть спостерігається подовження виживання алотрансплантованих нирок (АТН) завдяки вдосконаленню усіх складових ТН та особливо завдяки досягненням у вивченні трансплантаційного імунітету, створенню нових лікарських засобів для імуносупресивної терапії (ІСТ) [**6, 7**]. 5-річне виживанння реципієнтів АТН складає 95% та 92%, а трансплантатів – 87% та 81% від живих та трупних донорів відповідно [**8, 9**].

Досягнення ІСТ дозволили зменшити частоту та досягати зворотності гострого відторгнення трансплантата (ГВТ) протягом першого року після ТН до 8,4% у разі застосування антитіл до IL-2 або 6,6% – коли застосування ІСТ спрямоване на супресію функціонування Т-лімфоцитів [**10, 11**]. Згідно з даними Organ Procurement and Transplantation Network (OPTN), 10-річна виживаність АТН складає 55%-40% від живих або посмертних донорів відповідно [**12**]. Дослідження структури причин відторгнення продемонстрували, що саме аллоімунні фактори є визначальними для тривалості виживаності АТН (табл. 1) [**13**].

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Таблиця 1

Причини смерті - відторгнення трансплантата у пацієнтів, яким трансплантовано нирки з 2006 по 2018 рік

	Час після ТН			
Причини	**Загальні**	**Менше 1 р**	**1-5 р**	**Більше 5 р**
Загальні	553	131	235	188
Аллоімунні	38,7%	12,2%	49,8%	43,3%
Гломерулярні хвороби	18,6%	13,7%	17,4%	23,5%
Ураження ниркових канальців	13,9%	9,2%	17,4%	12,8%
Первинна дисфункція	14,3%	60,3%	0%	0%
ВК нефропатія	4,3%	3,1%	4,3%	5,3%
Невідомо/інше	10,1%	1,5%	11,1%	15,0%

Як гостре, так і хронічне відторгнення АТН реалізуються через ефекторні механізми активації клітинно- та антитілоопосередкованих реакцій імунної системи [**14, 15**].

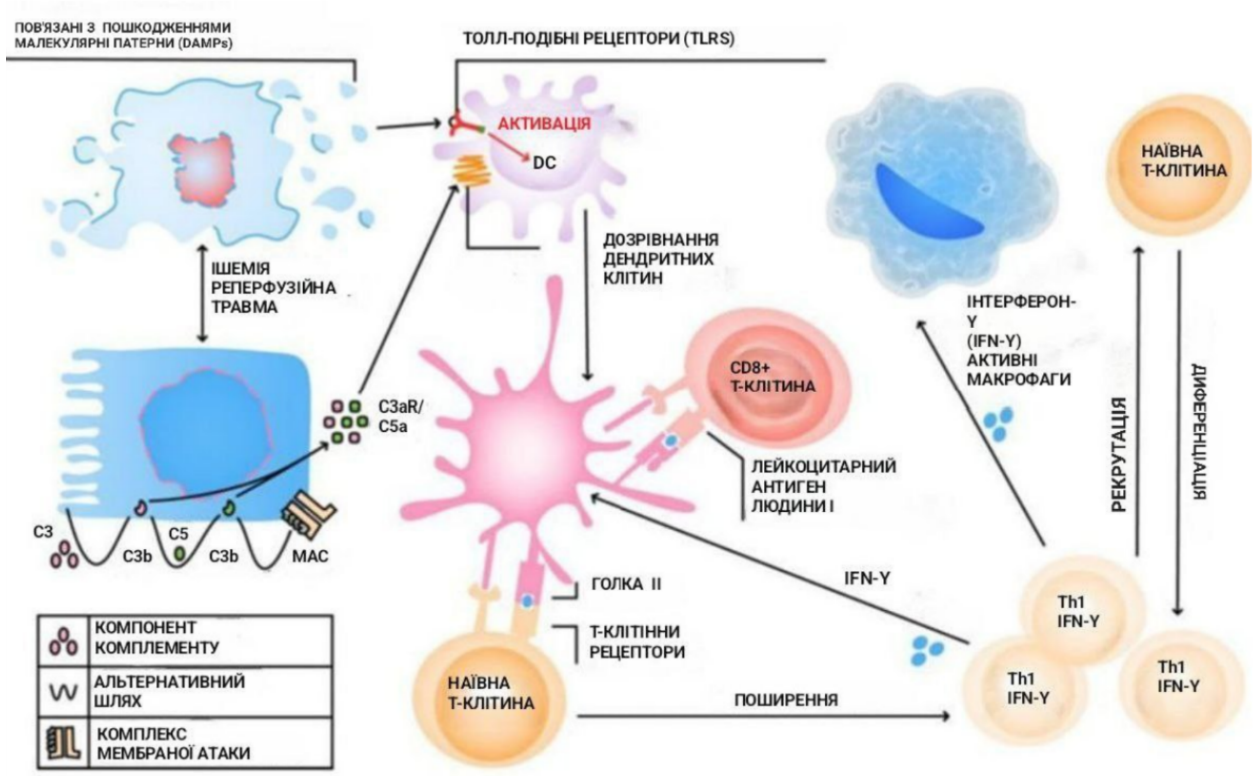

Рис. 1. Ефекторні механізми вродженого та адаптивного імунітету після ТН [16].

Ішемічно-реперфузійне ушкодження тканин донора запускає активацію клітин вродженого імунітету через DAMPs/PRR-сигналінг. Дендритні клітини дозрівають і мігрують до лімфатичних вузлів, де презентують антигени (HLA I/II) наївним Т-лімфоцитам. $CD8^+$ Т-клітини продукують IFN-γ, $CD4^+$ Th1-клітини активують макрофаги та сприяють синтезу цитокінів. У подальшому В-клітини формують ДСА, які взаємодіють з NK-клітинами та запускають ADCC. Активація комплементу (C3a, C5a) підсилює запалення.

Скорочення: DAMPs – молекулярні патерни, пов'язані з ушкодженням; DC – дендритні клітини; A – антигени головного комплексу гістосумісності; IFN-γ – інтерферон-гамма; ADCC – антитілозалежна клітинна цитотоксичність; NK – природні кілерні клітини; MAC – комплекс мембранної атаки.

Незалежними предикторами тривалого виживання АТН є його базова швидкість клубочкової фільтрації (eGFR), величина протеїнурії, наявність фіброзу інтерстицію, тубулярної атрофії, гломеруліту, перитубулярного капіляриту, інтерстиціального запалення, тубу літу та глибокого пошкодження клубочкових структур [17, 18].

Метою цієї роботи є встановлення імунологічних детермінант як терапевтичних мішеней тривалого виживання ниркового трансплантата.

