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Шановні колеги!

Пропоную вам вибрати свій слоган для цього номера.



*Яскраве — манить,
та в рядках дрібного шрифту
солодке — з гірким.*

Пролог: шепіт із піску

У вітрах за Межевими Горами подекуди про постать, яка з'являється на межі сутінку — ні день, ні ніч. Його звать Златоязикий Дух. Він не продає зброї, не торгує золотом. Він дарує мрії. Але жодна — не безцінна. Прави-телям він пропонує вплив. Вигнанцям — другий шанс. Поетам — безмежну натхненність. Але всі йдуть із тінню.

Одного разу молодий маг запитав:

— Чому кожне твоє бажання несе кайдани?

Златоязикий усміхнувся очима, що світилися, як монети у джерелі:

— Бо ніхто не читає напису на звороті бажання, — і розтанув у пилу забуття.

Фраза «Диявол у дрібниці» давно стала звичною. Але справжній її сенс розкривається лише тоді, коли перед нами не формальність, а вибір із необоротними наслідками.

Зустрінь я того, хто пропонує щедрі угоди без ціни, — можливо, я краще зрозумів би, чому «безкоштовне» часто виявляється найдорожчим. Але, якщо чесно, я волів би ніколи не знайомитися з такими.

Кожна, на перший погляд, добра пропозиція заслуговує на пильне око. Бо блиск — не завжди світло. Іноді це приманка. І часто ми розуміємо це запізно — коли кінець вже не солодкий, а солодко-гіркий.

Жити просто. Жити чесно. Жити без боргу перед темним — от що варто.

Післямова

Минуло багато років, і той юний маг став сивим мандрівником. Він так і не звернувся по бажання, не торкнувся жодної угоди. Але в його книзі з'явилися нові розділи: не про чари — про вибір.

Одного дня він зустрів учня. Той питав, чому маг такий сильний, хоча не має амулетів чи угод з духами.

— Тому що я навчився читати дрібним шрифтом, — відповів старий.

— А що там написано? — не вгавав учень.

— Завжди одне й те саме, — сказав маг і усміхнувся. — **Те, що здається коротким шляхом, найчастіше є колом.**

З повагою, проф. Д. Іванов ■



DOI: <https://doi.org/10.22141/2307-1257.14.3.2025.544>Maytham Abas Makki¹, Emaduldeen Hatem Abed², Adian Abd Alrazak Dakl¹¹College of Science, Al-Muthanna University, Samawah, Iraq²Environmental Research Center, University of Technology, Baghdad, Iraq

Investigation of some bacterial causes of urinary tract infection in diabetic patients in Al-Muthanna Province

Abstract. Background. Urinary tract infections (UTIs) are among the most common infections affecting diabetic patients, contributing to increased morbidity and healthcare burden. This study purposed to investigate the prevalence and bacterial causes of UTIs among diabetic patients in Al-Muthanna Province, Iraq, and to determine the antimicrobial susceptibility patterns of the isolated pathogens. **Materials and methods.** A total of 200 urine samples were collected from diabetic patients presenting with UTI symptoms. Bacterial identification and antibiotic susceptibility testing were conducted using standard microbiological methods. **Results.** The results showed that 123 (61.5 %) samples were positive for bacterial growth. *Escherichia coli* was the most prevalent organism (52 %), followed by *Klebsiella pneumoniae* (19.5 %), *Staphylococcus aureus* (13 %), *Proteus mirabilis* (8 %), and *Pseudomonas aeruginosa* (6.5 %). High resistance was observed against ampicillin and TMP-SMX, while nitrofurantoin and imipenem showed the highest sensitivity. **Conclusions.** These findings underscore the need for continuous surveillance and appropriate empirical therapy to manage UTIs in diabetic individuals.

Keywords: *Klebsiella pneumoniae*; *Staphylococcus aureus*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; urinary tract infections

Introduction

Urinary tract infections (UTIs) are a common and recurrent problem among diabetic patients due to immune dysfunction, poor glycemic control, and structural abnormalities in the urinary tract [1]. Diabetes mellitus, particularly type 2, has been identified as a significant risk factor for both asymptomatic and symptomatic bacteriuria [2]. The anatomical and physiological changes associated with diabetes such as autonomic neuropathy, increased glucose content in urine, and bladder dysfunction increase the risk of urinary stasis and subsequent infection. Furthermore, impaired neutrophil function and altered cytokine response in diabetic patients contribute to reduced host defenses against invading uropathogens [3].

The most commonly implicated uropathogens in diabetic patients include *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* [4, 5]. These pathogens may present with more severe symptoms or complications in diabetics, including pyelonephritis, renal abscesses, and emphysematous infections. In recent years, there has been an alarming rise in

antibiotic resistance among these pathogens, complicating the choice of empirical therapy and increasing the risk of treatment failure [6].

Understanding the local epidemiology and resistance patterns of uropathogens is crucial for effective treatment planning and for reducing the burden of recurrent infections and hospitalizations in diabetic individuals. However, limited data exist from the southern regions of Iraq, including Al-Muthanna Province. This study was therefore designed to fill this gap by identifying the bacterial agents responsible for UTIs in diabetic patients and analyzing their antibiotic resistance profiles.

The current research aims to determine the prevalence of UTIs in diabetic patients and to identify the bacterial pathogens responsible for UTIs, also to analyze the antibiotic resistance patterns of the isolated bacteria.

Materials and methods

Study design and population

A cross-sectional study was conducted from January to March 2025 at Al-Hussein Teaching Hospital in Al-Muthanna Province. A total of 200 diabetic patients (both type

1 and type 2) presenting with symptoms suggestive of UTI were included.

Sample collection

Midstream urine samples were collected in sterile containers and transported immediately to the microbiology laboratory. The samples were collected from different age groups years, 49 males and 74 females who at Al-Hussein Teaching Hospital for the period January to March 2025.

Bacterial isolation

All samples were cultured and activated in brain heart Infusion broth (BHI) for 24 hours at a temperature of 37 °C in order to stimulate the potential presence of bacteria, then transferred to the selective culture medium (chromogenic agar) for a period of 24 to 48 hours in order to diagnose and isolate the bacteria and assure it with biochemical examinations [7]. The media used are UTI chromogenic agar, *E.coliforms* chromogenic agar and CHROMagar *Pseudomonas*. Traditional media was also used to confirm the results of the cultures such as MacConkey agar, EMB agar. Biochemical tests as IMVIC tests [8].

Bacterial identification

Isolates diagnosed depending on Bergey's manual [9] and according to the methods used by [10].

Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method was used according to CLSI guidelines. Antibiotics tested included: ampicillin, TMP-SMX, ciprofloxacin, ceftriaxone, nitrofurantoin, imipenem, gentamicin, and vancomycin (for Gram-positive organisms).

Ethical approval

The Medical Ethical Committee of College of Science, Al-Muthanna University approved this study (No. 11 on 3/1/2025).

Statistic

In the present study, the following statistical methods were applied: descriptive statistics were utilized to summarize the distribution of cases by calculating frequencies and percentages. The Chi-square test (χ^2) was conducted to assess differences in the distribution of positive cases among distinct categories. The p-value was significance of associations at < 0.05.

Results

The data show a high prevalence of UTIs among diabetic patients, indicating that diabetes significantly increases susceptibility to urinary infections. This is consistent with previous findings linking impaired immune responses and glycosuria in diabetics to higher infection rates. Females accounted for 60 % of positive cases, aligning with the well-known anatomical predisposition due to a shorter urethra and its proximity to the anus. This also reflects global epidemiological patterns. The highest infection rate (45.5 %) was found in the 41–60 age group, suggesting that middle-aged

adults with diabetes are at increased risk. This could be due to a longer duration of illness, poor glycemic control, and age-related changes in immunity or bladder function. Type 2 diabetics had a significantly higher proportion of UTIs (72 %), likely due to the higher prevalence of type 2 diabetes in the general population and its stronger association with obesity and metabolic syndrome. The age group 41–60 years had the highest infection rate, likely due to longer disease duration and compromised immunity (Table 1).

Table 1. UTI prevalence and demographics

| Parameter | No. | % |
|-------------------------|-----|------|
| Positive cultures | 123 | 61.5 |
| Negative cultures | 77 | 38.5 |
| Sex | | |
| Female | 74 | 60 |
| Male | 49 | 40 |
| Age group, years | | |
| < 40 | 23 | 18.7 |
| 41–60 | 56 | 45.5 |
| > 60 | 44 | 35.8 |
| Type of diabetes | | |
| 1 | 35 | 28 |
| 2 | 88 | 72 |

E.coli was the most frequently isolated organism, accounting for more than half (52 %) of all UTI cases. This aligns with global data identifying *E.coli* as the predominant uropathogen, due to its ability to adhere to uroepithelial cells via fimbriae. *Klebsiella pneumoniae* (19.5 %) was the second most common pathogen, which is consistent with other reports in diabetic populations. This organism is also notable for its capacity to produce extended-spectrum beta-lactamases (ESBLs), leading to multidrug resistance. *Staphylococcus aureus* was identified in 16 % of cases, suggesting possible skin or catheter-related infections, or ascending infections in patients with poor hygiene or impaired immune systems. *Proteus mirabilis* and *Pseudomonas aeruginosa* were less common but are typically associated with complicated UTIs, recurrent infections, and prior antibiotic use (Table 2).

The resistance profile of *E.coli* revealed high resistance to ampicillin (83 %) and TMP-SMX (68 %), making these

Table 2. Frequency of bacterial isolates

| Bacterial species | No. | % |
|-------------------------------|-----|------|
| <i>Escherichia coli</i> | 64 | 52 |
| <i>Klebsiella pneumoniae</i> | 24 | 19.5 |
| <i>Staphylococcus aureus</i> | 16 | 13 |
| <i>Proteus mirabilis</i> | 11 | 9 |
| <i>Pseudomonas aeruginosa</i> | 8 | 6.5 |

Table 3. Antibiotic resistance patterns, %

| Antibiotic | <i>E.coli</i> | <i>K.pneumoniae</i> | <i>S.aureus</i> | <i>P.aeruginosa</i> |
|----------------|---------------|---------------------|-----------------|---------------------|
| Ampicillin | 83 | 100 | – | – |
| TMP-SMX | 68 | 60 | – | – |
| Ceftriaxone | 55 | 72 | – | – |
| Ciprofloxacin | 25 | 30 | – | 40 |
| Nitrofurantoin | 10 | 18 | – | – |
| Imipenem | 5 | 5 | – | 15 |
| Gentamicin | 15 | 18 | – | 22 |
| Vancomycin | – | – | 0 | – |

agents less favorable as empirical therapy options in diabetic patients. These rates are in line with global trends indicating increased resistance among Gram-negative uropathogens.

K.pneumoniae showed 100 % resistance to ampicillin and high resistance to third-generation cephalosporins (72 %), reinforcing the need for sensitivity testing before treatment. This organism is well-known for harboring ESBL genes. Fluoroquinolones like ciprofloxacin had lower resistance rates (25 % in *E.coli* and 30 % in *K.pneumoniae*), making them relatively effective, though still requiring cautious use to prevent resistance escalation. Nitrofurantoin and imipenem exhibited excellent activity against *E.coli* and *K.pneumoniae*, making them potential choices, especially for lower UTIs and multidrug-resistant infections, respectively. *P.aeruginosa* demonstrated moderate resistance to ciprofloxacin (40 %) and gentamicin (22 %), consistent with its known capacity to resist multiple drug classes. Importantly, *S.aureus* was fully sensitive to vancomycin, maintaining its status as the drug of choice for Gram-positive cocci in serious infections (Table 3).

Discussion

The prevalence of UTIs among diabetic patients was 61.5 %, aligning with findings from [11] in Ethiopia and [12] in Iraq. Female patients were more commonly affected, consistent with [2], who attributed this to anatomical factors.

Escherichia coli was the most common isolate (52 %), consistent with [4, 5], who also identified *E.coli* as the leading cause of UTIs in diabetics. *Klebsiella pneumoniae* was the second most prevalent pathogen, as reported by [13]. The presence of *Staphylococcus aureus* aligns with [14], suggesting possible contamination or secondary bacteremia. *Proteus mirabilis* and *Pseudomonas aeruginosa* were also isolated and are often associated with complicated UTIs, as reported by [13].

Given the increased use of antibiotics and the resulting increase in antibiotic resistance, identifying the antibiotic susceptibility pattern of this organism can be useful in treating most patients with UTI [15, 16].

High resistance to ampicillin (83 % in *E.coli* and 100 % in *K.pneumoniae*) is consistent with the global trends reported by [6, 15, 16]. TMP-SMX and ceftriaxone also showed reduced efficacy, supporting similar findings in [5]. Nitrofu-

rantoin and imipenem maintained high sensitivity, aligning with clinical reports on their retained effectiveness. *S.aureus* was fully sensitive to vancomycin, as confirmed by [17]. Moderate resistance by *P.aeruginosa* to ciprofloxacin and gentamicin agrees with studies by [18].

The results of this study were consistent with the findings of [19], which indicated that the predominant causative organism for complicated and uncomplicated urinary tract infections is *Escherichia coli*, followed by *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Proteus mirabilis*. The current study also agreed with the findings of [20], where it was found that the most common bacteria was *Staphylococcus aureus* (38.75 %), followed by *Enterobacter cloacae* (36.25 %), *Pseudomonas aeruginosa* (27.5 %), *Escherichia coli* (26.25 %), and *Klebsiella pneumoniae* (25 %). *Staphylococcus epidermidis* and *Streptococcus agalactiae* also appeared (21.25 % each), *Proteus mirabilis* (17.5 %), and *Enterococcus faecalis* (11.25 %), while *Staphylococcus saprophyticus* was the least (5 %).

Conclusions

UTIs are highly prevalent among diabetic patients in Al-Muthanna Province, with *E.coli* being the predominant pathogen. High levels of antimicrobial resistance, especially to first-line drugs, underline the importance of local antibiograms and tailored therapy. Nitrofurantoin and imipenem remain effective options for empirical treatment.

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Дослідження деяких бактеріальних причин інфекцій сечовивідних шляхів у пацієнтів із діабетом у провінції Аль-Мутанна

Резюме. Актуальність. Інфекції сечовивідних шляхів (ІСШ) є одними з найпоширеніших інфекцій, що вражають пацієнтів із діабетом, підвищуючи захворюваність і навантаження на охорону здоров'я. **Мета:** дослідити поширеність та бактеріальні причини ІСШ серед осіб із діабетом у провінції Аль-Мутанна (Ірак), а також визначити моделі чутливості виділених збудників до антимікробних препаратів. **Матеріали та методи.** Загалом було зібрано 200 зразків сечі в пацієнтів із діабетом, які мали симптоми ІСШ. Ідентифікацію бактерій і тестування на чутливість до антибіотиків проводили за допомогою стандартних мікробіологічних методів. **Результати.** Ріст бактерій виявлено в

123 (61,5 %) зразках. Найпоширенішим мікроорганізмом була *Escherichia coli* (52 %), далі йшли *Klebsiella pneumoniae* (19,5 %), *Staphylococcus aureus* (16 %), *Proteus mirabilis* (8 %) та *Pseudomonas aeruginosa* (6,5 %). Висока резистентність спостерігалася до ампіциліну й TMP-SMX, тоді як нітрофурантоїн та іміпенем показали найвищу чутливість. **Висновки.** Ці результати підкреслюють необхідність постійного спостереження та відповідної емпіричної терапії при інфекціях сечовивідних шляхів в осіб із діабетом.