Гостре відторгнення трансплантованої нирки. На сьогодні ГВТ є основною причиною ранньої втрати АТН. Цей тип відторгнення розвивається впродовж перших декількох тижнів та реалізується через Т-клітинні механізми. Пошкодженні АТН викликають реакції як залежні від Т-хелперних клітин (за участю Т-кілерів, макрофагів, еозинофілів), так і антитілозалежні (активація комплемента) [19-22]. Імунні механізми, що лежать в основі ГВТ, представлені на рис. 2.

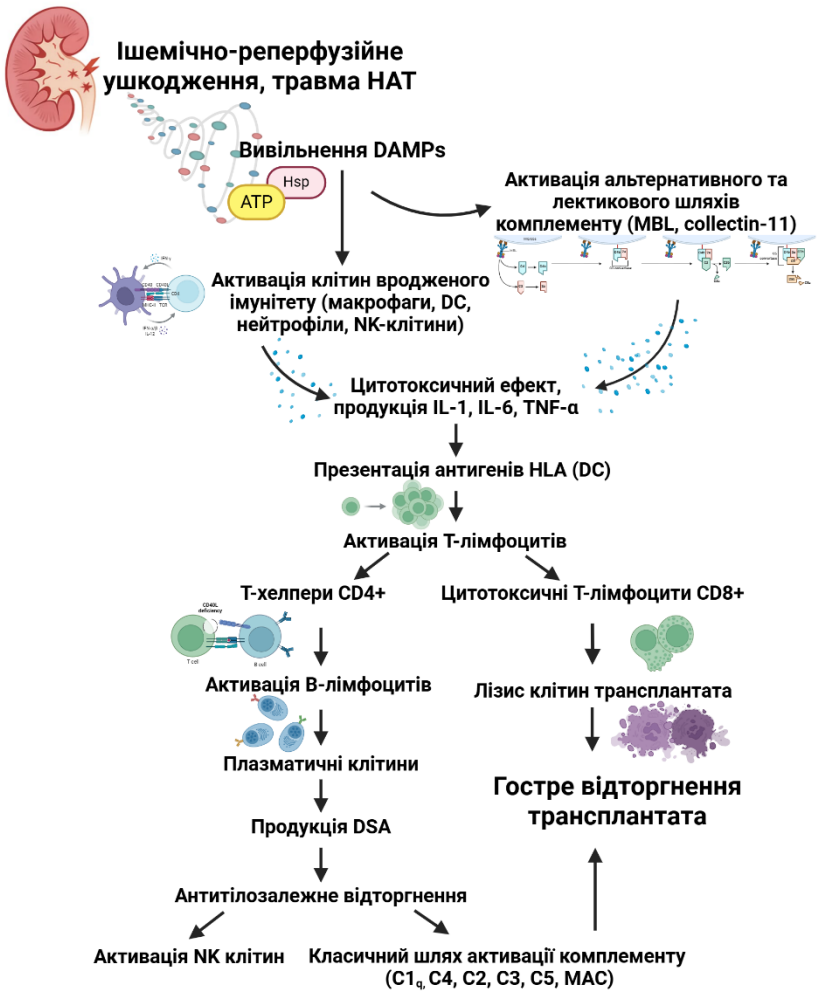

Рис. 2. Імунопатогенез гострого відторгнення трансплантованої нирки (створено за допомогою BioRender.com).

Схематичне зображення механізмів, що запускаються у відповідь на ішемічно-реперфузійне ушкодження або травму трансплантата. Вивільнення DAMPs активує клітини вродженого імунітету та систему комплементу, спричиняючи продукцію прозапальних цитокінів і презентацію HLA-антигенів. Це призводить до активації $CD4^+$ та $CD8^+$ Т-лімфоцитів, а також В-клітин із подальшим утворенням донорспецифічних антитіл (DSA). В результаті формується Т-клітинно- та антитілозалежне гостре відторгнення із залученням цитотоксичних клітин, системи комплементу та ефекторних механізмів гуморального імунітету.

Скорочення: НАТ – нирковий алотрансплантат, DAMPs – молекули, асоційовані з ушкодженням клітин, DC – дендритні клітини, IL – інтерлейкін, HLA – антиген лейкоцитів людини, MBL – манозозв'язувальний лектин, NK-клітини – природні клітини кілери, TNF-α – фактор некрозу пухлини альфа.

Головним індуктором ГВТ та його інтенсивності є ступінь відмінностей донора і реципієнта за антигенами HLA. Донорспецифічні антитіла (ДСА) є біомаркером прогнозу антитілоопосередкованого відторгнення (АОВ) [23, 24]. Існує кілька фенотипів АОВ, які визначаються часом виникнення гуморальної відповіді та характеристиками ДСА (специфічність, сила антитіл, підкласи IgG та здатність до зв'язування комплементу). ДСА у сенсибілізованих реципієнтів викликають надгостре

відторгнення, прискорене гостре відторгнення та гостре відторгнення. De novo ДСА пов'язані з пізнім гострим АОВ, хронічним АОВ, гломерулопатією трансплантата та зниженням його виживаності [25, 26]. ДСА з C1q, пов'язані з формуванням гострого АОВ, тоді як C1q-незв'язуючі ДСА спричинюють хронічне АОВ та пізню втрату трансплантата. Підкласи IgG мають різну здатність активувати комплемент та залучати ефекторні клітини через Fc-рецептор, ДСА IgG3, що зв'язують комплемент, пов'язані з гострим АОВ, тоді як незв'язуючі ДСА IgG4 викликають хронічне АОВ [27, 28].

Визначення складних характеристик ДСА допоможуть стратифікувати імунологічний ризик пацієнта, передбачити виникнення фенотипів АОВ і, таким чином, сприятимуть покращенню виживаності трансплантата Однак, ДСА до HLA виявляються лише у половині випадків АОВ [29].

Безклітинна ДНК донора у периферичної крові реципієнта, є неінвазивним маркером діагностики відторгнення АТН [30]. Оцінюючи діагностичну ефективність безклітинної ДНК плазми донора (cfDNA) у розрізненні АОВ, або de novo ДСА без гістологічних уражень у реципієнтів АТН було встановлено, що фракція безклітинної ДНК плазми донора сприяє визначенню АОВ або стабільної функції АТН [31], для моніторингу ефективності після лікування АОВ [32]. Таким чином, моніторинг de novo ДСА та безклітинної ДНК плазми донора є перспективним неінвазивним способом оцінки пошкодження трансплантата [33].

Отже, ГВТ є результатом складної взаємодії між імунними клітинами, розчинними молекулами та клітинами нирок [34] та класифікується як Т-клітинно-опосередковане відторгнення (ТКВО), яке характеризується тубулоінтерстиціальним запаленням або артеріїтом внаслідок активації Т-лімфоцитів, і АОВ, що проявляється мікросудинним запаленням викликанним активацією комплементу, індукованого зв'язуванням ДСА з донорським ендотелієм [15, 16, 35].

У дослідженні O'Leary et al. було показано, що АОВ частково опосередковується активацією комплементу та антитілозалежною клітинною цитотоксичністю, здебільшого індукованою ДСА підкласів IgG1 та 1Gg3 [36]. В іншому дослідженні Lefaucheur et al. встановили, що високі рівні IgG3 ДСАs були виявлені у пацієнтів з АТН і ГВТ порівняно з пацієнтами без ГВТ, і вважають, що IgG3 та C1q+ іДСАs є ефекторами втрати АТН [37].

Хронічне відторгнення трансплантованої нирки. В умовах ІСТ та за наявності відмінностей між донором і реципієнтом за слабкими (мінорними) антигенами HLA процес відторгнення АТН може затягуватися на багато місяців і навіть років. Воно характеризується поступовою втратою функцій АТН через хронічне запалення, фіброз і судинні зміни. Таке хронічне відторгнення (ХВТ) викликається як гуморальними, так і клітиноопосередкованими реакціями низької інтенсивності [25, 26, 38] (рис. 3).

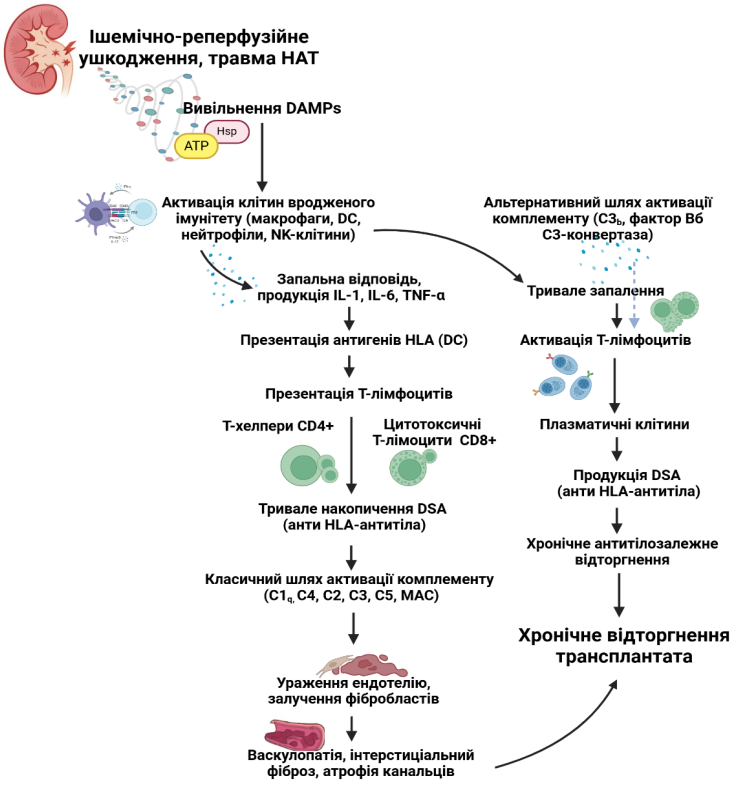

Рис. 3. Імунопатогенез хронічного відторгнення трансплантованої нирки (створено за допомогою BioRender.com).

Ішемічно-реперфузійне ушкодження або травма алотрансплантата активують вроджений імунітет та систему комплементу, ініціюючи хронічну запальну відповідь. Презентація HLA-антигенів антигенпрезентувальними клітинами призводить до активації $CD4^+$ і $CD8^+$ Т-лімфоцитів, а також В-клітин, що синтезують донорспецифічні антитіла (DSA). Тривала дія DSA спричиняє активацію класичного шляху комплементу, ендотеліальне ушкодження, активацію фібробластів і формування фіброзу. У результаті розвиваються васкулопатія, інтерстиціальний фіброз та атрофія канальців – морфологічні ознаки хронічного відторгнення трансплантата.

Скорочення: НАТ – нирковий алотрансплантат, DAMPs – молекули, асоційовані з ушкодженням клітин, DC – дендритні клітини, DSA – донорспецифічні антитіла, IL – інтерлейкін, HLA – антиген лейкоцитів людини, MAC – мембраноатакувальний комплекс, TNF-α – фактор некрозу пухлини альфа.

Пошкодження АТН пов'язане з інфільтрацією судин та тканин макрофагами, що на ранній стадії залучаються (у формі моноцитів) за допомогою хемокінів (CCL5-RANTES та ін.) алореактивними CD4T-лімфоцитами (Tx1) та активуються секретованими ними цитокінами (ІФН-γ), а пізніше їх рекрутування посилюється під впливом продуктів макрофагальних клітин (ІЛ-1, ІФН-α, хемокіну CCL2-MCP-1) [39]. Хронічне запалення надалі призводить до розвитку атеросклерозу кровоносних судин АТН - характерної ознаки ХВТ. Показано, що в ІФН-γ-дефіцитних реципієнтів атеросклероз судин не розвивається. Клітинна інфільтрація за умов ХВТ, на відміну від гострого, виражена слабко. Імунні реакції супроводжуються активацією і пошкодженням ендотелію та звільненням різних факторів росту (ТФР-β), з якими пов'язані розвиток і регуляція двох основних ознак ХВТ: рубцювання тканини (фіброз) та закриття просвіту судин (облітерація) трансплантата. Облітерація кровоносних судин відбувається внаслідок розмноження гладком'язових клітин інтими, що міґрували в судинну стінку, та відкладання компонентів матриксу. Поступове зменшення кровопостачання і заміщення паренхіми фіброзною тканиною призводить до повної втрати функції АТН [40-42].

На думку Berger M. et al. хронічне АОВ є найважливішою причиною пізньої втрати АТН та включає Т-клітини, комплемент, молекули ендотеліальної адгезії, нейтрофіли, моноцити/макрофаги та стимульовану IgG антитілозалежну клітинну цитотоксичність природними клітинами-кілерами [43]. До того ж, необхідно враховувати, що IL-6 сприяє активації Т-клітин, зниженню Treg, стимуляції Т-фолікулярних клітин-хеперів і зародкових центрів [44], а також відіграє роль у керуванні проліферацією, дозріванням і перемиканням класів В-клітин [45]. Крім того, IL-6 здатний індукувати реагенти гострої фази, ендотеліальні клітини та сприяє пошкодженню судин, а поліморфізм гена IL- 6 корелює з ГВТ та з виживаністю НАТ [46].