Ключові слова: *Klebsiella pneumoniae*; *Staphylococcus aureus*; *Proteus mirabilis*; *Pseudomonas aeruginosa*; інфекції сечовивідних шляхів

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Sharba method: innovative one-sided laparoscopic approach for continuous ambulatory peritoneal dialysis catheter placement

Abstract. Background. This study aims to evaluate whether modifications to the surgical technique can improve peritoneal catheter longevity. In our view, this longevity may be enhanced in two distinct ways. The proposed method facilitates the insertion of a second contralateral catheter and may offer advantages in kidney transplantation (although most procedures are now performed retroperitoneally) and other nephrological surgical interventions, due to the absence of lateral surgical access and reduced risk of adhesions, particularly in cases requiring intraperitoneal surgery. **Materials and methods.** The study includes 410 cases of one-sided laparoscopic continuous ambulatory peritoneal dialysis (CAPD) catheter placement between 2018 and December 2022, with data collected from our center in Najaf. Additionally, a comparative survey was conducted on 118 cases using the traditional two-sided laparoscopic CAPD technique, performed by different surgeons at another center in the same city. Our technique underwent periodic refinements to address complications observed over time, although the core procedural steps remained consistent. Techniques used by other surgeons, both domestically and internationally, vary in certain aspects while sharing similarities in others. Feedback from transplant surgeons was collected for patients who later underwent kidney transplantation, allowing comparison with other approaches and evaluation of postoperative complications potentially avoided with our method. **Results.** The study analyzed age distribution among patients undergoing laparoscopic CAPD, ranging from 8 months to 85 years, with the youngest excluded due to incomplete data. Comparative feedback showed slightly better outcomes for the one-sided technique, though without statistical significance ($p = 0.24530$). Early failure rates were higher in the two-sided group, while late catheter patency failures were more frequent in the one-sided group, mostly unrelated to the technique itself. Revision rates and success were also assessed, revealing a significantly higher success rate for the one-sided approach (95 %) compared to the two-sided method (33.33 %). **Conclusions.** Our findings suggest that the one-sided laparoscopic CAPD catheter insertion technique represents a promising alternative to the traditional two-sided approach. It demonstrates favorable late patency outcomes and significantly higher revision success rates. Although transplant surgeon feedback slightly favored the one-sided method, the difference was not statistically significant. Further long-term studies are needed to validate these results, but this technique may help reduce complications and improve catheter longevity.

Keywords: laparoscopic continuous ambulatory peritoneal dialysis; home peritoneal dialysis; peritoneal catheter insertion strategy; end-stage renal disease; peritoneal dialysis access

Introduction

Peritoneal dialysis (PD) is a well-established renal replacement therapy for patients with end-stage renal disease, offering advantages such as better preservation of residual renal function and improved quality of life [1]. The success of PD

largely depends on the proper placement and long-term functionality of the dialysis catheter. Traditionally, catheters have been inserted using open surgical techniques; however, laparoscopic methods have gained popularity due to their minimally invasive nature and potential for enhanced outcomes [2, 3].

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Recent advancements in laparoscopic techniques have led to the development of single-port approaches, which aim to further reduce surgical trauma and improve recovery times. Studies have demonstrated that single-port laparoscopic placement of PD catheters is both feasible and safe, with low complication rates and favorable long-term outcomes [4, 5]. However, we think it's used limited to those patients who did not need further intervention like omentopexy or colonopexy although some surgeons preferred it over others omitted in the seeking of additional procedures.

In our center, located in Najaf city, we have adopted a modified one-sided laparoscopic technique for continuous ambulatory peritoneal dialysis (CAPD) catheter insertion, we called it the *Sharba method* to differentiate from other procedures done by our colleagues. This approach involves placing all ports on one side of the abdomen, typically the left, unless contraindicated. The technique includes specific steps such as omentopexy and catheter fixation to minimize complications like catheter migration and obstruction.

Materials and methods

Ethics committee approval

The Medical Ethical Committee of the Surgical department, Medical College, Jabir Ibn Hayyan University for Medical & Pharmaceutical Sciences approved this study. This study was a retrospective cohort analysis based on routinely collected clinical data and was not prospectively registered. It received institutional ethics committee approval (Protocol No. 004, date 12/2/2018), and the requirement for patient consent was waived. No pre-specified analysis plan or registration protocol existed prior to data collection.

Study design and setting

Totally, 410 cases underwent laparoscopic CAPD from 2018 till December 2022. Of those patients 88 (21.46 %) lived in Najaf city (our center located) and the rest 322 (78.54 %) from other cities all over the country. Male 226 to female 184 (M : F 1.228). Different numbers may be shown in other our studies according to the time taken or different sample or surgeons done the procedures [6, 7].

Another dialysis center in our town (Najaf Teaching Hospital) where laparoscopic CAPD is done by the ordinary method where two sides are used, the aiding port on the right side instead of the left as in our technique done for 118 cases.

We will study here the two techniques that differ in two main legs.

1st leg. 24 patients (4 %) of all laparoscopic CAPD patients transferred to kidney transplant. 19 of them from our center and the remaining 5 from the neighboring center and

feedback obtained from the transplanted surgeons regarding favorability measured to either approach (one side versus two sides) which was measured by a scale of 4.

2nd leg. We assessed all these cases for a minimum of one year. In some cases, their patients exceed six years. An early failure for the one-side technique was 6 while 8 were for the two-side technique, all underwent revision with no difference in time difference or technique the same catheter with omentopexy or fixation or both was done for all. On the other hand, late patency differed in that 78 failed (7 early so a total of 85), of those (78) twenty underwent revision while those with the two-sided technique (21). Six of them underwent revision as seen in the two tables below.

Our surgical approach (one-sided laparoscopic technique): Sharba method

Anesthesia. For patients over 40 years of age, as well as any patient with a history of cardiac conditions, a preoperative echocardiographic evaluation is advised. The ejection fraction (EF) is a critical determinant in guiding the anesthetic plan (Fig. 1).

This proposed framework underscores the necessity of individualized anesthetic management, emphasizing integrating each patient's medical history, physical status, and

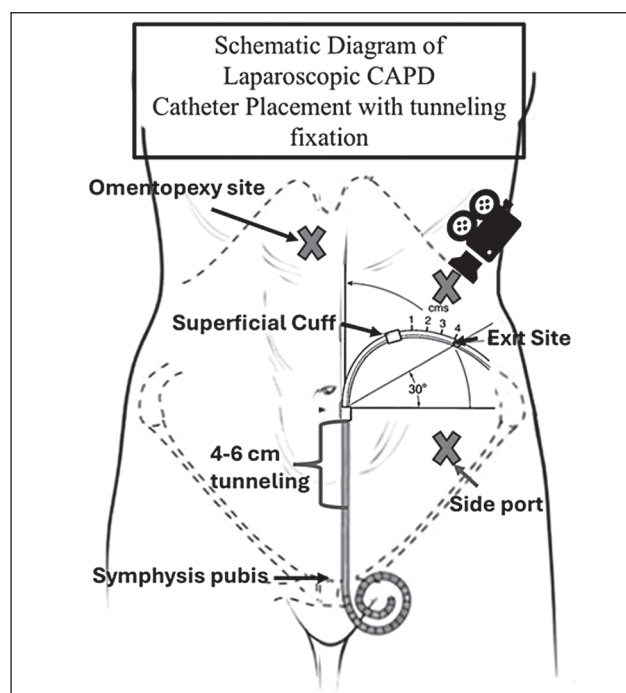


Figure 1. Sharba method

Table 1. Scale feedback of transplanted surgeons' opinions about two modalities

| Scale | Responses |
|-------|---|
| 1 | No difference |
| 2 | Mild (accessible better but equal time) |
| 3 | Moderate (accessible and time better however never mind if other modality cases are received in the future) |
| 4 | Severe (accessible better and time shorter and rejected the other modality in future) |

diagnostic findings to inform the most suitable anesthetic approach. In the study population, 150 patients received general anesthesia with an endotracheal tube, 156 underwent spinal anesthesia, and the remaining 104 were managed with local anesthesia and spontaneous ventilation. The outlined criteria serve as practical guidelines for anesthesiologists in managing these patients.

Surgical technique. One important fact when doing the surgery was looking for future need for further intervention as they mostly hope of kidney transplant or a prolonged need for PD that two-sided were need it to use the catheter for an extended time so from this vision and another reason, we think modification of the ordinary approach of laparoscopic CAPD surgery may need to develop with time and not only the methods.

All openings and ports are on one side, and it is usually the left side unless there is an obstacle that prevents left paramedian catheter insertion so the catheter will be on the right side first camera site we use the left hypochondrial site (Palmer site). It is the first site to get in so rare time Veress needle used before the use of a Safety port especially in an infant or multiple abdominal surgeries especially at the site of entrance that we suspect severe adhesion.

Veress needles were used in 120 cases for those with a previous history of multiple abdominal surgeries and adhesion was suspected sometimes in young children with small abdominal contours, however, sometimes direct insertion of a safety port was done without this step in patients with previous abdominal surgeries in those with ascites.

Usually, a 10 mm camera is used except in children where 5 mm cameras were suitable, however in the last cases even in adults, we used angled 5 mm cameras, and hence port was smaller which is favorable but not for the surgeon. So, the camera port is almost always in the left hypochondrial region except in three cases on the right side and, in one case a 10-year-old boy, although started on the left side the adhesion was so severe there that we shifted to the right side using 8 mmHg pressure, which gives me good vision, in addition, it is safer than other sites even if injuries could happen to the liver (two cases) it was easy to achieve hemostasis through pressure effect with electrical coagulopathy or gauze introduced through a 10 mm port.

Five mm port cameras were used in 60 cases and 4 cases started with 5 mm and then shifted to a 10 mm camera to improve our vision while the rest used a 10 mm camera from the beginning. Although all pediatric age groups used a 5 mm angled camera, lastly, we used it even for the adult age group to avoid a 10 mm incision. However, the angled 5 mm camera proved sufficient view.

Artificial pneumoperitoneum through CO₂ insufflation through a camera port or Veress needle if used, usual pressure will decrease when O₂ saturation decreases in patients with spontaneous breathing. The pressure inside is determined by the age of patients and the type of anesthesia. Hence, we use low pressure in the pediatric age group and when patients are under spontaneous breathing anesthesia so that higher pressure interferes with the patient's breathing most commonly 12 mmHg and for the childhood category 10 mmHg as shown in Fig. 2.

Omentopexy is done at the right paramedian site (not far on the right side to keep it untouched) with a simple few millimeters of skin and use nylon (first loop suture and lastly, we prefer non-loop one with number 1 size) then by the aid of stich passer except in some cases where it has been put in the left side when catheter in the right side paramedian instead of the usual left side. Rarely does it need to be done on another site which may be necessary when changing the site of the catheter or when adhesion is so severe that omentopexy has been used rather than the Parda technique.

The descriptions of omentopexy and catheter fixation have been carefully revised and standardized for clarity and precision. Specifically:

— **omentopexy.** Clearly described as fixation of the omentum via minimal skin incision using nylon suture (1 size) aided by a stitch passer;

— **catheter fixation.** Although we did catheters fixation in our methods in two consistent methods used:

— **suprapubic fixation** using nylon (#1) via stitch passer;

— **subcutaneous tunneling** approximately 4–6 cm using a trocar sheath included in the percutaneous catheter set.

While in ordinary method used only the subcutaneous tunneling so we omitted all cases that we used suprapubic fixation cases that not included in this study so that not interfere with the result of catheter patency so that all cases in both groups with same fixation method.

One of the major complications that caused serious problems related to catheter patency and function, especially in the long term was the migration of the catheter this will be overcome through fixation which was done in two ways in our study, some with suprapubic fixation via the aid of stich passer using nylon 1 or recently using tunneling for about 4–6 cm via sheath with trocar which already provided in the set of Covidien catheter (percutaneous set) or use of 7.5 mm port subcutaneously.

The standardized clinical definitions have now been explicitly included:

— **early failure.** Defined as catheter dysfunction or failure (poor flow, obstruction, or leakage) occurring within 30 days post-insertion;

— **late patency failure.** Defined as catheter dysfunction or obstruction occurring more than 30 days after insertion;

— **revision success.** Defined as successful restoration of catheter functionality following a revision procedure, without requiring subsequent intervention within six months post-revision.

Statistical analysis

Use of statistics to show the correlation between each type of catheter fixation in laparoscopic CAPD with the P-value significant if it is < 0.05. The chi-square (χ^2) and P-values were calculated using the interactive calculation tool [8].

Results

Age distribution varies between two extremities youngest age in our study was recorded as an 8-month-old boy from Baghdad, Iraq (although we did CAPD for a few days his data was incomplete, so he was excluded from our study).

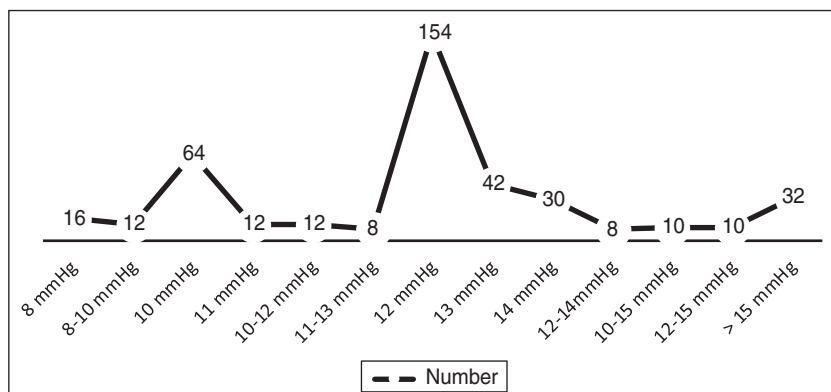


Figure 2. Intraperitoneal pressure used in our laparoscopic technique

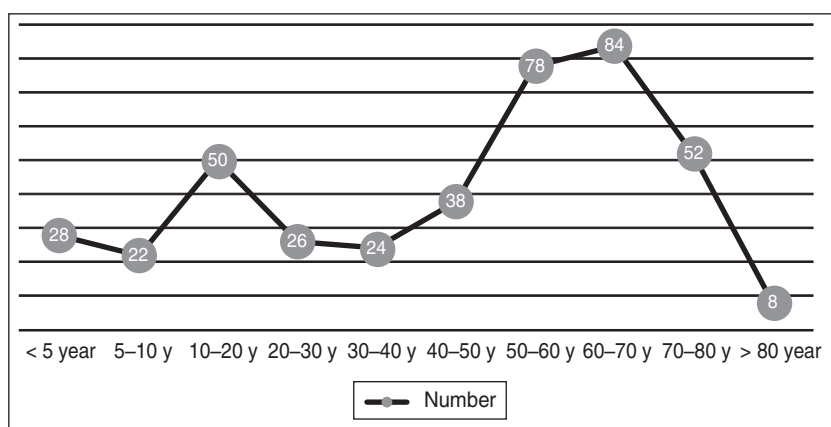


Figure 2. Age distribution in our patients that underwent the one-sided technique

The oldest age in our study was 85 years. Fig. 2 shows the age distribution.

While baseline patient characteristics were nearly similar between the two groups (one-sided (Sharba method) and two-sided laparoscopic CAPD catheter insertion), enabling meaningful comparison and robust interpretation of results, we have added a detailed Table 2 to transparently document these demographic and clinical parameters. Table 2 demonstrates the homogeneity of the patient cohorts, thereby ensuring that observed differences in outcomes, such as catheter patency and transplant surgeon preference, are more confidently attributed to differences in surgical techniques rather than baseline variability.

These data demonstrate clearly that both patient cohorts were well-balanced across demographic characteristics, comorbidities, lab parameters, and anesthesia methods. Therefore, any differences observed, particularly in catheter patency, ease of subsequent surgical procedures, and reduced postoperative complications, can be confidently attributed to differences in the surgical technique itself, notably the one-sided Sharba method.

A detailed summary (Table 3) of adverse events — including intraoperative

Table 2. Baseline demographic and clinical characteristics of patients undergoing one-sided versus two-sided laparoscopic CAPD

| Characteristic | One-sided technique (Sharba method) (n = 410) | Two-sided technique (n = 118) | P-value |
|---|---|-------------------------------|---------|
| Age (years), mean (range) | 42.6 (0.7–85) | 44.2 (2–79) | 0.86 |
| Sex Male : female | 226 : 184 (55.1 : 44.9 %) | 67 : 51 (56.8 : 43.2 %) | 0.87 |
| Comorbidities (%) | | | |
| Diabetes mellitus | 31.7 | 30.9 | 0.92 |
| Hypertension | 55.6 | 54.2 | 0.89 |
| Cardiovascular diseases | 12.9 | 14.1 | 0.81 |
| Previous abdominal surgery (%) | 29.3 | 27.8 | 0.84 |
| Relevant labs | | | |
| Hemoglobin (g/dL, mean ± SD) | 10.6 ± 1.4 | 10.5 ± 1.5 | 1 |
| Serum creatinine (mg/dL, mean ± SD) | 7.8 ± 2.3 | 8.1 ± 2.1 | 0.93 |
| CRP (mg/L, mean ± SD) | 40.2 ± 4.3 | 40.5 ± 4.7 | 0.97 |
| Serum albumin (g/dL, mean ± SD) | 2.6 ± 0.6 | 2.5 ± 0.5 | 0.96 |
| GFR (ml/min/1.73 m ² , mean ± SD) | 9.2 ± 3.1 | 9.0 ± 2.8 | 0.96 |
| Anesthesia distribution, % (cases) | | | |
| General anesthesia | 7.3 (30) | 7.5 (9) | 0.95 |
| Spinal anesthesia | 18.5 (76) | 18.8 (22) | 0.96 |
| Local anesthesia with spontaneous ventilation | 74.2 (304) | 73.7 (87) | 0.96 |

complications, postoperative infections, hospital stay duration, readmissions, and mortality — has been incorporated into the manuscript. However, it is important to emphasize that our primary study endpoints, notably catheter patency and ease of subsequent kidney transplantation surgery, revealed significant differences favoring the one-sided approach (Sharba method). Specifically:

— **significantly better catheter patency.** Our findings clearly demonstrate a statistically significant higher success rate of catheter revision with the one-sided technique (95 vs. 33.33 %; P-value = 0.0126). This is clinically relevant because it maintains the contralateral abdominal side free of adhesions or previous surgical trauma, thereby preserving a clear surgical field for subsequent interventions if needed;

— **superior ease for transplant surgeons.** Feedback from transplant surgeons consistently indicated that the one-sided technique provided easier surgical access and fewer adhesions, directly facilitating kidney transplantation surgery. Even though surgeon feedback was subjective, it clearly underscores a practically meaningful clinical advantage of the one-sided method in preserving a surgical field free from catheter-induced adhesions. We appreciate the editor's emphasis on clearly reporting adverse events. A structured, detailed summary (Table 3) highlighting intraoperative complications, postoperative infections, hospital stay duration, readmissions, and mortality is provided below. The structured account clearly illustrates the favorable profile of the laparoscopic (one-sided) technique compared to traditional methods, based on our previous comprehensive retrospective analysis.

This structured analysis clearly demonstrates the superiority of the one-sided laparoscopic CAPD technique (Sharba method) regarding:

— **significantly higher early and late catheter patency rates** (99 vs. 93 % early patency);

— **reduced postoperative complications**, including notably lower incidences of infections, migration, and leakage;

— **readmissions:** Fewer readmissions, reflecting clinical and economic advantages;

— **higher success rates of revision interventions** (95 vs. 33.33 %), ensuring continued functionality and preserving the contralateral abdomen from surgical trauma and adhesions, beneficial for potential future interventions (e.g., kidney transplantation);

— **intraoperative complications.** Minimal (rare vascular injuries, mild bleeding events controlled intraoperatively, organ injuries very rare) because in our method camera site at left palmer site which is highly safe area in contrast to ordinary method.

Thus, our data robustly support the one-sided laparoscopic method as safer and more efficient, substantially decreasing patient morbidity compared to the traditional two-sided laparoscopic technique.

To assess the difference between the two modalities in the two main comparisons:

1) transplanted surgeon feedback as in Table 1. As a result our approach gets better feedback by one more scale however with no significant difference ($p = 0.245$);

2) an early failure for the one-side technique was 6 while 8 were for the two-side technique, all underwent revision with no difference in time difference or technique the same catheter with omentopexy or fixation or both was done for all. On the other hand, late patency differed in that 78 failed (7 early so a total of 85), of those (78) twenty of them underwent revision while those with the two-sided technique (21), six of them underwent revision as seen in Tables 4, 5.

Table 3. Detailed structured account of adverse events comparing CAPD techniques

| Adverse events | One-sided laparoscopic CAPD (Sharba method) | Traditional two-sided laparoscopic CAPD | P-value |
|--|--|--|----------|
| Early postoperative complications (within 30 days) | Patency: 99 % | Patency: 93 % | 0.66 |
| | Early closure: ~ 1 % | Early closure: ~ 1 % | 1 |
| | Infection: 11.38 % | Infection: ~ 18 % | 0.22 |
| | Catheter migration: 11.84 % | Catheter migration: ~ 17 % | 0.33 |
| | Fluid leakage: 0 % | Fluid leakage: ~ 5 % | 0.025 |
| | Obstruction: 13.7 % | Obstruction: ~ 20 % | 0.27 |
| | Peritonitis: 0.5 % | Peritonitis: ~ 3 % | 0.18 |
| | Complications: 57.4 % | Complications: ~ 70 % | 0.26 |
| Late postoperative complications (after 30 days) | Lower incidence of obstruction, infection, migration, fluid leakage success rate of 95 % | Higher rate of late catheter dysfunction and lower revision success rate of 33.33 % | < 0.0001 |
| Hospital stay duration (mean days ± SD) | 1.2 ± 0.9 days | 1.6 ± 1.2 days | 0.81 |
| Readmissions (within first 6 months) | 8 % (primarily due to catheter malfunction requiring revision) | 16 % (primarily due to catheter-related infection and migration) | 0.1 |
| Mortality (during study period) | 3 deaths intraoperatively overall (among 410 patients), 0.73 % , primarily from medical causes unrelated directly to CAPD technique | 1 death among 118 intraoperatively, 0.85 % . Mortality similar to the one-sided technique, not directly attributable to technique | 0.92 |

Table 4. Comparison between Sharba method and two-sided technique laparoscopic CAPD in relation to patency

| Technique | Total No. | Early patency | Late patency |
|----------------|-----------|---------------|--------------|
| Sharba method | 410 | 403 | 325 |
| Two-sided | 118 | 110 | 89 |
| p-value | | 0.72 | 0.75 |

Table 5. Comparison between Sharba method and two-sided technique laparoscopic CAPD in relation to success of revision

| Technique | Total No. | Another side | Same side | Failed to revision | Success, % |
|-------------------------------------|-----------|--------------|-----------|--------------------|--------------|
| Sharba method | 20 | 17 | 2 | 1 | 95 |
| Two-sided | 6 | 1 | 1 | 4 | 33.33 |
| Chi-square: 6.128; p = 0.012 | | | | | |

Discussion

In the discussion of our study comparing the one-sided laparoscopic CAPD catheter insertion technique to the traditional two-sided approach, several key findings have emerged that merit deeper analysis. Our results indicate that the one-sided technique, which offers a simplified, minimally invasive approach, is associated with a comparable if not superior outcome in terms of catheter longevity, complication rates, and revision success when compared to the two-sided approach.

Previous studies have highlighted the benefits of laparoscopic approaches in PD catheter placement, including enhanced visualization and reduced postoperative complications [9–11]. However, our findings suggest that the one-sided method further simplifies the procedure by reducing the number of ports, which in turn lowers the risk of adhesions and subsequent complications, an issue that has been well documented in the literature.

In terms of early and late catheter patency, our study found no significant difference in early and late failure rates between the one-sided and two-sided techniques ($p = 0.72367361$ and 0.75424304 , respectively). Although patency rates are no significant difference between the two methods, the significantly higher success rate of catheter revision in the one-sided group (95 versus 33.33 %, highly significant statistically P -value 0.01264579) underscores the potential of this technique to enhance patient outcomes, particularly in those requiring long-term peritoneal dialysis. Feedback from nephrological surgeons involved in kidney transplants further supports the advantages of the one-sided approach. While the difference in surgeon preference was not statistically significant ($p = 0.24530$), the trend suggests a slight favorability toward the one-sided technique, possibly due to the ease of accessibility and reduced procedural time. This is consistent with earlier studies that have shown that simplified surgical approaches can lead to better patient outcomes and surgeon satisfaction [12].

Additionally, the one-sided approach may offer distinct advantages in terms of reducing postoperative adhesion formation, which is a significant concern in patients who may require future abdominal surgeries or kidney transplants.

This is particularly relevant for patients undergoing PD for prolonged periods, as adhesion-related complications can severely affect catheter function and patient morbidity [13].

Our study adds to the growing body of evidence supporting minimally invasive approaches to CAPD catheter placement. The one-sided laparoscopic technique simplifies the procedure and improves long-term outcomes, reduces the need for catheter revision failure, and potentially enhances the experience for both surgeons and patients. Further studies, particularly randomized controlled trials, must confirm these findings and assess their generalizability across different patient populations.

Recent guidelines, meta-analyses, and landmark studies comparing single-port, traditional two-port, and multi-port techniques have been cited explicitly [1, 3, 4, 12].

The discussion now explicitly outlines why the one-sided approach (Sharba method) is potentially superior, emphasizing:

- **reduced operative complexity** (fewer ports, less manipulation, lower risk of adhesions);

- **enhanced long-term catheter patency** and significantly better revision success rates (statistically significant findings demonstrated in our study);

- **improved cost-effectiveness**: fewer procedural materials, potentially shorter operative times, and reduced postoperative complications or readmissions.

This justification has been explicitly articulated to demonstrate clear clinical utility and cost benefits, supported by references to recent global literature.

The retrospective design and lack of protocol registration are acknowledged limitations that may introduce bias. The findings should be considered hypothesis-generating and require prospective validation.

We acknowledge the subjectivity concern raised by the editor. Initially, we utilized a four-point subjective scoring system. To address this concern, we have now clarified the scoring mechanism by adding clear definitions for each response category (1–4), including objective criteria related to accessibility, surgical time, and ease of dissection. Additionally, we suggest in our limitations section that future studies may benefit from structured external validation by blinded

assessment from independent transplant surgeons to reduce subjectivity bias. For further information about other techniques of peritoneal dialysis read recently published works [14–22].

Conclusions

Our study demonstrates that the one-sided laparoscopic CAPD catheter insertion technique offers a viable and potentially advantageous alternative to the traditional two-sided approach. The one-sided technique provides several benefits, including fewer postoperative complications, better feedback from transplant surgeons, and higher success rates in catheter revision, as evidenced by the comparison of patency rates and revision success between the two methods.

Key findings include:

— Transplanted surgeon feedback slightly favored the one-sided technique, though the difference was not statistically significant ($p = 0.24$).

— The one-sided approach resulted in a lower early failure rate (6 vs. 8 in the two-sided technique) and demonstrated superior late patency outcomes. However, we could not improve it statistically or practically that it is related.

— The revision success rate was significantly higher in the one-sided technique (95 %) compared to the two-sided technique (33.33 %) ($P = 0.012$)

Practical recommendation

While further long-term studies are needed to confirm these findings, our results suggest that this innovative approach may improve outcomes for patients undergoing laparoscopic CAPD, particularly in minimizing complications and enhancing catheter longevity.

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Метод Шарби: інноваційний односторонній лапароскопічний підхід до встановлення катетера при безперервному амбулаторному перитонеальному діалізі

Резюме. Актуальність. Мета дослідження: оцінити, чи можуть модифікації хірургічної техніки підвищити довговічність перитонеального катетера. На нашу думку, цей показник може бути поліпшений двома різними шляхами. Запропонований метод полегшує введення другого катетера контрлатерально і може мати переваги у сфері трансплантації нирок (хоча більшість процедур нині виконується ретроперитонеально), а також при інших нефрологічних хірургічних втручаннях завдяки відсутності бокового хірургічного доступу та зниженому ризику утворення спайок, особливо за потреби внутрішньочеревних втручань. **Матеріали та методи.** Дослідження охоплює 410 випадків одностороннього лапароскопічного введення катетера для безперервного амбулаторного перитонеального діалізу (БАПД) у період з 2018 року по грудень 2022 року з даними, зібраними в нашому центрі в м. Наджаф (Ірак). Додатково проведено порівняльне опитування щодо 118 випадків застосування традиційного двостороннього лапароскопічного методу БАПД різними хірургами в іншому центрі того ж міста. Наша техніка періодично вдосконалювалася для усунення ускладнень, які виникали з часом, хоча основні етапи залишалися незмінними. Методики інших хірургів як в Україні, так і за кордоном мають певні відмінності, хоча в окремих аспектах є схожими. Було зібрано відгуки хірургів-трансплантологів стосовно пацієнтів, які згодом перенесли трансплантацію нирки, що дозволило порівняти підходи й оцінити післяопераційні ускладнення, яких вдалося уникнути завдяки нашому методу. **Результати.** У до-

слідженні проаналізовано віковий розподіл пацієнтів, яким лапароскопічно встановлено катетер для БАПД (діапазон від 8 місяців до 85 років); наймолодші пацієнти були виключені через неповні дані. За відгуками, дещо кращі результати отримано при використанні одностороннього методу, хоча статистично значущої різниці не виявлено ($p = 0,24530$). Рівень ранніх невдач був вищим у групі двостороннього методу, тоді як пізні порушення прохідності катетера частіше траплялися при односторонньому методі, що здебільшого не було пов'язано з технікою. Також оцінювали показники ревізій та їх успішність — продемонстровано значно вищий рівень успіху для одностороннього підходу (95 %) порівняно з двостороннім (33,33 %). **Висновки.** Наші результати свідчать про те, що односторонній лапароскопічний метод введення катетера для БАПД є перспективною альтернативою традиційному двосторонньому підходу. Він демонструє кращі показники пізньої прохідності та значно вищу успішність ревізій. Хоча за відгуками хірурги-трансплантологи дещо схиляються на користь одностороннього методу, статистично значущої різниці не виявлено. Для підтвердження цих висновків необхідні подальші довгострокові дослідження, однак запропонована техніка може сприяти зменшенню ускладнень та поліпшенню довговічності катетера.