Загалом, імунологічні механізми є ключовим фактором втрати АТН. Ці механізми у реципієнтів реалізуються шляхом активації вродженого імунітету через Toll-like рецептори, NK клітини, систему комплемента; активації Т-клітинно опосередкованих реакцій за участі CD4+ і CD8+ Т-клітин; ініціації імунної відповіді і утворенням ДСА- предиктора АВО; ДСА зв'язуються з ендотелієм судин трансплантата, активують систему комплемента і викликають ушкодження ендотеліальних клітин [34]. Крім того, це супроводжується розвитком запалення і продукцією прозапальних цитокінів (IFN-γ, TNF-α) та запальних хемокінів CXCL9, CXCL10, що сприяє фіброзу та тубулоінтерстиціальному пошкодженню трансплантата, а також формуванню імунної пам'яті з утворенням перехрестно реактивних клітин [47]. Постійна активація імунної системи призводить до проліферації фібробластів і надмірного відкладення позаклітинного матриксу, що, в свою чергу, викликає рубцювання тканини, звуження судин і порушення кровопостачання [14].

Таким чином, ТН може бути успішною і запезпечувати довгострокове функціонування АНТ за умови адекватного імунологічного моніторингу з метою збереження функціонування НАТ в довгостроковій перспективі.

Біомаркери ушкодження трансплантованої нирки. На сьогодні в практиці трансплантаційних центрів широко застосовується низка лабораторних маркерів для імунологічного моніторингу функції АТН, що використовуються для прогнозування затримки функції трансплантата (ЗФТ); для оцінки, ідентифікації та характеристики ГВТ; для диференціальної діагностики між хронічним відторгненням та дисфункцією АТН; для довгострокового моніторингу та прогнозування виникнення пошкодження АТН. Найбільш досліджувані сучасні імунологічні детермінанти [16, 22, 48-55], як маркери відторгнення подані у таблиці 1.

Таблиця 1

Імунологічні детермінанти як маркери відторгнення АТН

Детермінанта	Біологічна роль	Клінічне значення
CXCL9	Хемокін, індукований IFN-γ	Ранній маркер клітинного відторгнення. У сечі може передбачати гостре клітинне відторгнення за 3–30 днів до клінічних проявів. Рівні CXCL9/CXCL10 у сечі мають високу діагностичну точність, AUC до 0.84.
CXCL10	Хемокін, індукований IFN-γ	Маркер антитілозалежного відторгнення. Підвищені рівні в сечі та крові пов'язані з ризиком АОВ.
DSA (IgG3)	Антитіла проти донорських антигенів	Маркер хронічного відторгнення. Наявність IgG3-DSA асоціюється з високим ризиком.

Продовження таблиці 1

Детермінанта	Біологічна роль	Клінічне значення
dd-cfDNA	Фрагменти ДНК донора в крові	Маркер пошкодження трансплантата.
TruGraf / AlloMap	Генетичні профілі	Маркер субклінічного відторгнення.
sCD30	Розчинний рецептор CD30	Маркер ризику відторгнення.
TLR4, ICAM-1	Молекули активації вродженого імунітету	Маркер ендотеліальної активації.
CIRBP	Холод-індукований РНК-зв'язуючий білок	Прогноз ЗФТ.
Інтегрини (LFA-1, α4β1)	Трансмембранні глікопротеїни	Маркери ризику відторгнення.
Екзосоми та РНК	Екзосомні біомолекули	Визначення типу відторгнення.
міРНК (miR-182-5p, miR-21-3p, miR-25, miR-181a, miR-204, miR-192, miR-10b, miR-142-3p, miR-215, miR-342-3p, miR-615-3p, miR-210, miR-99)	Некодувальні РНК	Прогнозування ГВТ, ТКОВ, ЗФТ.
Генні сигнатури (CXCL9, CD3ε, LCK, Foxp3, ... інші списки генів)	Генна експресія	Рання діагностика, прогноз ТКОВ, АОВ, ГВТ.
SLPI	Інгібітор лейкоцитарної пептидази	Маркер гострого ушкодження.
GZMB, PRF1	Цитотоксичні білки	Маркери гострого ТКОВ.
FASLG	Fas-ліганд	Маркер ГВТ.
TNFR2	Рецептор до TNF	Предиктор ЗФТ.
CCR2, MCP-1	Рецептори хемокінів	Маркери прогнозу ЗФТ.
BCL-2, BCL-xL	Інгібітори апоптозу	Маркери виживаності.
TNF-α, IL-6, TGF-β	Гени стресу/запалення	Прогноз гострого/хронічного відторгнення.
IL-6, IL-18, sIL-6R, gp130	Цитокіни	Маркери ЗФТ.

Рівні мРНК CXCL9 сечі після ТН виявилися предикторами пошкодження НАТ. Кілька біомаркерів сечі корелювали з пошкодженням АТН, включаючи CXCL9, CXCL10, ліганд хемокіну 2 мотиву CC (CCL2), NGAL, IL-18, CYC, KIM-1 та білок 3, що містить імуноглобулін/муцинові домени Т-клітин. Рецептор хемокіну CXCR3 сечі є перспективним кандидатом для виявлення субклінічного запалення [48, 49]. Визначення dd-cfDNA крові дозволяє раніше ідентифікувати розвиток ГВТ та контролювати відповідь на лікування [31, 32].

Профілювання транскриптома генома виявило унікальні та спільні ознаки генів гострого ТКОВ та АОВ. Профілі мРНК клітин сечі є діагностичними та прогностичними показниками ГВТ та можуть служити критеріями імунного статусу in vivo [52]. Секвенування РНК дає змогу зрозуміти механізми відторгнення та допомагає визначити пріоритети терапевтичних цілей. Секвенування РНК, революційний інструмент молекулярного профілювання, допоможе визначити локалізацію мРНК ГВТ в АТН з безпрецедентним рівнем точності. Гостре ТКОВ в АНТ передбачають рівні мРНК перфорину та мРНК гранзиму В у клітинах сечі, інгібітора серинової протеїнази-9 (PI-9), природний антагоніст гранзиму В; рівень мРНК CD103, рівні мРНК IP-10, мРНК CXCR3 мРНК CD3e в клітинах сечі, рівень FOXP3 в клітинах сечі однозначно прогнозує реверсію гострого ТКОВ [56, 57].