Ключові слова: лапароскопічний безперервний амбулаторний перитонеальний діаліз; домашній перитонеальний діаліз; стратегія введення перитонеального катетера; термінальна стадія ниркової недостатності; доступ для перитонеального діалізу

DOI: <https://doi.org/10.22141/2307-1257.14.3.2025.540>Mohammed Hussam Mohammed Saeed¹, Zainab Abdul Hammed Ibrahim²¹College of Medicine, University of Tikrit, Iraq²College of Pharmacy, University of Tikrit, Iraq

A comparative study between N-acetylcysteine and L-carnitine in the management of male infertility (placebo-controlled trial)

Abstract. Background. Male infertility is a globally recognised health condition, which is mainly reported in the age range of 35–39 years. Males from at least three in six couples are impacted by infertility on a global scale. Research evidence reveals the therapeutic benefits of L-carnitine and N-acetylcysteine for infertile males; however, the current evidence is limited by high heterogeneity in contemporary studies. Therefore, this study aimed to investigate the influence of these treatments on sperm parameters and the semen's malondialdehyde level and total antioxidant capacity. **Materials and methods.** This randomised placebo-controlled clinical trial deployed 180 male patients with normal female factor and idiopathic oligoasthenoeratozoospermia from January 2021 to December 2024. The patients in the age group of 25–40 years were randomised into L-carnitine (1000 mg oral dose), N-acetylcysteine (oral dose), and placebo (sugar sachets) treatments. The baseline data included the patient's age and sperm parameters (including sperm motility, sperm concentration, sperm morphology, and semen volume). The semen parameters in the study groups were re-investigated after six months of the study intervention. Additionally, total antioxidant capacity and malondialdehyde levels in the semen were evaluated before and after the treatment administration. **Results.** The six-monthly analysis revealed that the sperm parameters, including sperm motility (38 and 38 vs. 4%), morphology (30 vs. 29 vs. 7%), and concentration (25 and 24 vs. 2%), significantly improved with the administration of N-acetylcysteine and L-carnitine, respectively, in comparison to the placebo. However, semen volume (6 and 5 vs. 4%) was not impacted by either treatment and did not differ significantly from the placebo group ($p > 0.05$). Compared to pretreatment, N-acetylcysteine monotherapy improved the total antioxidant capacity (1.92 ± 0.12 vs. 2.61 ± 0.12 ; $p = 0.01$) and reduced the level of malondialdehyde (2.46 ± 0.11 vs. 1.85 ± 0.10 ; $p = 0.01$) in the semen. However, in comparison to the placebo, these improvements were not observed with L-carnitine monotherapy. **Conclusions.** The oral L-carnitine and N-acetylcysteine treatments effectively improved sperm concentration, morphology, and motility in male patients with infertility. However, as a single-agent therapy, no statistically significant differences were observed between the outcomes of N-acetylcysteine and L-carnitine. In addition, N-acetylcysteine appeared superior to L-carnitine in reducing the oxidative stress and malondialdehyde levels in the seminal plasma. Prospective studies should identify the mechanisms underlying the efficacy of L-carnitine/N-acetylcysteine and evaluate the safety and effectiveness of combination antioxidant treatments against male infertility.

Keywords: male infertility; N-acetylcysteine; L-carnitine; motility; morphology; total antioxidant capacity; malondialdehyde; sperm; semen

Introduction

Male infertility is globally reported in at least three in six couples and is predominantly impacted by societal and cultural attributes [1, 2]. Nearly 3×10^5 disability-adjusted life years were observed in 55 million infertile males across the

world in 2021 [1]. Male infertility is mainly reported in the age range of 25–40 years [1, 3]. While a significant increase in age-standardised rates of male infertility is specifically reported in Eastern Europe, high-severity cases are observed in Eastern European and African regions. Male infertility

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increases the risk of deterioration in psychological well-being, reduction in family/community integrity, and an overall decline in population health [4]. The prevalence of male infertility reciprocates with sociodemographic index, age, and location. The regions such as Southeast Asia, South Asia, and Saharan Africa with reduced sociodemographic index have an elevated burden of male infertility [1, 3]. The past three decades have witnessed an unprecedented increase in the worldwide occurrences of male infertility due to insufficient assisted reproductive interventions, unhealthy lifestyles, sexually transmitted diseases, and health-inappropriate environments [5]. The psychosocial outcomes of male infertility include selective sharing of infertility diagnosis, relationship crises, emotional turbulence, and changes in perspectives toward society [6].

A decline in male reproductive health is indicated by low semen quality [3]. The abnormal semen attributes include the increased time of sperm liquefaction, reduced volume of ejaculation, low motility of the sperm, and decline in semen count and motility. However, the sperm of the infertile males may still appear normal in morphology. It is important to note that the aetiology of > 30 % of male infertility cases is not yet determined [7, 8]. Furthermore, nearly 30 % of males with infertility are impacted by idiopathic oligoastheno-teratozoospermia (iOAT) [9]. The predominant causes of iOAT include hormonal changes, environmental effluents, and mutations in the mitochondrial DNA and the genome of the gamete [10]. The morphology, motility, and concentration of the sperm in infertile males are predominantly impacted by apoptosis and in scenarios when the seminal plasma and tubules are overburdened with reactive oxygen species [8]. Infertile males with iOAT are affected with less than 4 % of normal sperm (teratozoospermia), less than 32 % of progressively motile sperm (asthenozoospermia), and less than 15 million/ml sperm concentration (oligozoospermia) [7].

Additionally, male infertility is associated with reduced sperm function under the influence of oxidative stress [11]. The sperm of infertile men lose their motility and fluidity due to the deterioration of their membrane via lipid peroxidation. The deterioration of offspring outcomes and infertility are also triggered by a significant decline in the genetic integrity of the sperm due to its DNA fragmentation via reactive oxygen species [12]. The impacted sperm fails to fertilise the egg due to low motility and disfigurement of its structured proteins, caused by protein oxidation. Pollution and smoking (environmental factors), dysfunction of the mitochondria, and activity of the leukocyte are the potential causes of the sperm's oxidative stress in infertile males [13]. Of note, since the repair processes in the spermatozoa are limited, it is prone to deterioration in spite of the antioxidant defences in the body [14]. This is why lifestyle modification and antioxidant therapies are highly recommended to subdue the adverse impact of oxidative stress on the function of the sperm. The natural antioxidants with the ability to counter oxidative stress in the sperm include copper, selenium, zinc (micronutrients), carnitine, coenzyme Q10, pantothenic acid, glutathione, vitamin B complex, vitamin C, vitamin E, and vitamin A

[15]. In asthenozoospermic men, the administration of oral antioxidants helps minimise oxidative damage by neutralising the reactive oxygen species [16]. They also normalise the seminal plasma's oxidation-reduction potential, reverse leukocytospermia, and minimise the DNA fragmentation in the sperm [17, 18].

The antioxidant potential of the glutathione and cysteine precursor, N-acetylcysteine, is due to its ability to facilitate antioxidant signalling, replenishment of glutathione, and scavenging of oxidants [19]. The orally administered N-acetylcysteine (11 g admixed with H₂O (300 ml)) reaches its maximum plasma concentration (27 µg/ml) in two hours. N-acetylcysteine has a protein binding capacity of 66–87 % and a steady state distribution volume of 0.47 L/kg [20]. This drug has the ability to form conjugates, disulfide, and cysteine [20]. The mean clearance and mean half-life of N-acetylcysteine are 0.11 L/hour/kg and 7 hours, respectively [20]. Research evidence from a meta-analysis reveals that in idiopathic infertile males, the morphology of the sperm, sperm motility, ejaculate volume, and concentration of the sperm can be enhanced with the daily administration of oral N-acetylcysteine [21]. Another piece of evidence from a randomised-controlled study states that orally administered N-acetylcysteine resulted in a noticeable post-treatment decline in protamine deficiency, DNA fragmentation, abnormal sperm morphology, and a marked elevation in sperm motility and sperm count [22].

Similarly, the antioxidant L-carnitine is known for its ability to act on the inside of the mitochondrial membrane and facilitate long-chain fatty acid transport [23]. It also supports recovery in athletes after exercise and helps to enhance their performance. Research has revealed the possible role of L-carnitine in improving testicular function by minimising the concentration of reactive oxygen species [24]. Findings from a recent retrospective analysis revealed the potential of oral L-carnitine (2000 mg) in improving embryo quality and sperm attributes. L-carnitine also helps to treat astheno-teratozoospermia, which may minimise the use of assisted reproduction technology [25]. A recent case study analysed the outcomes of asthenozoospermic patients after modifying their lifestyles, adding antioxidants to their diets, and administering oral L-carnitine (3000 mg) per day for a month's duration [26]. The posttreatment assessment indicated a 3–7 % improvement in normal sperm morphology, elevation in sperm count (from 25 to 49 million/ml), and a significant enhancement in sperm motility (from 15 to 50 %) [26]. These results restated the possible role of L-carnitine in improving fertility parameters, oxidative stress, and the function of mitochondria in the sperm.

The contemporary studies have not acquired any consensus on the comparative evidence concerning the efficacy of N-acetylcysteine and L-carnitine against male infertility. A meta-analysis of studies focusing on idiopathic asthenozoospermia indicated a significant elevation in the volume of the ejaculate and concentration of the sperm in infertile males who were treated with N-acetylcysteine [27]. In addition, L-carnitine treatment enhanced the volume of the ejaculate and the concentration of the sperm. Contrarily,

another umbrella analysis of clinical trials and real-world studies revealed that N-acetylcysteine lacks the potential to enhance the rates of pregnancy and sperm count and can only increase the concentration of sperm cells with normal shape along with their swimming patterns [28]. A recent meta-analysis revealed yet another contradictory evidence regarding the role of both N-acetylcysteine and L-carnitine in improving pregnancy outcomes as well as morphology, motility, and concentration of the sperm (i.e., all sperm attributes) in idiopathic infertile males [29]. These gaps in the highly heterogeneous contemporary evidence warrant the organisation of randomised controlled studies to investigate the clinical effectiveness of L-carnitine and N-acetylcysteine in infertile adult men. Accordingly, this study aimed to evaluate the efficacy and outcomes of orally administered N-acetylcysteine and L-carnitine in young adult males with a definitive diagnosis of infertility.

Materials and methods

Participants

This randomised placebo-controlled clinical trial began in January 2021 and continued till December 2024 in the Saladin province of Iraq. One hundred eighty male participants with a laboratory-confirmed diagnosis of infertility were recruited in this study. The patients were randomised into three equal groups (n = 60 each). The first group of patients (n = 60) received a 1000 mg oral dose of L-carnitine. The second group was treated with N-acetylcysteine; however, sugar sachets (or placebo treatment) were administered to the third group. Each of the study groups received their respective treatments exclusively for a duration of six months.

Inclusion and exclusion criteria

Adults aged 25–40 years with normal female factor and iOAT were included in this study. Alternatively, those with obstructive azoospermia, varicocele, scrotal tumours, genital trauma, or genital infection were excluded from this analysis. Notably, patients below 25 years and above 40 years of age were also excluded from this clinical trial.

Data collection and statistical analysis

At the start of the study (or day 0), the baseline data were collected after analysing the semen samples from all participants. Age, marriage time, job type, and habits were additionally recorded from each patient through interactive interviews. Following the initial six months of the study

treatments, the semen samples were recollected for subsequent assessment. The laboratory analysis of the study samples required a maximum time of 15 minutes. The WHO 2021 parameter guided the interpretation of the study samples [30]. The Kruger criteria were utilised to investigate the semen morphology [31]. The authors used Windows SPSS (version 26.0) for data analysis. The significance of the results from the chi-square, Kruskal-Wallis, and Mann-Whitney U tests was determined with the probability value reference ($p < 0.05$) [32, 33].

Ethical parameters

All study procedures and interventions were thoroughly explained to each of the study participants. The objectives and concerns regarding the study were categorically explained to the enrollees. Interactive discussions were organised to address and resolve the concerns and questions of the participants regarding the research study. All the research participants provided written informed consent for the study. The ethical approval for the study was obtained from the local Institutional Review Board.

Study procedures

A Doppler study was performed on all study participants to rule out varicocele. Pre- and post-treatment assessments of seminal plasma were undertaken through precipitation and centrifugation techniques. Malondialdehyde and total antioxidant capacity were evaluated to reveal the oxidative stress and the antioxidant defence of the semen in each participant.

Results

Table 1 depicts age, sperm motility, sperm concentration, sperm morphology, and semen volume in each of the study groups before the study treatments. The mean age of the participants was 30 years, and the percentage of normal motility (grade a + grade b) ranged from 23 to 24 %. The sperm concentration varied from 47.2 to 48.4 million/ml across the study groups. The percentage of normal morphology as per Kruger criteria ranged from 7 to 8 % between the N-acetylcysteine, L-carnitine, and placebo groups. The semen volume fluctuated from 2.4 to 2.5 ml across the participants. Notably, no statistically significant differences were observed in each of the patient domains between the study groups before the study initiation ($p > 0.05$).

Table 2 provides post-intervention data regarding the sperm parameters after six months of the study initiation.

Table 1. Patient domains before the start of the therapy

| Domains | N-acetylcysteine (n = 60) | L-carnitine (n = 60) | Placebo group (n = 60) | P value |
|--|---------------------------|----------------------|------------------------|---------|
| Age (years) | 30.2 ± 9.2 | 30.1 ± 9.6 | 30.2 ± 8.6 | > 0.05 |
| % of normal motility (grade a + b) | 23 | 24 | 23 | > 0.05 |
| Sperm concentration (million/ml) | 48.4 ± 12.3 | 47.2 ± 10.1 | 47.5 ± 8.2 | > 0.05 |
| % of normal morphology (Kruger criteria) | 7 | 8 | 8 | > 0.05 |
| Semen volume (ml) | 2.54 ± 1.23 | 2.51 ± 0.85 | 2.42 ± 0.92 | > 0.05 |

Compared to the placebo group, N-acetylcysteine and L-carnitine groups had statistically significant improvements in sperm motility ($p = 0.01$), morphology ($p = 0.03$), and concentration ($p = 0.01$). However, improvements in the semen volume did not significantly differ between the treatment and placebo groups ($p > 0.05$). Furthermore, the findings did not reveal statistically significant differences in outcomes between the N-acetylcysteine monotherapy and the L-carnitine monotherapy.

Table 3 and the corresponding Fig. 1 depict malondialdehyde levels and total antioxidant capacity in the semen of the study participants before and after the administration of study treatments. The results revealed a significant increase in total antioxidant capacity in patients treated with N-acetylcysteine ($p = 0.01$). They also indicated a significant decline in malondialdehyde levels after N-acetylcysteine treatment. Alternatively, L-carnitine therapy did not significantly influence the semen malondialdehyde levels and total antioxidant capacity in the respective patients ($p \geq 0.05$).

Discussion

The six-monthly analysis revealed that the sperm parameters, including sperm motility, morphology, and concentration, significantly improved with the administration of N-acetylcysteine and L-carnitine to male patients with infertility. However, semen volume was not impacted by either treatment and did not differ significantly from the placebo group. These outcomes indi-

cate the effectiveness of N-acetylcysteine and L-carnitine monotherapies in improving the overall quality of semen in infertile males. Another noticeable finding from this study was that N-acetylcysteine monotherapy improved the total antioxidant capacity of the semen and reduced the level of malondialdehyde in the semen. However, in comparison to the placebo, these improvements were not observed with L-carnitine monotherapy.