У багатоцентровому дослідженні розглянуто когорту реципієнтів НАТ з метою визначення незалежної від імуносупресії генної сигнатури для прогнозування толерантності. Вони ідентифікували дев'ять генів, включаючи атаксин 3 (ATXN3), білок A1, пов'язаний з BCL2 (BCLA1), фактор елонгації еукаріотичної трансляції 1 альфа 1 (EEF1A1), білок 9, пов'язаний з Gem (GEMIN7), константу лямбда 1 імуноглобуліну (IGLC1), мембранний 4-домен A4A (MS4A4A), ген-енхансер поліпептиду ядерного фактора каппа-легкого в В-клітинах, інгібітор альфа (NFκBIA), RAB40C, член родини онкогенів RAS, та білок 3, індукований TNF, α (TNFAIP3). Крім того, програма тесту спонтанної операційної толерантності нирок (kSPOT) ідентифікувала 21 ген, задіяний в ОТ. Серед них, для розробки три-

генного аналізу з високою точністю для виявлення ОТ були використані Kruppel-Like Factor 6 (KLF6), Basonuclin 2 (BNC2) та Cytochrome P450 Family 1 Subrodina B Member 1 (CYP1B1) [48].

Міжнародне дослідження «Геноміка хронічного відторгнення алотрансплантата» (GoCAR), проспективний мікрочиповий аналіз профілів експресії генів у зразках тканини алотрансплантата від 159 реципієнтів променевої терапії зі стабільною функцією трансплантата через 3 місяці після ТН, виявило набір з 13 генів, які незалежно передбачали фіброз алотрансплантата через 12 місяців після ТН. Ця генна сигнатура, тобто анкіриновий повтор та SOCS-бокс, що містить 15 (ASB15), домен спіральна спіраль-спіральна спіраль, що містить 10 (CHCHD10), чотириз'єднаний бокс 1 (FJX1), член родини Kelch-подібних 13 (KLHL13), антиген, асоційований з нирками 1 (KAAG1), протоонкоген Met (MET), рецептор ретиноїду X альфа (RXRA), білок безкінечного пальця 149 (RNF149), інкорпоратор серину 5 (SERINC5), гомолог Sprouty 4 (SPRY4), супресор пухлиногенності 5 (ST5), гомеобокс 1 фактора, індукованого TGF-β (TGIF1), та член родини сайтів інтеграції MMTV типу 9A (WNT9A), мала вищу прогностичну цінність для розвитку фіброзу АНТ, перевершуючи клінічні та патологічні змінні [58].

Дослідження Leng Q, та співаторів показало, що підвищений рівень CIRBP у плазмі донора перед трансплантацією є незалежним предиктором розвитку ЗФТ у плазмі донора перед трансплантацією є незалежним предиктором розвитку ЗФТ. Це відкриває можливість використання CIRBP як нового біомаркера для оцінки функції трансплантата [59]. В іншому дослідженні було продемонстровано, що вміст Проенкефаліну А в плазмі реципієнта в день ТН може передбачати розвиток ЗФТ [60].

Завдяки імунному моніторингу з вимірюванням експресованих генів (геноміка) або білків (протеоміка), менеджмент реципієнтів ниркових трансплантатів може повністю змінитися. Хоча в даний час деякі неінвазивні імунологічні макери обмежено використовуються в клінічній практиці і потребують валідації, в майбутньому вони можуть стати корисними для діагностики, для моніторингу відповіді на ІСТ або стати потенційною терапевтичною мішенею.

Ці аналізи також можуть бути використані для розробки та оцінки нових імуносупресивних препаратів.

Управління з контролю за продуктами і ліками США схвалило аналіз ImmuKnow для оцінки загального стану імунної системи у пацієнтів з ослабленим імунітетом. Він базується на здатності клітин CD4 відповідати на мітогенну стимуляцію фітогемаглютиніном-L *in vitro* шляхом кількісного визначення кількості аденозинтрифосфату, що виробляється і вивільняється з цих клітин після стимуляції. Однак, досі не має даних про прогностичну та проспективну цінність цього механізму у реципієнтів трансплантатів та про вплив вижначення даних імунних біомаркерів на прогнозування довгострокового виживання НАТ [61]. Виявлення та валідація біомаркерів, які корелюють з раннім відторгненням трансплантата, можуть передбачити його виникнення, або спрогнозувати виживаність трансплантата. Інформація про біомаркери також може допомогти диференціювати пацієнтів з високим та низьким імунологічним ризиком, сприяючи індивідуалізації ІСТ.

Прогнозування виживаності трансплантованої нирки. Koukoulaki M. et al. досліджуючи детермінанти виживаності НАТ показали, що виживаність НАТ на перший, п'ятий і десятий рік після ТН становила 89%, 76% і 67%, а виживаність пацієнтів становила відповідно 95%, 89% і 83%. Автори відзначили, що лише вік донора впливає на виживання НАТ ($P < 0,05$). Невідповідності HLA не корелювали з виживанням трансплантата (log rank $P = 0,495$), але ідентифікація панелі реактивних антитіл (PRA) I та II класу після ТН мала статистично значущий вплив на тривале виживання НАТ (log rank $P < 0,001$ і $P = 0,021$, відповідно). Визначеня PRA після трансплантації нирки вплинув на довгострокове виживання трансплантата. HLA-сумісність принаймні з одним HLA-DR асоціювалася з тривалим виживанням трансплантата та пацієнта [62].

В іншому дослідженні автори дослідили частоту виникнення, фактори ризику, відповідь на лікування та вплив на результати АОВ серед 3131 реципієнта нирки було зареєстровано 194 випадки АОВ (6,2%) протягом (середнє ± стандартне відхилення) 4,85 ± 1,86 років спостереження. Час до АОВ становив 0,97 ± 1,17 (медіана, 0,48) років. Фактори ризику АОВ включали молодший вік реципієнта, невідповідність антигенів лейкоцитів людини за локусом DR, позитивні панель-реактивні антитіла (>0%), позитивний перехресний лімфоцитотоксичний тест (Т- або В-клітин) та ВФТ. Порівняно з відсутністю АОВ, скоригований залежний від часу коефіцієнт ризику для відторгнення трансплантата з ризиком смерті становив 10,1 (95% довірчий інтервал, 6,5-15,7) для всіх пацієнтів з АОВ, 4,0 (2,5, 9,1) для раннього АОВ (<90 днів після трансплантації) та 24,0 (14,0-41,1) для пізнього АОВ (≥90 днів після трансплантації) [63].

Застосування штучного інтелекту, передбачає створення алгоритмів, які автономно розпізнають закономірності у великих вибірках даних. У цьому контексті його застосування у трансплантології доможе прогнозувати виникнення ускладнень після трансплантації, аналізувати складні взаємодії між параметрами здоров'я реципієнта, донора та їх імунологічними характеристиками [64].

Дослідження застосування штучного інтелекту у ТН переважно зосереджені на таких трьох ключових напрямках: прогнозування виживання трансплантата, оптимізація дозування імуносупресив-

них лікарських засобів та підвищення ефективності підбору пари донор-реципієнт [65].

Моделі, які розробляються наразі, здебільшого спираються на такі змінні, як вік та стать донора/реципієнта, невідповідність HLA, час холодної/теплої ішемії, тривалість діалізу до трансплантації, супутні захворювання, рівень креатиніну сироватки крові та протоколу ІСТ [66]. В останні роки Євротрансплант запровадив віртуальний перехресний аналіз для розподілу нирок та підшлункової залози як кращу альтернативу фізичним перехресним аналізам комплементзалежної цитотоксичності, які були пов'язані з довшим часом холодової ішемії та хибнопозитивними реакціями. Крім того було впроваджено розрахунок віртуальної панельної реактивної антитіл за 11 локусами, електронна передача даних HLA-типування з використанням формату файлу мови гістоімуногенетичної розмітки та фактичний віртуальний перехресний аналіз на основі неоднозначного HLA-типування другого поля донора за всіма 11 локусами [66, 67].

Останнім часом все частіше застосовують алгоритми прогнозування дострокої вижживаності НАТ:

1. Модель iBox, використовує штучний інтелект для прогнозування короткострокових, середньострокових та довгострокових результатів трансплантації. Цей інструмент аналізує експресію генів у трансплантаті та інші клінічні дані для точного прогнозування відторгнення [68].
2. Сучасні дослідження застосовують алгоритми машинного навчання, такі як випадкові ліси (Random Forest), штучні нейронні мережі та методи глибокого навчання для прогнозування виживаності трансплантата. Ці моделі аналізують великі обсяги даних, включаючи інформацію про донора та реципієнта, для точного прогнозування результатів трансплантації [69].
3. Створено номограми, які поєднують кілька клінічних та лабораторних параметрів для прогнозування 20-річної виживаності трансплантата. Ці моделі використовують методи, такі як LASSO-регресія та випадкові ліси, для відбору предикторів і побудови точних прогнозів [70].
4. Динамічні моделі, такі як баєсівські моделі, використовують траєкторії eGFR для прогнозування довгострокової виживаності трансплантата та пацієнта. Ці моделі дозволяють оновлювати прогнози в реальному часі на основі нових даних [71].

Таким чином, для забезпечення кращих результатів ТН необхідно визначити імунологічні детермінанти виживаності НАТ щонайменше основні: сумісність за системою АВ0, панельно-реактивні антитіла реципієнта, антитіла до HLA донора та перехресної проби на індивідуальну сумісність з потенційним донором [48, 72, 73].

Реципієнтів з високим імунологічним ризиком, які мають в анамнезі гемотрансфузії, вагітність або попередню трансплантацію органів та, відповідно, підвищений рівень панельно-реактивних антитіл відносять до високосенсибілізованих кандидатів [72], які можуть потребувати проведення десенсибілізації перед ТН та індивідуального вибору режиму ІСТ [73].

Отже, основними імунологічними детермінантами, які негативно впливають на тривалість функціонування АТН є: високий ступінь HLA-несумісності, несумісність за HLA-DR, високий рівень ДСА та реактивних антитіл. Ці фактори слід враховувати при плануванні ТН та підборі донора, щоб мінімізувати ризики та забезпечити довготривалу функціональність АТН. Результат вивчення імунологічних детермінант формування різноманітних варіантів відторгнення дозволили виокремити більше 1 з них, які відповідно до сьогоднішніх можливостей можна розглядами як тератевтичні мішені (табл. 2).

Таблиця 2

Імунологічні детермінанти як потенційні терапевтичні мішені

Приклади детермінант	Патогенна роль	Потенціал терапевтичного впливу
Гуморальна відповідь	DSA, IgG3-DSA	Блокада комплементу (e.g. eculizumab), плазмаферез
Клітинна відповідь	Allo-специфічні CD8+ Т-клітини, CD4+ TEM, CD4+ Tfh	Інактивація mTOR, інгібітори JAK/STAT
Запальні хемокіни	CXCL9, CXCL10, CCL5	Інгібіція хемокінів або їх рецепторів (наприклад, блокада CXCR3)
Молекули активації	ICAM-1, TLR4, CD40/CD40L	Антагоністи ICAM-1 або CD40/CD154
Генні сигнатури	IFN-γ-асоційовані транскрипти	Ідентифікація пацієнтів для таргетної терапії

Таким чином, можна зробити висновок, що аналіз досліджень, присвячених цій проблемі протягом останнього десятиріччя, продемонстрував значимість як класичних, так і нових імунологічних детермінант, частина яких є терапевтичними мішенями та/або біомаркерами.

Переконливо продемонстровано, що алоімунний конфлікт є багатовимірним і включає епітопне навантаження, спектр non-HLA антитіл, активацію комплементу, механізми «trained immunity», які ідентифікуються визначенням маркерів ушкодження (cfDNA, транскриптоміка).

Інтеграція цих факторів відкриває можливість персоналізованої імуносупресії й проактивного імунного моніторингу пацієнта для підвищення виживаності АНТ, бо сучасне «плато» 10- і 15-річної виживаності АНТ є прямим наслідком того, що клінічна практика досі спирається майже виняткова на класичні HLA-показники й емпіричні схеми імуносупресії. Тому в майбутньому необхідні комплексні дослідження з пошуку нових детермінант та ідентифікація нових терапевтичних мішеней. Нові підходи до стратифікації пацієнтів для персоналізованої імуносупресії, шляхом застосування епітоп-/eplet-matching і мультибіомаркерних панелей дозволить виділити групи високого ризику, де доцільно посилювати десенситизацію чи додавати комплемент- або AT1R-інгібітори, а також групи низького ризику, які можна безпечно «декомпресувати» від токсичних препаратів, а також моделювання довгострокового прогнозу виживаності, перспективним виглядає залучення програм машинного навчання та штучного інтелекту.

Таким чином, комплексне врахування HLA-, non-HLA- та інфламасом-залежних факторів, поєднаних з новими біомаркерами та імунологічними детермінантами відкриває шлях до істотного подовження функції АНТ і формує напрямки майбутніх досліджень. Комплексне, мультиомне дослідження імунологічних детермінант — необхідна умова для переходу від «лікування відторгнення після факту» до проактивного, точного запобігання імунному ушкодженню, що сприятиме пошуку нових терапевтичнії мішеней, оптимізації імуносупресії, суттєвому подовженню життя АНТ.

Конфлікт інтересів. Автори декларують відсутність конфлікту інтересів.

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Інформація про внесок кожного учасника.