Findings from this study support the outcomes of an umbrella assessment of the randomised controlled studies that indicated the effectiveness of orally administered N-acetylcysteine in terms of enhancing normal morphology, sperm motility, and sperm concentration [21]. However, the results contradicted the outcome, stating that

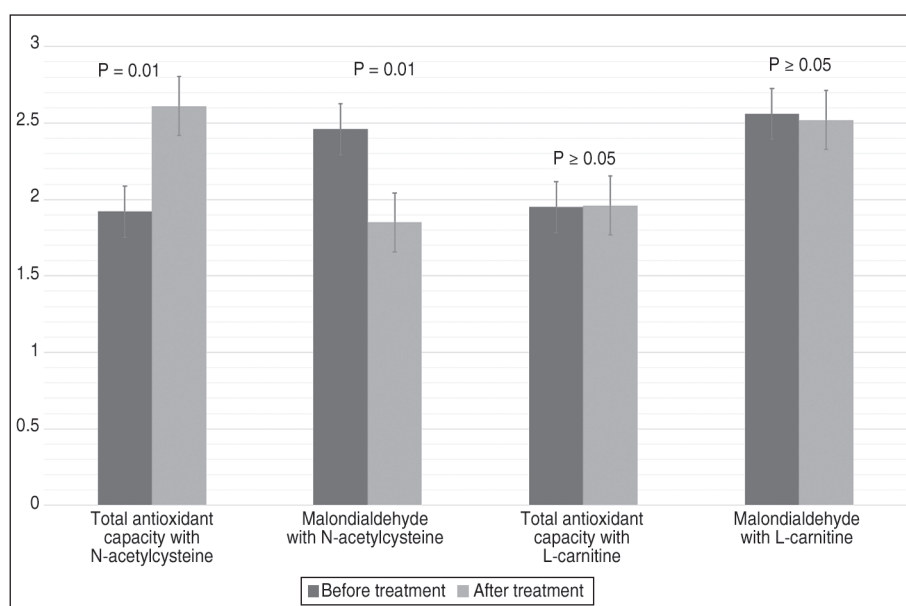


Figure 1. Malondialdehyde and total antioxidant capacity before and after treatment

Table 2. Improvements in motility, morphology, and concentration of the sperm by the end of the six months of the study, %

| Domains | N-acetylcysteine (n = 60) | L-carnitine (n = 60) | Placebo group (n = 60) | P value |
|---------------------------|---------------------------|----------------------|------------------------|---------|
| Motility improvement | 38 | 38 | 4 | 0.01 |
| Morphology improvement | 30 | 29 | 7 | 0.03 |
| Concentration improvement | 25 | 24 | 2 | 0.01 |
| Volume improvement | 6 | 5 | 4 | > 0.05 |

Table 3. The levels of malondialdehyde and total antioxidant capacity before and after treatment

| Domains | Before treatment | After treatment | P value |
|-------------------------------|------------------|-----------------|---------|
| TAC with NAC (n = 60) | 1.92 ± 0.12 | 2.61 ± 0.12 | 0.01 |
| MAD with NAC (n = 60) | 2.46 ± 0.11 | 1.85 ± 0.10 | 0.01 |
| TAC with L-carnitine (n = 60) | 1.95 ± 0.12 | 1.96 ± 0.14 | ≥ 0.05 |
| MAD with L-carnitine (n = 60) | 2.56 ± 0.12 | 2.52 ± 0.11 | ≥ 0.05 |

N-acetylcysteine successfully improved the ejaculate volume in males with a diagnosis of infertility. A randomised clinical study by Jannatifar et al. aligned with the current findings, stating that marked improvements in sperm count and sperm motility were observed in infertile males following their treatment with N-acetylcysteine [22]. However, this study emphasised the therapeutic benefits of N-acetylcysteine for infertile males with a month's treatment duration. Another randomised study by Ciftci et al. contradicted the current finding by revealing a significant increase in semen volume in infertile males after treating them with 600 mg oral N-acetylcysteine per day. The results from this study, however, supported our outcome concerning the improvement in sperm motility with N-acetylcysteine therapy. The additional findings revealed significant improvement in semen viscosity and a decline in serum reactive oxygen species in infertile male patients who underwent N-acetylcysteine treatment. The result contradicted our finding by indicating that oral N-acetylcysteine failed to improve sperm count and sperm morphology in males with infertility. Another randomised analysis by Safarinejad et al. advocated the possible role of the combination therapy of N-acetylcysteine and selenium in enhancing the overall semen quality, indicated by improved normal morphology of the sperm, sperm motility, and mean sperm concentration [34]. However, these results did not substantiate the sperm quality improvement ability of N-acetylcysteine when administered exclusively in the absence of combination therapy. The observations from this study support the outcomes from the systematic review of 84 studies that substantiate the role of N-acetylcysteine in elevating testicular function and spermatogenesis [35]. In contrast to the current belief, an *in vitro* experimental study indicated the possible role of prophylactically administered N-acetylcysteine in elevating the DNA fragmentation and immature chromatin level in the sperm of males with infertility [36]. The findings from this study also revealed an elevation in cellular antioxidant glutathione and oxidative levels following the N-acetylcysteine therapy, which also altered the cytotoxic effects of etoposide and other similar chemotherapeutic agents in sperm. These results indicate the possibility of an alternative mechanism of action (other than a DNA-based mechanism) of N-acetylcysteine on the human sperm.

A contemporary prospective analysis by Nazari et al. revealed an improvement in semen quality (i.e., sperm morphology) in infertile men who were treated with L-carnitine (1500 mg)-based supplementation of antioxidants [37]. However, contrary to our findings, the antioxidant therapy did not significantly improve sperm motility. Observations from another contemporary randomised clinical trial indicated that three-monthly treatment with L-carnitine-based antioxidants improved sperm motility and reduced the DNA fragmentation index [38]. However, the results negated any influence of L-carnitine treatment on DNA decondensation in the sperm and enhancement in the sperm parameters, such as vitality, count, and volume. Furthermore, L-carnitine-oriented therapy also enhanced the live

birth and clinical pregnancy rates in the participants. A meta-analysis of seven studies indicated that L-carnitine can effectively enhance sperm morphology, progressive sperm motility, and total sperm motility [28]. However, findings from this assessment denied any role of L-carnitine in improving the overall concentration of sperm in males with idiopathic infertility. These findings are supported by a review article that revealed the ability of L-carnitine to minimise oxidative damage of the sperm by the reactive oxygen species, enhance the functional and conventional sperm parameters, and safeguard the testes [39]. Another randomised-controlled study revealed a progressive decline of the sperm DNA fragmentation index with three months of treatment with L-carnitine and enhancement of sperm vitality, progressive motility, and volume with six months of therapy [40]. The authors of this study linked progressive motility of the sperm with elevated concentrations of alpha-glucosidase and seminal carnitine. They further correlated > 10 % sperm motility with a reduced DNA fragmentation index [40]. They suggested the positive role of micronutrients and metabolic attributes in improving fertility rates in male populations. Except for the literature findings concerning the semen volume improvement, our study reaffirmed the effectiveness of L-carnitine in enhancing sperm parameters.

A review paper supports our findings concerning the potential of N-acetylcysteine in improving the sperm's total antioxidant capacity, which helps to minimise oxidative damage of DNA and restore the sperm's motility [41]. A similar result was revealed by a network meta-analysis that provided evidence concerning the role of N-acetylcysteine and L-carnitine combination in reversing oxidative stress [42]. However, this finding contradicts our results that negate the improvement in total antioxidant capacity of the semen with N-acetylcysteine, while excluding the L-carnitine treatment. Of note, the authors of this study also revealed the beneficial effects of other antioxidants, such as vitamin C, vitamin E, zinc, selenium, omega-3 fatty acids, and coenzyme Q10, on sperm parameters. However, this analysis was beyond the scope of the current study. Other contemporary studies describe carnitines as potential energy sources with the capacity to neutralise free radicals in the semen [43]. Our results dispute these findings by refuting any significant influence of L-carnitine monotherapy on the semen's total antioxidant capacity.

The reduction of malondialdehyde with N-acetylcysteine in our study correlates with the plausible role of this supplement in minimising epididymal lipid peroxidation, thereby improving lipid metabolism in the testes. The possible mechanisms of this improvement include the enhancement of the level of oxidative enzymes and the NRF2 gene with oral N-acetylcysteine treatment [44]. These findings reaffirm the antioxidant capacity of N-acetylcysteine that helps to reverse the sperm's oxidative deterioration and enhance the sperm's membrane integrity, chromatin consistency, morphology, and viability [45]. The findings from this study are against the general perception of the ability of L-carnitine to reduce the malondialdehyde levels in the testes. Contemporary studies advocate the role

of L-carnitine in improving the overall activity of the antioxidant system and minimising oxidative stress responses in the testicular environment [46, 47]. Since our results do not contradict the protective effect of L-carnitine on the sperm parameters, no impact of L-carnitine monotherapy on malondialdehyde level in the current study warrants further investigation.

The oxidative stress in the testes develops with a significant decline in the antioxidant enzymes/antioxidants, which increases the risk and incidence of the deterioration of the sperm's DNA, motility, membrane integrity, and membrane fluidity [48]. The oxidative stress compromises the DNA structure and function by increasing the concentration of reactive oxygen species. L-carnitine is known to minimise sperm apoptosis, enhance mitochondrial function, protect the integrity of the plasma membrane, and improve lipid metabolism [49]. The antioxidant properties of L-carnitine and N-acetylcysteine indicate their ability to alter the oxidation mechanism based on the transport of the products of beta-oxidation toward the Krebs cycle via mitochondria. Both oral treatments possibly minimise the intracellular reactive oxygen species concentration in the testes [48]. Scientific literature depicts the role of N-acetylcysteine in improving the antioxidant potential of catalase, glutathione peroxidase, and other similar antioxidant enzymes that help to counter the rising levels of reactive oxygen species in the male reproductive environment [48, 50]. Notably, the reactive oxygen species are produced with increased mitochondrial oxidative phosphorylation. The N-acetylcysteine and L-carnitine combination controls the intracellular reactive oxygen species accumulation that eventually minimises the mitochondrial membrane potential and improves sperm motility [22]. The literature results emphasise the need to adjust and optimise the dosages of N-acetylcysteine and L-carnitine to improve sperm parameters [51]. However, to date, there is no consensus on the dosage and duration of these treatments for male patients with infertility. The possible mechanism concerning sperm motility improvement with L-carnitine and N-acetylcysteine is that the combination treatment ceases the superoxide anion reduction, reduces DNA deterioration, and enhances spermatogenesis and sperm metabolism [48].

The International Society of Sports Nutrition dietary supplement classification's third category includes L-carnitine as an antioxidant supplement [52]. The acetyl L-carnitine and L-carnitine exist in free form in the male epididymis. The maximum concentration of L-carnitine is found in the lower epididymis, and it facilitates fertilisation and maturation of the sperm. In infertile males, the seminal plasma's L-carnitine concentration and content are reduced in comparison to those of fertile males [23]. Literature provides evidence regarding a positive association between sperm concentration and L-carnitine levels [53]. Importantly, glutathione is produced from the increased accumulation of L-carnitine, which is transformed from the orally administered N-acetylcysteine. This glutathione plays a pivotal role in minimising oxidative stress by reducing the accumula-

tion of free radicals [54]. Additionally, the sulfur group in the untransformed N-acetylcysteine effectively neutralises the free radicals, which further lowers the oxidative stress. The contemporary evidence also advocates the role of N-acetylcysteine in reducing stress in the endoplasmic reticulum and countering the infiltration of neutrophils [52]. This exogenous antioxidant utilises these mechanisms to sustain the epididymis' antioxidant properties and maintain the oxidative balance in the testes. However, the acetylated form of L-carnitine not only controls lipid peroxidation and DNA damage but also enhances the mitochondrial activity and the ATP content in the sperm [55]. The L-carnitine achieves these benefits by improving beta-oxidation in mitochondria through the increased transport of fatty acids across its inner membrane. The increased provision of energy eventually enhances the motility of the sperm. The mechanisms governing the mitochondrial activity and ATP production improvement by L-carnitine relate to the entrapment of additional acetyl-coenzyme A by acetyl-L-carnitine [52]. The deficit of acetyl-CoA helps to improve the citric acid cycle and pyruvate dehydrogenase capacity. L-carnitine uses its sodium-potassium pump and balances the energy production and homeostasis in mitochondria by facilitating the partial exclusion of sodium chloride [56]. Future studies are warranted to further understand and unravel the intricate mechanism of action of N-acetylcysteine and L-carnitine in relation to their sperm parameter/quality improvement potential.

Limitations

Despite its randomised placebo-controlled design, this study is not devoid of noticeable limitations. First, the single-centre analysis and limited sample size restrict the generalizability of outcomes across wider male populations with infertility diagnoses. Second, the limited (i.e., six months) follow-up duration and no assessment of the fertility hormones such as prolactin, follicle-stimulating hormone, luteinizing hormone, and testosterone in patients with N-acetylcysteine/L-carnitine and placebo treatments impact the reliability of outcomes. Finally, this study did not evaluate the dose-dependent outcomes and mechanism of action of N-acetylcysteine and L-carnitine, which require further assessment through prospective studies.

Conclusions

This study revealed the effectiveness of orally administered L-carnitine and N-acetylcysteine in improving sperm parameters, including sperm concentration, morphology, and motility. The findings revealed no significant statistical difference between the use of N-acetylcysteine and L-carnitine as a single-agent therapy. Compared to L-carnitine treatment, N-acetylcysteine had a significantly greater role in achieving total antioxidant capacity and lowering the seminal plasma malondialdehyde levels. Future studies should evaluate the mechanisms underlying the therapeutic effects of oral L-carnitine and oral N-acetylcysteine in infertility and reinvestigate the role of these treatments in improving semen volume.

Recommendations

The findings from this study advocate the use of L-carnitine/N-acetylcysteine monotherapy in improving sperm quality in patients with iOAT. N-acetylcysteine can be the preferred adjunctive treatment for infertility in males based on its greater potential to manage oxidative stress. Future randomised-controlled studies should aim at evaluating the role of combination antioxidant therapies in improving fertility rates across the male population.

Ethical approval

All ethical requirements and supporting documentation were fully adhered to and applied as part of the research process.

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Порівняльне дослідження N-ацетилцистеїну та L-карнітину в лікуванні чоловічого безпліддя (плацебо-контрольоване дослідження)

Резюме. Актуальність. Чоловіче безпліддя — це всесвітньо визнаний стан здоров'я, який спостерігається переважно у віковому діапазоні 35–39 років. У глобальному масштабі чоловіки шонайменше з трьох із шести пар страждають від безпліддя. Дослідницькі дані показують терапевтичні переваги L-карнітину та N-ацетилцистеїну в безплідних чоловіків, однак наявні дані обмежені високою гетерогенністю сучасних досліджень. Тому **метою** було вивчити вплив цих методів лікування на параметри сперми, рівень малонового діальдегіду в ній та загальну антиоксидантну здатність. **Матеріали та методи.** У цьому рандомізованому плацебо-контрольованому клінічному дослідженні з січня 2021 року по грудень 2024 року взяли участь 180 пацієнтів чоловічої статі з нормальним жіночим фактором та ідіопатичною олігоастенотератозооспермією. Особи вікової групи 25–40 років були рандомізовані для лікування L-карнітином (1000 мг перорально), N-ацетилцистеїном (перорально) та плацебо (цукрові пакетики). Базові дані включали вік пацієнта та параметри сперми (рухливість, концентрацію, морфологію сперматозоїдів та об'єм сперми). Параметри сперми в групах були повторно вивчені через шість місяців дослідження. Крім того, загальну антиоксидантну здатність та рівень малонового діальдегіду в спермі оцінювали до та після лікування. **Результати.** Шестимісячний аналіз показав, що параметри сперми, включаючи рухливість сперматозоїдів (38 та 38 проти 4 %), їхню

морфологію (30 і 29 проти 7 %) та концентрацію (25 і 24 проти 2 %), значно поліпшилися при застосуванні відповідно N-ацетилцистеїну та L-карнітину порівняно з плацебо. Однак об'єм сперми (6 та 5 проти 4 %) не зазнав впливу жодного з методів лікування й суттєво не відрізнявся від групи плацебо ($p > 0,05$). Порівняно з попереднім лікуванням монотерапія N-ацетилцистеїном поліпшила загальну антиоксидантну здатність ($1,92 \pm 0,12$ проти $2,61 \pm 0,12$; $p = 0,01$) та знизила рівень малонового діальдегіду ($2,46 \pm 0,11$ проти $1,85 \pm 0,10$; $p = 0,01$) у спермі. Однак порівняно з плацебо ці поліпшення не спостерігалися при монотерапії L-карнітином. **Висновки.** Пероральне лікування L-карнітином та N-ацетилцистеїном ефективно поліпшувало концентрацію, морфологію та рухливість сперматозоїдів у чоловіків із безпліддям. Однак при використанні N-ацетилцистеїну та L-карнітину як монотерапії статистично значущих відмінностей не було. Крім того, N-ацетилцистеїн виявився кращим за L-карнітин у зниженні оксидативного стресу та рівня малонового діальдегіду в плазмі сперми. Проспективні дослідження повинні визначити механізми, що лежать в основі ефективності L-карнітину/N-ацетилцистеїну, та оцінити безпеку й ефективність комбінованого антиоксидантного лікування чоловічого безпліддя. **Ключові слова:** чоловіче безпліддя; N-ацетилцистеїн; L-карнітин; рухливість; морфологія; загальна антиоксидантна здатність; малоновий діальдегід; сперматозоїди; сперма

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The level of blood lead, zinc and relationship with the metallothionein gene polymorphism in chronic kidney failure

Abstract. Background. Chronic kidney disease is defined by renal damage or an estimated glomerular filtration rate less than 60 ml/min/1.73 m². Lead is a ubiquitous environmental factor that can contribute to lengthy clinical complications in individuals with chronic kidney disease. They can be exposed to changes in zinc homeostasis. The MT2A gene also expresses a wide range of physiological and pathological effects.