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ВИМОГИ ДО РОБІТ, ЩО ПОДАЮТЬСЯ ДО ПУБЛІКАЦІЇ В "УКРАЇНСЬКИЙ ЖУРНАЛ НЕФРОЛОГІЇ ТА ДІАЛІЗУ"

У зв'язку з індексуванням журналу міжнародними пошуковими системами та підвищенням вимог до оформлення наукового матеріалу, редколегія формалізує вимоги до видання для ефективного розповсюдження матеріалів у світовій мережі Інтернет та приєднання до міжнародних наукових баз даних.

Правила для авторів складені відповідно до «Єдиних вимог до рукописів, що подаються у біомедичні журнали» (Uniform Requirements for Manuscripts Submitted to Biomedical Journals), які сформульовані Міжнародним комітетом редакторів медичних журналів (International Committee of Medical Journal Editors) та містяться на ресурсі ICMJE.org.

ЖУРНАЛ СТРУКТУРОВАНО ЗА 5 ОСНОВНИМИ РОЗДІЛАМИ:

1. Точка зору
2. Проблеми організації та економіки нефрологічної допомоги
3. Оригінальні наукові роботи
4. Школа нефролога
5. Редакційна інформація, інформація про наукові форуми, коментарі, рецензії, знаменні дати.

Перший розділ. В цьому розділі друкуються статті, які відображають точку зору на конкретну проблему автора чи авторів.

Другий розділ висвітлює можливі шляхи покращення організаційної складової діяльності нефрологічної служби в Україні на всіх етапах надання спеціалізованої медичної допомоги та її економічний аналіз.

У третьому розділі розміщуються статті, які знайомлять з результатами оригінальних досліджень.

Розділ "Школа нефролога" друкує роботи, метою яких є підвищення нефрологічної грамотності читачів.

Останній розділ інформує про основні науково-практичні події, публікує рецензії, редакційну інформацію і т.п.

Рукопис разом з дозволом на його використання направляється до редакції тільки в електронному варіанті через он-лайн систему, яка міститься на сайті журналу. У редакції здійснюється двостороннє сліпе (анонімність рецензента та автора) наукове рецензування і літературне редагування статей.

Дозвіл на використання рукопису можна завантажити за посиланням

Статті, оформлені без додержання правил не приймаються, авторам не повертаються.

У разі негативної наукової рецензії, статті не публікуються, авторам ел. поштою надсилається відгук з можливістю доопрацювання статті чи заміни її іншим матеріалом.

До публікації приймаються оригінальні роботи, огляди літератури, лекції, короткі повідомлення, рекомендації практичним лікарям, опис випадків з практики, інформація про наукові форуми.

Редакція не приймає раніше опубліковані роботи або статті, прийняті до друку в інших виданнях.

Статті публікуються українською, російською та англійською мовами. Файл зі статтею представляти у форматі Microsoft Word (розширення *.doc, *.docx, *.rtf).

ПОСЛІДОВНІСТЬ РОЗМІЩЕННЯ МАТЕРІАЛУ НАСТУПНА:

1. Ініціали та прізвища авторів англійською мовою;
2. Назва статті англійською мовою;
3. Назва установи та організації, в якій працюють автори англійською мовою;
4. Резюме статті англійською мовою;
5. Ключові слова (8-10 слів чи словосполучень, що розкривають зміст статті) англійською мовою;
6. УДК;
7. Ініціали та прізвища авторів мовою, якою написана стаття;
8. Назва статті (мовою оригіналу);
9. Назва установи та організації, в якій працюють автори, місто (мовою статті);
10. Ключові слова (8-10 слів чи словосполучень, що розкривають зміст статті) мовою статті;
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12. Текст статті;
13. Розкриття потенційних конфліктів інтересів;
14. Інформація про внесок кожного учасника;
15. Список використаних джерел під назвою «Література (References)», оформлений відповідно до стандарту Vancouver style;
16. Відомості про відповідального автора: ПІБ, наукове звання, посада та місце роботи; e-mail (обов'язково) та робочий телефон.

Формат тексту рукопису. Текст статті друкується шрифтом Times New Roman, розмір 14 pt, з полуторним міжрядковим інтервалом. Відступи з кожного боку сторінки 2 см. На всі ілюстрації, графіки і таблиці мають бути посилання в тексті.

Виділення в тексті можна робити курсивом або напівжирним шрифтом, НЕ підкресленням. З тексту слід видалити всі перенесення, повторювані пропуски, зайві розриви рядків (в автоматичному режимі через сервіс Microsoft Word "знайти і замінити").

Файл з текстом статті містить всю інформацію для публікації, у тому числі рисунки і таблиці після їх першого згадування.

Структура рукопису має відповідати наведеному шаблону (залежно від типу роботи).

УДК розміщується у верхньому лівому куті.

Автори. Прізвище авторів вказувати після ініціалів (**О. С. Іванов, С. І. Петров**), жирним шрифтом, мовою оригіналу та англійською мовою, вирівнювання за центром. ПІБ авторів англійською необхідно вказувати відповідно з закордонним паспортом, або як в раніше опублікованих зарубіжних журнальних статтях. Автори, які публікуються вперше і не мають закордонного паспорта, мають скористатися стандартом транслітерації КМУ—2010.

Бажано додати посилання на власні офіційні інтернет сторінки автора(ів) (https://.....), Scopus ID, Researcher ID або ORCID ID для розміщення гіперпосилання на сайті журналу та pdf-версії статті.

Назва статті англійською мовою та мовою оригіналу розміщується за центром після прізвищ авторів жирним шрифтом (**«Оцінка резидуальної функції нирок у хворих на ХХН VД СТ. та підходи до її збереження»**). Англомовна назва має бути грамотною з точки зору англійської мови та повністю відповідати україно/російськомовній назві за змістом.

Установа. Необхідно наводити офіційну повну назву установи (без скорочень). Після назви установи через кому зазначити назву міста та країни. Якщо у дослідженні брали участь автори з різних установ, слід співвіднести назви установ і прізвища авторів за допомогою цифрових індексів у верхньому регістрі. Під назвою необхідно додати інтернет адресу (https://.....) офіційної сторінки установи.

Реферат (якщо робота оригінальна) має бути структурованим: мета, матеріали і методи, результати, висновки. Реферат має повністю відповідати змісту роботи, **обсяг тексту не менше 1 800 знаків** (з пропусками). Резюме до публікацій, що подаються в інші розділи журналу (1,2,4,5) оформляється довільно, але з таким самим обсягом.

Англомовна версія резюме статті за змістом і структурою (Aim, Methods, Results, Conclusions) повністю відповідає україно/російськомовній.

Ключові слова. Необхідно вказати ключові слова — від 3 до 10 для індексування статті в пошукових системах. Ключові слова повністю відповідають українською/російською та англійською мовою. Для вибору ключових слів англійською використовують тезаурус Національної медичної бібліотеки США (Medical Subject Headings — MeSH).

Текст статті (українською або російською мовою) структурований за розділами: вступ (актуальність), мета, матеріали і методи, результати, обговорення, висновки. **Розділ «Обговорення» є обов'язковим та має включати 3000-3500 знаків без пробілів.**

Розкриття потенційних конфліктів інтересів

Автори повинні розкривати всі відносини або інтереси, які можуть мати прямий або потенційний вплив чи надати упередженість в роботі. Хоча автор, можливо, не відчуває, що існує який-небудь конфлікт, розкриття відносин і інтересів забезпечує більш повний і прозорий процес, що призводить до точної і об'єктивної оцінки роботи.

Усвідомлення реального або передбачуваного конфлікту інтересів – це перспектива, на яку мають право читачі. Це не означає, що фінансові відносини з організацією, яка спонсорувала дослідження або компенсацію, отриману за консультаційну роботу є недоречними.

Приклади потенційних конфліктів інтересів, які прямо або побічно пов'язані з дослідженням, можуть включати, але не обмежуються наступним:

- Наукові гранти від фінансових агентств (прохання надати дані про спонсора дослідження та номер гранту)
- Гонорари за виступи на симпозіумах
- Фінансова підтримка участі в симпозіумах
- Фінансова підтримка освітніх програм
- Зайнятість або консультації
- Підтримка з боку спонсора проекту
- Посада в консультативній раді або раді директорів або в інших відносинах управління
- Кілька філій
- Фінансові відносини, наприклад, пайову участь або інвестиційний інтерес
- Права інтелектуальної власності (наприклад, патенти, авторські права і роялті від таких прав)
- Утримання чоловіка і / або дітей, які можуть мати фінансовий інтерес до роботи

Крім того, слід розкривати інтереси, що виходять за рамки фінансових інтересів і компенсації (нефінансові інтереси), які можуть бути важливі для читачів. Вони можуть включати, але не обмежуються, особисті відносини або конкуруючі інтереси, прямо або побічно пов'язані з цим дослідженням, або професійні інтереси або особисті переконання, які можуть вплинути на ваше дослідження.

Відповідальний автор збирає форми розкриття конфлікту інтересів від усіх авторів. В авторській співпраці, де допускаються формальні угоди про представництво, для відповідного учасника досить підписати форму розкриття від імені всіх авторів.

Приклади розкриття інформації

Дослідження фінансувалося X (грант № X).

Конфлікт інтересів: автор А отримав дослідні гранти від компанії А. Автор В отримав гонорар доповідача від компанії X і володіє акціями в компанії Y. Автор С є членом комітету Z.

Конфлікт інтересів: автори заявляють, що у них немає конфлікту інтересів.

Подяка. Автори можуть висловити подяку особам та організаціям, що сприяли публікації статті, але не є її авторами.

Інформація про внесок кожного учасника (і осіб, зазначених у розділі "подяка").

Приклад: О.С. Іванов – концепція та дизайн дослідження, І.П. Петров – аналіз отриманих даних, оформлення тексту роботи.

Автори висловлюють подяку (Прізвище І. Б.) за оформлення ілюстрацій.

Список літератури. У бібліографії (пристатейному списку літератури) кожне джерело зазначають з нового рядка під порядковим номером. Вимоги до оформ-

лення літературних джерел за Vancouver style детально представлено на сайті журналу. **Після кожного джерела ОБОВ'ЯЗКОВИМ є додавання його інтернет адреси** (https://ukrjnd.com.ua/index.php/journal/article/view/22).

Перед відправкою автори мають здійснити **самоперевірку тексту наукової статі** на:

1. **Плагіат.** Україно та російськомовні тексти статті перевіряють на плагіат за допомогою програми eTXT Антиплагиат https://www.etxt.ru/antiplagiat, що є необхідною умовою для передачі статті для подальшого рецензування. Рівень індивідуальності дослідження має бути не нижчий 80%.
2. Науковий стиль викладення матеріалу.
3. Тавтологію – повторювання у тексті.
4. Універсальність викладення матеріалу (читабельність). Текст статті має легко та просто сприйматися, не бути переобтяженим абревіатурами, спеціальною вузькопрофільною термінологією або такою, що не набула міжнародної адаптації. Речення мають бути простими, лаконічними і нести завершений зміст.
5. Кількість посилань на статті та наукові матеріали з ідентифікатором DOI (не менше 80 %).
6. Відповідність вимогам видання.

ПОМИЛКИ, ЩО НАЙЧАСТІШЕ ВИНИКЮТЬ У ПОДАНИХ РУКОПИСАХ:

1. Використовують у реченнях «зайві» слова і вирази. Усього зайвого треба уникати. Керуйтеся правилом: «Якщо слово з речення можна викинути і при цьому зміст не втрачено – слово треба викинути». Це саме стосується і більших за обсягом фрагментів тексту.
2. Не вірно вказують одиниці виміру. Системні одиниці виміру системи СІ наводять без крапки (м, г, га, моль), а нестандартизовані одиниці – за скороченнями.
3. Треба розрізняти символи «—», «–» та «-». Перший із них у рукописах не використовують.
4. Більшість редакторських правок обумовлені невірним вживанням слів «в», «у», «і», «та», «з», «із», «зі».
5. У статтях не використовують вирази «на протязі» – заміняємо «протягом», «найбільш потужний» – «найпотужніший», «при» – «у разі» і т. п.
6. Зайве використання слова «було». Треба уникати слова «було»: без нього, зазвичай, зміст речення не зміниться.
7. Скорочення наукових термінів у статті треба звести до мінімуму.
8. Назви таблиць і рисунків (та примітки до них) повинні бути «вичерпними». Читач не повинен додатково перечитувати «Матеріал і методи досліджень» або назву роботи, щоб розібратися у змісті таблиці чи рисунка.
9. Статті найчастіше відхиляються редколегією через відсутність статистичного опрацювання первинних даних (загальні вимоги до фахових публікацій).
10. Не рекомендовано вживати в тексті пасивний залог: «проби відбиралися», замість цього – «проби відбирали»; «дослідження здійснювалися» – «дослідження здійснили».
11. Якщо виникають питання щодо оформлення чи представлення певних даних у статті – можна брати зразок останнього номеру журналу.
12. Відсутня можливість редагування рисунків, таблиці, формул, наведених у роботі. Необхідно надати редакції змогу їх редагувати, тобто не використовувати нестандартні програми.

Статті, оформлені без додержання правил не приймаються, авторам не повертаються.

У разі негативної наукової рецензії, статті не публікуються, авторам ел. поштою надсилається відгук з можливістю доопрацювання статті чи заміни її іншим матеріалом.