Materials and methods. This study involved 60 blood samples from individuals with kidney disease on hemodialysis, and 60 samples from apparently healthy individuals as a control. The purpose was to identify the molecular character of the genotype of the MT2A gene SNP (A>G) (rs28366003) in a cohort of chronic kidney disease subjects and apparently healthy controls. **Results.** Blood lead and zinc serum levels were compared between patients and healthy controls by flame atomic absorption spectrophotometry. Lead contents were significantly and considerably higher, with significant differences ($p > 0.01$) between the patient cohort and the healthy controls, while serum zinc was significantly decreased. Males are more affected than females with chronic kidney disease, and individuals older than 40 years had a greater risk of complications. Hypertension has a meaningful positive relation to chronic kidney disease, and it is therefore considered a possible risk factor. The rs28366003 A>G genotype associated with increased risk of kidney disease in Iraqi patients demonstrated considerable variation. The median age of kidney disease patients was 20 to 69 years. Genotypes and allele frequencies of rs28366003, A>G in the kidney disease population: 51.7 % ($n = 31$) were wild-type (AA), 33.3 % ($n = 20$) were heterozygous (AG) and 15 % ($n = 9$) were homozygous (GG). The allele frequencies of A and G were 68.3 and 31.7 %. **Conclusions.** Thus, the drop in zinc levels and the harmful increase in blood lead in chronic kidney failure patients who possess SNP variants of the MT2A gene, specifically rs28366003, may be involved in kidney disease susceptibility.

Keywords: chronic kidney disease; metallothionein gene polymorphism; lead exposure, zinc deficiency; gene SNP rs28366003; hemodialysis

Introduction

Chronic kidney disease (CKD) is a serious threat to global health as more than two million of us get CKD annually [1]. It is a primary source of illness and mortality globally and is becoming more acknowledged as a global public health concern, particularly in developing nation [2]. There are five stages of chronic renal failure (CRF) based on glomerular filtration rate (GFR), and stage 5, is also called end-stage renal disease (ESRD). CKD is present when damage to the kidney exists with persistence for approximately three months or longer; it also poses an es-

pecially large burden in low- and middle-income countries [3]. It is now recognized that even slight changes in kidney structure and function are associated with increased risk of mortality and implications for other organ systems [4]. Dialysis therapies replace some functions of the healthy kidney by removal of fluid and waste products [5]. Maintenance hemodialysis is considered a life sustaining treatment for patients with such disease. It also requires adherence to recommended attendance for hemodialysis, guidelines for dietary and fluid intake, and adherence to medication regimens to perpetuate its benefits [6]. In the population

of patients receiving maintenance hemodialysis, nutritional therapy focuses on ensuring an appropriate intake of protein and calories [7]. Major causes of CKD are diabetes, hypertension, chronic glomerulonephritis, chronic pyelonephritis, chronic use of anti-inflammatory medications, autoimmune diseases, polycystic kidney disease and long-term acute renal disease [8]. Zinc (Zn) is a trace element involved in multiple physiological functions in the body. It is essential for cell viability, growth, and replication, and the activation of 300 or more enzymes [9]. A deficiency of trace elements, particularly Zn, can lead to cardiovascular disease; lower serum Zn levels were associated with increased premature death and diminished physical activity [10]. Zinc stimulates the production of metallothionein, which are proteins that are effective in diminishing hydroxyl radicals and sequestering any reactive oxygen species (ROS) produced during stress conditions [11]. Lead has a high affinity for low-molecular-weight proteins which are easily filtered through the glomerulus then reabsorbed in proximal tubules, establishing primary tubular toxicity. This may culminate into albuminuria and progressive kidney disease towards ESRD [12]. The first identified case of Pb-related nephrotoxicity was reported in the 19th century. Since then, exposure to high concentrations of Pb has been regarded as an environmental risk factor for hypertension and kidney injury [13]. Metallothionein is a protein rich in cysteine with large amounts of metals that can be found in all organisms. Apart from heavy metal detoxification, metallothionein is one of the most powerful antioxidants where it is capable of regulating and mediating several cellular processes [14]. Any mutation of this gene will affect the function metallothionein proteins increasing the effects of heavy metals. ROS, the SNP A-G (rs28366003) in promoter of metallothionein gene, A to G allele conversion appearance, thereby decrease transcription qualification [15]. The location of the SNP A-G (rs28366003) in promoter of a metallothionein gene, this polymorphism exists away of five base pairs upstream of their initiation site; this polymorphism includes changing an A nucleotide to G nucleotide in promoter of MT gene, later caused a decrease in production of metallothionein and a decrease in the role of metallothioneins to protect the cell from dangerous products of metabolism, exercise or cellular injury [16].

Materials and methods

Study design

From December 2020 to March 2021, blood samples were collected from patients with CKD under hemodialysis and a healthy control group. A total of 7 ml of blood was taken from each patient, 5 ml in plain vacutainer tubes and 2 ml in EDTA. DNA was extracted from the Iraqi Dialysis Center and the Medical City Center. The study consisted of

120 blood samples from two groups as follows: group 1, 60 samples of Iraqi patients with chronic kidney disease, and group 2, 60 samples from healthy controls which included no history of kidney disease. We prepared a questionnaire with information including age, chronic diseases, blood pressure, and gender.

Ethical commission

The study was approved by Scientific Research Commission, Research and Technology Center of Environment, Water and Renewable Energy and Institute of Genetic Engineering and Biotechnology, University of Baghdad (No. 335 in 22/1/2025).

Survey administration

Verbal consent was obtained from patients undergoing hemodialysis sessions and they were asked whether they agreed to participate in the current study. If they agreed, the survey questions were explained to the patients as they completed the research questionnaire.

Exclusion criteria

Patients under 20 years old, patients who need immunosuppressive treatment for kidney disease and patient with polycystic kidney disease

Lead and zinc measurement

5 ml of blood collected in plain vacutainer tubes were used to determine the concentrations of zinc and the blood lead with flame atomic absorption spectrophotometry (FAAS), an analytical technique used to determine the concentration of metal. It utilizes the principle of atomic absorption, where excited atoms in a flame absorb light at specific wavelengths, leading to a decrease in the intensity of the transmitted light.

Molecular methods

DNA extraction. 2 ml in EDTA (Ethylene Diamine Tetracetic Acid) blood samples from patients and controls were kept at -20° C. ReliaPrep™ Blood gDNA Miniprep System was utilized to extract genomic DNA. In the case of DNA purity genotyping of polymorphism metallothionein (rs28366003) by using high resolution melting (HRM). Used master mixes were containing EVA-Green, HRM Master Mix Synthetic SNP sequences. The DNA was extracted, using DNA extraction kit EasyPure® Genomic (TransGen Biotech, EE101-01) (Fig. 1). Primer sequences were designed according to their reference sequence (rs) in the National Center for Biotechnology Information database (NCBI) in Table 1 forward-primer CTTGGGATCTC-CAACCTCAC and the reverse-primer ACTTCTCTGAT-GCCCCTTTG the thermal cycle in Table 2.

Table 1. Specific primers for MT single nucleotide polymorphisms

| | Primer sequence (5'-3') | Primer size, bp | Product size, bp |
|--------------|-------------------------|-----------------|------------------|
| F-rs28366003 | CTTGGGATCTCCAACCTCAC | 20 | 200 |
| R-rs28366003 | ACTTCTCTGATGCCCTTTG | 20 | 200 |

Analysis of data

The Statistical Package for the Social Sciences (2019) program was utilized to determine the effect of difference groups on study variables [17]. For the normally distributed quantitative data of study groups, parametric (independent samples t-test, ANOVA, standard error) were used. Qualitative data (categorical variables) were presented as the frequency (percentage), and the significant differences between their distributions in study groups were evaluated by a chi-square test of independence (χ^2 -test) or Fisher's exact tests, where applicable. The allelic and genotypic frequencies were calculated using direct gene counting method. The differences were determined by chi-square test of independence, odds ratios (ORs) and its 95% confidence interval (CI) were estimated to determine the association MT2A SNPs with chronic kidney failure and treatment response using WINPEPI program for epidemiologists (2002, 2020).

Ethics statement

This study was approved by the Ethics Committee of the Institute of Genetic Engineering and Biotechnology for postgraduate studies, all participants gave informed consent, the study followed the Declaration of Helsinki principles.

Results

Age and hypertension

Table 3 illustrates the chronic kidney patients aged 50 to 59 had the highest prevalence of 43.33 % when compared with other groups while only 1.67 % of patients within age group 20–29 and 6.67 % with in age group 30–39 and 15 % of patients within age group 40–49 while in age group above 60 years, 33.33 %. There are highly significant differences between the incidences of the different age groups among chronic kidney disease patients ($p < 0.01$) (Table 2).

Another study found there was no difference between the ages of patients compared to healthy people, and this study does not agree with our study [18]. Also, Table 3



Figure 1. Genomic DNA gel electrophoresis for 9 samples on agarose gel with a concentration of 1 % for 70 min and 70 V

Table 2. The cycling protocol

| Step | Temperature | Time, sec | Cycles |
|-------------------|-------------|-------------|--------|
| Enzyme activation | 94 | 30 | 1 |
| Denaturation | 94 | 10 | 40 |
| Annealing | 60 | 15 | |
| Extension | 72 | 20 | |
| HRM | 55–95 | 0.5 for 1 ° | |

Table 3. Distribution of sample study according to age, gender and hypertension in different groups

| Variables/factors | | Patients (N = 60) | | Controls (N = 60) | |
|-------------------|----------|-------------------|-------|-------------------|-------|
| | | Abs. | % | Abs. | % |
| Age (years) | 20–29 | 1 | 1.67 | 9 | 15.00 |
| | 30–39 | 4 | 6.67 | 13 | 21.67 |
| | 40–49 | 9 | 15.00 | 22 | 36.67 |
| | 50–59 | 26 | 43.33 | 9 | 15.00 |
| | ≤ 60 | 20 | 33.33 | 7 | 11.67 |
| | Total | 60 | 100 | 60 | 100 |
| | p-value | 0.0001** | | 0.0174* | |
| Sex | Male | 31 | 51.67 | 36 | 60.00 |
| | Female | 29 | 48.33 | 24 | 40.00 |
| | p-value | 0.796 NS | | 0.121 NS | |
| Hypertension | Positive | 48 | 80.00 | 14 | 23.33 |
| | Negative | 12 | 20.00 | 46 | 76.67 |
| | p-value | 0.0001** | | 0.0001** | |

Notes: * — $p \leq 0.05$; ** — $p \leq 0.01$; NS — non-significant.

showed there was a very significant ($p \leq 0.0001$) difference between the same group 96 % of patients in CKD group had hypertension patently. and this result concurs with [19] who recorded hypertension prevalence (80 %) in CKD.

Lead and zinc

Table 4 showed significant decrease between the level of zinc in patients and control group the mean of the Zn in the patients 63.86 ± 1.06 , while the control 99.41 ± 2.01 and this result agree with [20]. Also, the result shown the high significant increase in lead in the CKD patients ($p \leq 0.01$) the mean of the CKD patients 23.05 ± 0.75 while the control group 16.50 ± 0.42 and this result agree with [21].

SNP polymorphism MT2A gene

A single nucleotide polymorphism of *MT2A* gene in this study the genotypes and allele frequencies of SNP rs28366003 A>G in Hardy-Weinberg equilibrium (HWE) in patient with kidney disease groups 51.7 % ($n = 31$) wild (AA), and 33.3 % ($n = 20$) heterozygous (AG) and 15 %

($n = 9$) homozygous (GG). The genotypes and allele frequencies (HWE) in healthy control was 36 % ($n = 60$) wild (AA), and 40 % ($n = 24$) heterozygous (AG) and 0 % ($n = 0$) homozygous (GG). Allele frequencies for A and G were 68.3 and 31.7 %, respectively, as shown in Table 5.

DNA samples of all study groups were genotyped of *MT2A* SNP (rs28366003), detection was achieved by using HRM real-time PCR. The resulting output of thermocycler of the HRM analysis process for SNP (rs28366003) three genotypes is shown in Fig. 2.

The relationship between *MT2A* rs28366003 and blood lead and zinc

As shown in Table 6, there was no significant difference in Zn in the group of patient, control and the rs28366003 while observed group of patients is a significant difference in the value of lead.

Discussion

Age and hypertension

CKD is a primary source of illness it is slow, steady progression characterizes it and is irreversible [22]. Age, sex and socioeconomic status are considered to be influential in the development, progression and outcomes from CKD [23]. The prevalence of chronic kidney disease increases markedly with old age. The reason for this because the patients with advanced age may suffer from systemic and chronic diseases, stress, and an increasing rate of catabolism. Moreover, elderly patients suffer from a decrease in the glomerular filtration rate (GFR) due to diseases that complicate aging, such as atherosclerosis and high blood pressure. In addition, there is an increase in catabolism rates and a decrease in metabolism

Table 4. Comparison between different groups in Zn and Pb

| Group | Mean \pm SE | |
|----------|------------------|------------------|
| | Zn | Pb |
| Patients | 63.86 ± 1.06 | 23.05 ± 0.75 |
| Controls | 99.41 ± 2.01 | 16.50 ± 0.42 |
| T-test | 15.6 | 7.6 |
| p-value | 0.0001* | 0.0001* |

Note: * — $p \leq 0.01$.

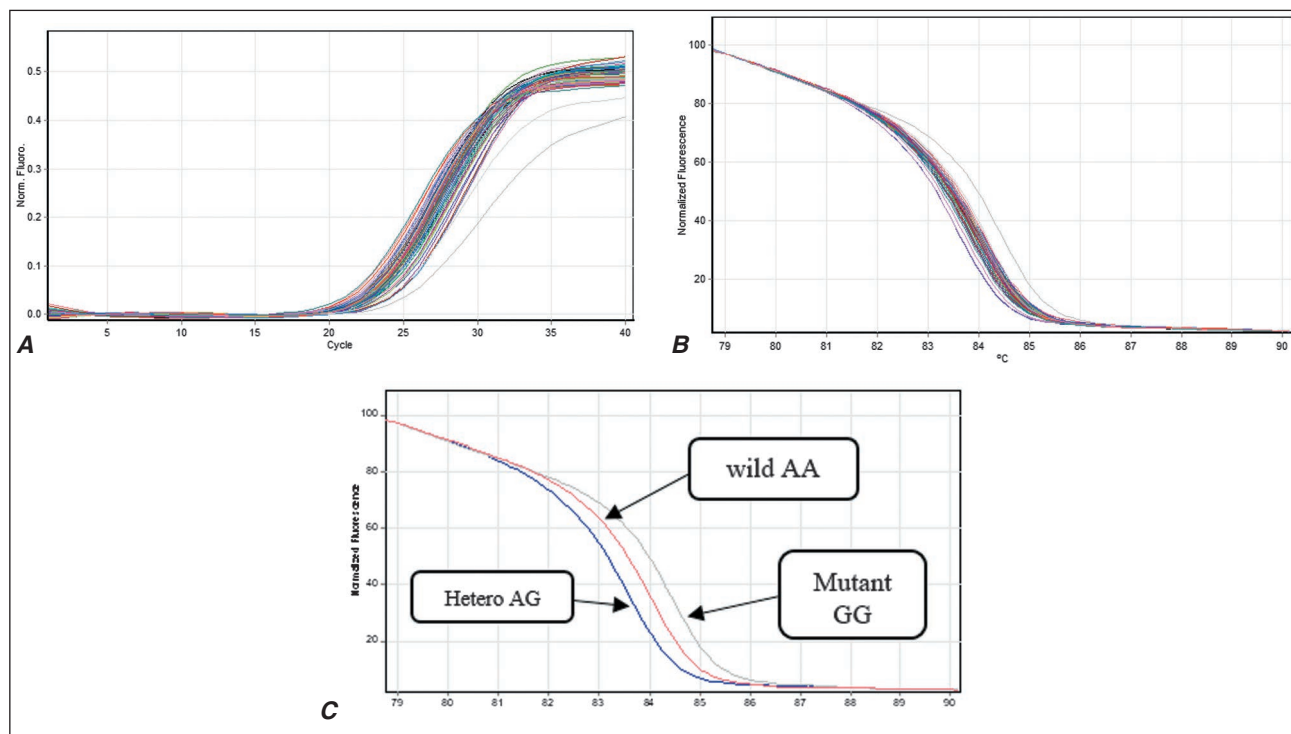


Figure 2. The genotype result for SNP rs28366003: A, B — the HRM result, C — the wild, heterozygous and mutant genotypes

rates, which makes the elderly vulnerable to many chronic diseases and health problems [24]. The encompassing factors of male sex hormones, gender neutrality of occupation role, stress expressed as performance, and chronic disease distribution all contribute to the fact that males are at a higher risk of CKD than female gender. In addition, differences in lifestyle (e.g. smoking cigarettes; drinking alcohol) might also help explain this gender gap [25]. Of the highest prevalence of chronic kidney disease, the female gender was the greatest risk factor for chronic kidney disease. This could be based on biological differences between men and women in glomerular structure; glomerular hemodynamics; muscle mass; and hormone metabolism [26]. Hemodialysis patients had high blood pressure (hypertension) immediately after their first time experiencing hemodialysis. Patients undergoing dialysis often experience high blood pressure — difficult to treat and causes a range of adverse effects including increased risk of heart disease [27]. Hypertension is one of the leading factors for the development of CKD, given the adverse effects of enhanced blood pressure on kidney vasculature. Over the long-term, uncontrolled high blood pressure translates into high intraglomerular pressures that lead to impairment in glomerular filtration. Damage to the glomeruli lead to an increase in protein filtration, resulting in abnormally increased amounts of protein in the urine [28].

Lead and zinc

Zn, the second among the most critical trace elements in the human body, is crucial in regulating cellular and

subcellular processes across various tissues. Zn deficiency is linked to the advancement of CKD and related consequences. As CKD progresses to ESRD [29]. Zinc deficiency is a risk factor for ESRD and indicate association between zinc deficiency and organ damage due to fibrosis. Thus, zinc deficiency may affect kidney function via oxidative stress and fibrosis. Zn concentrations are decreased in CKD [30]. Increased zinc excretion causes zinc deficiency in patients with kidney diseases as well as in those on hemodialysis. CKD patients have higher urinary zinc excretion, which tends to increase as the CKD stage progresses [31]. Chronic kidney disease is susceptible to zinc deficiency, which may be caused by an inadequate dietary intake due to uremia-related anorexia and dietary restriction, reduced gastrointestinal zinc absorption, adsorption of zinc by phosphate binders, and removal of zinc by dialysis procedure [32] lead (Pb) binding to low-molecular-weight proteins, probably enters kidney proximal tubule cells through endocytosis. It seems to inhibit kidney mitochondria’s respiratory function inside the cells, which causes the formation of oxidative stress, reactive oxidative species, and intracellular depletion of glutathione [33]. Exposure to Pb is linked to an elevated CKD risk as assessed by proteinuria, and a greater risk of decreased GFR [34].

SNP polymorphism MT2A gene

Single nucleotide polymorphisms (SNPs) are the most common type of variation in the human genome. The vast majority of SNPs identified in the human genome do not

Table 5. Comparison of the genotype and allele frequency of SNP (rs28366003) between patients and controls, n (%)

| | Genotype | Controls | Patients | χ^2 | OR (95% CI) | p-value |
|----------|--------------|----------|-----------|----------|--------------------|---------|
| Genotype | AA reference | 36 (60) | 31 (51.7) | 0.03 | 1.00 (reference) | 0.9 NS |
| | AG | 24 (40) | 20 (33.3) | 0.47 | 0.9 (0.4471–2.065) | 0.9 NS |
| | GG | 0 (0) | 9 (15) | 9.1 | 22.02 (1.2315–.59) | 0.03* |
| | Total | 60 | 60 | | | |
| Allele | A reference | 96 (80) | 82 (68.3) | 0.011 | 1.00 (reference) | 0.92 |
| | G | 24 (20) | 38 (31.7) | 4.3 | 1.8 (1.044–3.273) | 0.03* |
| | Total | 120 | 120 | | | |

Notes: * — $p \leq 0.05$; NS — non-significant.

Table 6. Relationship between genotype of rs28366003 SNP with Zn and Pb in patients and control groups

| Group | Genotype | Mean ± SE, ppm | |
|----------|------------------|-------------------|----------------------------|
| | | Zn | Pb |
| Patients | AA | 65.16 ± 1.45 | 22.19 ± 1.21 ^a |
| | AG | 63.30 ± 2.04 | 17.85 ± 0.81 ^b |
| | GG | 61.55 ± 1.62 | 19.67 ± 1.20 ^{ab} |
| | L.S.D. (p-value) | 5.839 NS (0.4549) | 3.948 (0.0261)* |
| Controls | AA | 96.94 ± 2.17 | 18.05 ± 0.55 |
| | AG | 99.62 ± 3.23 | 17.67 ± 0.61 |
| | L.S.D. (p-value) | 7.491 NS (0.4767) | 1.678 NS (0.6445) |

Notes: means with the different letters in same column differed significantly; * — $p \leq 0.05$; NS — non-significant.

have any effect on the phenotype; however, some can lead to changes in the function of a gene or the level of its expression. Moreover, determination of associations of genetic variants with a disease does not provide information about the functionality of these variants, which is necessary to elucidate the molecular mechanisms of the development of pathology and to design effective methods for its treatment and prevention. Further scrutiny into the functionality of such SNPs will assist us in better understanding the associated differences between individuals and facilitate the development of alternative therapies targeting different groups of individuals with different SNP profiles [35]. Chronic kidney disease is a progressive disease that results from kidney damage and results in loss of kidney function. Genetic factors, such as allelic variants, can contribute to this disease. [36]. As is known, this mutation in the promotor of the metallothionein gene in the site A-G (rs28366003) lead to a defect in the function of the main protein that coding by metallothionein gene, this protein have able to combined the different type of heavy metals together by thiol group, and then remove it's from the body by filtration in the kidney and secreted with the urine, and because this mutation the heavy metals accumulate in the body of the patient and causes many health problems [37]. The effect of *MT2A* A-5G polymorphism in a general Japanese population, we observed significant associations with CKD the GG genotype was identified as a risk factor for CKD. The ORs for *MT2A* A-5G genotypes were statistically significant independently of age, sex and other potential confounders. This suggests that the vulnerability to these diseases due to *MT2A* A-5G polymorphism is independent of their major risk factors. *MT2A* A-5G may be associated with CKD and this polymorphism is a promising target for evaluations of CKD and with possible involvement of low-dose chronic exposure to environmental pollutant [38].

The relationship between *MT2A* rs28366003 and blood lead and zinc

Environmental pollution causes an increase in levels of heavy metals in the organism. Due to this mutation in the promotor of the metallothionein gene, the protein cannot remove all the amount of heavy metals from the body and consequently increase the toxicity of heavy metals [39]. Highly statistically significant associations were detected between the -5 A/G core promoter region SNP in the *MT2A* gene and Pb Individuals with the GG genotype had statistically lower Zn level and higher Pb levels in the blood samples than individuals with AA and AG genotypes. This study suggests that having the GG genotype individuals may be more sensitive for the metal toxicity and they should be more careful about protecting their health against the toxic effects of the heavy metals no detect changes in serum Zn level for the GA or GG genotype in population consider that *MT2A* A-5G polymorphism likely affects intracellular homeostasis rather than contributing to excretion of related molecules outside the cells [40].

Conclusions

This study showed that decreased zinc levels in patients with CKD are correlated with an elevated concentration of lead as a result of kidney's diminished ability to eliminate

lead because of the decreased activity of metallothionein genes. In our study there was positive correlation between the GG genotype and the kidneys disorders; this shows us that male carried GG genotype have higher risk to suffer kidney problems.

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Рівень свинцю й цинку в крові та зв'язок із поліморфізмом гена металотіонеїну при хронічній нирковій недостатності

Резюме. Актуальність. Хронічна хвороба нирок визначається пошкодженням нирок або розрахунковою швидкістю клубочкової фільтрації менше 60 мл/хв/1,73 м². Свинець є повсюдним фактором навколишнього середовища, що може призводити до тривалих клінічних ускладнень у людей із хронічною хворобою нирок. Вони можуть зазнавати змін у гомеостазі цинку. Ген MT2A також має широкий спектр фізіологічних та патологічних ефектів. **Матеріали та методи.** У цьому дослідженні вивчено 60 зразків крові від осіб із захворюваннями нирок, які перебувають на гемодіалізі, та 60 від практично здорових осіб (контрольна група). Мета: визначити молекулярний характер генотипу SNP (A>G) (rs28366003) гена MT2A у пацієнтів із хронічною хворобою нирок та в контрольній групі. **Результати.** Рівні свинцю в крові та цинку в сироватці крові порівнювали в обох групах за допомогою полум'яної атомно-абсорбційної спектроскопометрії. Уміст свинцю в крові був значно вищим, із суттєвими відмінностями ($p > 0,01$) між когортою пацієнтів та здоровими особами, тоді як рівень цинку в сироватці крові був значно знижений. Чоловіки частіше страждають на хронічну

хворобу нирок, ніж жінки, а пацієнти старше 40 років мали більший ризик ускладнень. Виявлено значущий позитивний зв'язок гіпертензії із хронічною хворобою нирок, тому її вважають можливим фактором ризику розвитку останньої. Генотип rs28366003 A>G, пов'язаний із підвищеним ризиком захворювання нирок в іракських пацієнтів, продемонстрував значну варіабельність. Медіанний вік осіб із хворобами нирок становив від 20 до 69 років. Генотипи та частота алелів rs28366003, A>G у популяції із захворюваннями нирок: 51,7 % ($n = 31$) були дикого типу (AA), 33,3 % ($n = 20$) — гетерозиготними (AG) та 15 % ($n = 9$) — гомозиготними (GG). Частота алелів А та G становила 68,3 та 31,7 %. **Висновки.** Таким чином, зниження рівня цинку та шкідливе підвищення рівня свинцю в крові пацієнтів із хронічною нирковою недостатністю, які мають варіанти SNP гена MT2A, зокрема rs28366003, можуть бути пов'язані зі схильністю до хвороб нирок.

Ключові слова: хронічна хвороба нирок; поліморфізм гена металотіонеїну; вплив свинцю; дефіцит цинку; SNP гена rs28366003; гемодіаліз

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The role of shilajit in reducing the toxicity of monosodium glutamate on liver enzyme and kidney functions in albino mice

Abstract. Background. A flavor enhancer that sees extensive usage in the food business is monosodium glutamate (MSG). While many studies have shown that long-term consumption of MSG can cause oxidative stress in animals, especially in their liver and kidneys, it was the goal of this study to examine the biochemical effects of hepatitis and kidney inflammation caused by different doses of MSG and the protective effect of shilajit water extract in albino mice. This research is designed to assess the biochemical toxicity of various dosages of MSG on the kidney and liver function in albino mice. **Materials and methods.** Fifty adult mice were randomly assigned to one of five groups (10 animals each). In contrast to the experimental group (G2) that received MSG at a dose of 2 g/kg body weight, the control group (G1) received pure water. The third group (G3) received the same amount of MSG plus 100 mg/kg of shilajit extract. In contrast to the fourth group (G4), which received a higher dose of MSG (4 g/kg body weight), the fifth group (G5) received the same amount of MSG in addition to 200 mg/kg of shilajit. The oral medications were maintained daily for a period of 14 days. On day 15, the animals were euthanized after being put to sleep. Following that, biochemical analysis was performed on the collected samples. This included testing for renal function indicators (such as creatinine and urea) and liver enzymes (such as AST, GGT, ALP, and ALT). **Results.** Compared to the control group, groups G2 and G4, which received just MSG, had a significant rise ($P \leq 0.05$) in liver enzyme levels (ALP, AST, and ALT), suggesting substantial liver damage. On the other hand, shilajit extract showed a significant decrease in these levels, suggesting that it may provide some protection against the toxicity caused by MSG. **Conclusions.** The current study found that when high doses of monosodium glutamate were administered, it caused significant disturbances in the function of both the liver and the kidneys. They were manifested by a significant increase in the levels of liver enzymes (AST, ALT, ALP, and GGT), as well as an increase in renal function indicators (urea and creatinine), which indicated that these organs had suffered tissue and functional damage as a result of excessive oxidative stress.

Keywords: monosodium glutamate; shilajit; liver; kidney; liver enzymes; renal function

Introduction

People are increasingly going against the recommendations of healthy eating programs like the DASH diet by consuming more processed foods and fast food [1]. Artificial sweeteners, hydrogenated fats, and flavor enhancers like monosodium glutamate (MSG), which is used widely in the food industry, have all seen an uptick in use due to this development [2]. Glutamic acid is abundant in both plants and animals, and it is one of the most prevalent amino acids that are not considered essential. On the other hand, Ajinomoto

is one of the terms for MSG. Magnesium stearate, or MSG, is essentially the sodium salt of glutamic acid [3]. Of its total composition, 78 % is glutamic acid and 22 % is salt and water [4]. Naturally occurring glutamate is found in a wide variety of foods, including tomatoes, milk, cheese, mushrooms, and seafood. Animal tissues may also contain glutamate. Glutamate is not only produced by the body but also plays an essential role in metabolic processes [5, 6].

MSG is a prominent component in many Asian cuisines, particularly those of China, Thailand, and Japan [7, 8]. Its

presence increases taste and stimulates appetite, making it a popular choice for cuisines in these countries. In spite of the fact that the United States Food and Drug Administration (FDA) has classified MSG as Generally Recognized as Safe (GRAS) [9], there is still a great deal of controversy among medical professionals and scientists over the possible adverse effects that it may have on human health. Investigations conducted by other researchers have shown a connection between it and harmful effects on the central nervous system, liver, and kidneys, in addition to the potential adverse effects on reproductive function. The oxidative stress, calcium imbalance, and glutamate receptor activation that occur in the brain are the mechanisms that are responsible for these consequences [10, 11].

One possible mechanism by which MSG causes neurotoxicity is via increasing the activity of N-methyl-D-aspartate (NMDA) receptors. The cascade of events begins with an overabundance of calcium ions entering neurons, which triggers cell-destructive enzymes [8, 11]. The organic component shilajit, on the other hand, is found in nature and is harvested from rocks in hilly areas like the Himalayas. Because of its many biological and pharmacological properties, shilajit has been a staple of traditional medicine for hundreds of years [12]. Among the compounds found in shilajit are those that possess anti-inflammatory effects. These chemicals have the potential to decrease the pain and other symptoms associated with inflammatory illnesses, such as arthritis and chronic musculoskeletal pain [13, 14]. With its benefits on cardiovascular health, which include improved blood circulation and management of blood cholesterol levels, shilajit may also help protect the liver from dangerous compounds by reducing oxidative stress and enhancing cell repair mechanisms, according to recent study [15]. Shilajit also has the potential to protect the liver from toxic substances. Due to the powerful antioxidant and immunomodulatory capabilities that they possess, fulvic acids and other active compounds are responsible for the majority of these effects [16].

Shilajit is an appealing natural medical drug [17], and this is due to the fact that it can lessen the negative effects that some chemical chemicals, such as monosodium glutamate (MSG), have on the kidneys, liver, and central nervous system. In light of the aforementioned, the purpose of the current inquiry is to evaluate, using albino mice serving as a model, the biochemical effects of MSG at different doses and the effectiveness of an aqueous shilajit extract in mitigating these effects, with a specific focus on markers of liver and kidney function.

Materials and methods

Methodology for the synthesis of monosodium glutamate and shilajit

In accordance with the procedures outlined in the research, distilled water was used to dissolve the monosodium glutamate (MSG) powder to achieve two concentrations: 2 and 4 g/kg body weight [18]. The two quantities of shilajit extract (100 and 200 mg/kg body weight) were achieved by dissolving tablets in distilled water [19].

Chemical composition of the shilajit extract

Shilajit is a complex mixture composed of several minerals, organic compounds, and bioactive substances. The primary component of shilajit is fulvic acid, which accounts for its unique properties. The chemical structure of shilajit can be described as follows:

1. Fulvic acid. A humic substance with a molecular weight of 5,000–10,000 Da, it is a significant part of shilajit and contributes to its therapeutic properties.

2. Minerals. Shilajit contains over 80 minerals, including iron, zinc, magnesium, copper, manganese, calcium, and potassium. These minerals are present in their ionic forms, making them more bioavailable.

3. Dibenzo- α -pyrones. These organic compounds are found in small quantities and are thought to contribute to the antioxidant properties of shilajit.

4. Other compounds. Includes amino acids, vitamins (like B-complex), and fatty acids.

Shilajit, due to its mineral and organic complexity, is known to act as a natural adaptogen and bioenhancer, improving the absorption and bioavailability of other nutrients in the body (Fig. 1).

Animal experiment design

From the National Center for Drug Control and Research's Experimental Animal Center, we procured albino mice weighing 20–30 g. Mice were kept in a typical laboratory setting with access to food and water at all times, in a moderately heated environment with adequate ventilation and a regular light/dark cycle [20].

There were 50 mice, and they were randomly put into five groups of ten mice each group:

- group 1. This group serves as the control, administered solely with distilled water;
- group 2. Administered MSG at a dosage of MSG 2 g/kg;
- group 3. Administered MSG at a dosage of 4 g/kg of body weight;
- group 4. Administered MSG at a dosage of 2 g/kg alongside shilajit at a dosage of 100 mg/kg;
- group 5. Administered MSG at a dosage of 4 g/kg in conjunction with shilajit at a dosage of 200 mg/kg.

The medicines were given orally for 14 days in a row using an oral pipette.

Sample collection and biochemical analysis

After the beginning of the 14-day treatment period, blood samples were taken from the mice and placed in tubes that

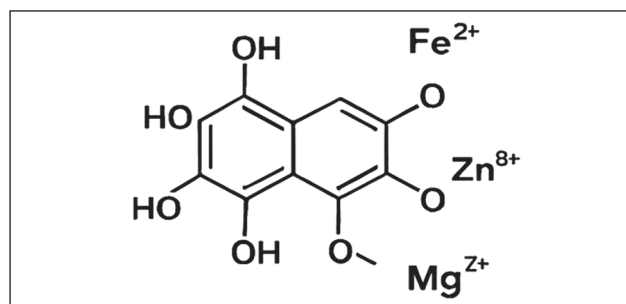


Figure 1. Chemical structure of shilajit

contained anticoagulant medication. In order to separate the serum, the samples were centrifuged for five minutes at a speed of 4,000 revolutions per minute [21]. In order to evaluate the function of the liver, the levels of the following enzymes were measured: ALT, AST, ALP, and GGT. In addition, assessments of kidney function included the measurement of urea and creatinine. Every test was carried out using ELISA kits purchased from Cusabio in the United States [22].

Statistical analysis

The results were represented using both the mean and the standard error (mean \pm SE). A one-way analysis of variance (ANOVA) was conducted to determine the least significant difference (LSD) among the groups. The LSD was found to be statistically significant at a probability threshold of $P < 0.05$ [23], suggesting that the observed difference holds statistical relevance.

Results

Liver functions

Efficacy of shilajit against the impact of monosodium glutamate on AST and GGT

The treated groups differed significantly from the control group (G1) at the probability level ($P < 0.05$), as indicated in Table 1. The second group (G2), which got 2 g/kg of MSG, and the fourth group (G4), which got 4 g/kg of MSG, both had significantly elevated AST levels. Two groups that received shilajit treatment — group 3 (G3: MSG 2 g + Shi 100 mg) and group 5 (G5: MSG 4 g + Shi 200 mg) — exhibited a marked reduction in enzyme levels when contrasted with the groups that received MSG alone. This suggests that shilajit protects against hepatotoxicity caused by MSG.

Comparing the treated groups with the control group revealed significant differences at a significance level of $P < 0.05$, according to the findings in the same table. In the fifth group (G5, which consisted of 4 grams of MSG and 200 milligrams of Shi), enzyme levels dropped significantly,

eventually matching those in the control group (G1). In contrast, the enzyme levels in the third group (MSG 2 g + Shi 100 mg, G3) were significantly higher than in the other groups, proving that medium dosages of shilajit were helpful and that low doses were ineffective in this setting.

Effectiveness of shilajit in counteracting the impact of monosodium glutamate on ALT and ALP levels

Table 2 indicates notable differences that are statistically significant at the $P \leq 0.05$ level when the treated groups are compared to the control group (G1). The table demonstrated that the fifth group (MSG 4 g + Shi 200 mg, G5) showed a significant decrease in the measured index, closely matching the values of the control group (G1). The third group (MSG 2 g + Shi 100 mg, G3) exhibited a significant increase in the same index relative to the other groups. The findings indicate that the low dose of shilajit failed to demonstrate a protective effect when compared to the effects of MSG ($P \leq 0.05$).

Table 2 presents a significant rise in the indicators for the treated groups relative to the control group, reaching a significance level of $P \leq 0.05$. Upon comparison of the treated groups, it was noted that the fifth group (MSG 4 g + Shi 200 mg, G5) demonstrated a significant reduction when contrasted with the fourth group, which received only MSG at a dosage of 4 g (G4). The third group (MSG 2 g + Shi 100 mg, G3) exhibited a significant increase relative to the second group (MSG 2 g, G2), while preserving the same level of significance ($P \leq 0.05$). The previous statistical evaluation highlights the notable protective effect of shilajit at the highest dosage (200 mg) in reducing the negative effects of MSG.

Renal function

Urea and creatinine

The information shown in Table 3 reveals significant variations at $P \leq 0.05$ when analyzing the treated groups

Table 1. Effect of shilajit versus MSG on AST and GGT levels in rat serum (mean \pm SE)

| Groups | N | AST | GGT |
|--------------------------------|----|------------------|--------------------|
| G1 (control — distilled water) | 10 | 31.03 \pm 1.20 | 161.12 \pm 6.42 |
| G2 (MSG 2 g/kg) | 10 | 34.30 \pm 1.09 | 295.67 \pm 19.40 |
| G3 (MSG 2 g + Shi 100 mg) | 10 | 33.09 \pm 0.51 | 385.33 \pm 27.40 |
| G4 (MSG 4 g/kg) | 10 | 34.72 \pm 1.15 | 193.64 \pm 5.14 |
| G5 (MSG 4 g + Shi 200 mg) | 10 | 33.31 \pm 0.91 | 159.73 \pm 9.32 |

Table 2. Effect of shilajit versus MSG on ALT and ALP levels in rat serum (mean \pm SE)

| Groups | N | ALT | ALP |
|--------------------------------|----|------------------|-------------------|
| G1 (control — distilled water) | 10 | 18.73 \pm 0.28 | 101.72 \pm 2.18 |
| G2 (MSG 2 g/kg) | 10 | 33.64 \pm 1.47 | 149.54 \pm 3.13 |
| G3 (MSG 2 g + Shi 100 mg) | 10 | 36.63 \pm 0.75 | 151.46 \pm 5.74 |
| G4 (MSG 4 g/kg) | 10 | 25.60 \pm 0.95 | 127.60 \pm 1.01 |
| G5 (MSG 4 g + Shi 200 mg) | 10 | 18.93 \pm 0.57 | 111.56 \pm 3.15 |

in relation to the control group, with urea levels showing a marked increase in the MSG-treated groups. An in-depth analysis of the groups revealed that the fifth group (MSG 4 g + Shi 200 mg, G5) exhibited a significant decrease in urea concentration relative to the other groups, reaching levels similar to those found in the control group (G1). The third group (MSG 2 g + Shi 100 mg, G3) demonstrated a significant increase in urea levels compared to the second group (MSG 2 g, G2), with consistent statistical significance ($P \leq 0.05$).

The findings presented in Table 3 indicate notable alterations in kidney function indicators when the experimental groups are compared to the control group (G1), with a significance level of $P \leq 0.05$. Both the second group (MSG 2 g, G2) and the fourth group (MSG 4 g, G4) exhibited a significant increase in the studied indicator relative to the control group, suggesting a distinct toxic effect of MSG. The third group (MSG 2 g + Shi 100 mg, G3) and the fifth group (MSG 4 g + Shi 200 mg, G5) demonstrated a significant decrease in the same indicator relative to the two MSG-only groups (G2 and G4), while preserving the same significance level ($P \leq 0.05$).

Discussion

Glutamate is an amino acid that occurs naturally in many foods in different levels. However, there is a difference between free glutamate and glutamate that is attached to proteins. Protein-bound glutamate, which is included in foods like meat and tomatoes, is not as harmful as free glutamate because it is absorbed into tissues, particularly muscle, over a longer period of time and breaks down more slowly in the gut. Compared to protein-bound glutamate, free glutamate which is included in taste enhancers like MSG — is more dangerous because it is quickly absorbed and causes a dramatic increase in blood glutamate concentrations [24]. This study's findings suggest that MSG inhibits antioxidant defenses, speeds up glucose metabolism, and increases cellular reactive oxygen species (ROS) generation, all of which harm DNA, proteins, and lipids. One of the long-term effects of MSG exposure is apoptosis, which occurs when cell membranes undergo lipid peroxidation due to the oxidation of unsaturated fatty acids. This, in turn, disrupts the structure and function of cell membranes, leading to cell death or permanent damage. This compound's free radicals degrade mitochondrial function and tamper with genetic information inside cells [9].

Exposure to environmental and dietary chemicals, such as MSG, makes the liver particularly vulnerable to damage

[25]. The liver is one of the most affected vital organs by food poisoning because of its central role in regulating metabolism, storing glycogen, synthesizing plasma proteins, producing bile (essential for fat digestion), and filtering toxins and harmful chemicals from the blood.

Consistent with other studies, this one also utilized two dosages of MSG (40 and 120 mg/kg), which had similar outcomes [26]. Functional damage to the liver was indicated by a rise in ALT and AST levels and a significant drop in total protein levels. After 28 days of MSG administration, another research found that laboratory rats' liver enzymes (AST, ALT, GGT) increased. This was thought to be because MSG exposure caused alterations in the liver's histology. Within the same framework, research [27], shown that male mice given a daily oral gavage dosage of MSG (2 g/kg) for four weeks had significantly higher body weight and blood ALT and AST levels than the control group, with a statistically significant difference at ($P < 0.001$) as recorded in [28].

Thirty found that ALT, AST, ALP, and GGT levels were significantly elevated after four weeks of treatment with MSG at a dosage of 1 mg/kg. Oxidative stress, DNA damage, and detrimental effects on liver function from PCNA and p53 protein gene expression were established. The activity of the liver cell membrane damage markers ALT and AST in serum may be used to measure MSG-induced hepatotoxicity, according to scientific research. The breakdown of cell membranes containing unsaturated fatty acids causes oxidative stress, which in turn causes enzymes normally contained in mitochondria and plasma membranes to seep into the circulation [29, 30]. Several studies have shown that MSG exposure, whether with a single large dosage [31–33], or with repeated low doses [34–36], significantly elevates ALT and AST enzymes. Our results are in line with these previous findings. All of this research showed that MSG is bad for your liver because it alters enzyme markers.

Chronic exposure to MSG causes physiological changes in the liver and kidneys, according to a study [8], which found that mice given two doses of MSG (0.6 and 1.6 mg/g of body weight) for 14 days had a marked increase in body weight and relative weight of the organs. In addition to its hepatotoxic effects, the present investigation demonstrated that MSG negatively impacts kidney function. This is shown by a significant rise in blood urea and creatinine levels, which suggest a decrease in renal efficiency [37]. Hypothesized that this rise in creatinine was due to either a decrease in renal tubular function or an interference between creatinine metabolism and MSG, which caused the latter to accumulate in the blood.

Table 3. Effect of shilajit versus MSG on urea and creatinine levels in rat serum (mean \pm SE)

| Groups | N | Urea | Creatinine |
|--------------------------------|----|------------------|-------------------|
| G1 (control — distilled water) | 10 | 25.51 \pm 1.08 | 0.390 \pm 0.009 |
| G2 (MSG 2 g/kg) | 10 | 31.29 \pm 1.32 | 0.590 \pm 0.020 |
| G3 (MSG 2 g + Shi 100 mg) | 10 | 32.63 \pm 2.18 | 0.490 \pm 0.012 |
| G4 (MSG 4 g/kg) | 10 | 28.25 \pm 1.16 | 0.520 \pm 0.018 |
| G5 (MSG 4 g + Shi 200 mg) | 10 | 23.62 \pm 0.89 | 0.490 \pm 0.010 |

A number of studies have linked the oxidative stress that monosodium glutamate (MSG) induces in renal tissue to negative impacts on kidney function. Research has shown that consuming MSG on a regular basis might lead to renal fibrosis, with oxidative stress playing a major role in kidney damage [38, 39]. The overproduction of free radicals, especially reactive oxygen species (ROS), or a breakdown in their intracellular elimination mechanisms is known as oxidative stress [40].

The development of oxidative stress inside the body is facilitated by a multitude of physiological and pathological processes, including metabolic pathways, cellular and noncellular components such as hormones and cytokines, and detoxification systems [41–43]. To put it another way, prolonged MSG exposure raises renal glutamate metabolism, which in turn increases ROS generation. Research in rats has shown that long-term exposure to MSG causes a decline in antioxidant enzyme levels and an increase in the buildup of lipid peroxidation products in the kidneys [44, 45]. High amounts of glutamate cause immediate cytotoxicity, as shown in experiments with cultivated kidney cells *in vitro* [46]. Kidney tissue is especially vulnerable to oxidative stress damage because it has a high concentration of long-chain polyunsaturated fatty acids [47]. Cell death results from a cascade of events that begin with lipid peroxidation and progress via protein modification, DNA damage, and cell death itself [48–50]. Reactive oxygen species are known to have a crucial role in producing pathological alterations in the kidneys, namely in the glomeruli, tubules, and interstitium [51, 52].

One of the main aims of the research was to find strategies to lessen or eliminate the harmful effects of MSG after the findings indicated that the fifth group did better than the control group in minimizing liver and kidney damage. According to the data, the shilajit aqueous extract is efficient because it contains physiologically active compounds such as fulvic acids, over 40 minerals, and the conjugated alpha-pyrone it releases [12]. Shilajit is a mineral supplement that includes over 20 different elements, including fulvic and humic acids, as well as minerals like calcium, magnesium, salt, iron, chromium, and lead. In addition to minerals accounting for around 15–20 % of its composition, it also includes organic substances such as hydrocarbons, proteins, carbs, fatty acids, amino acids, and alcohols. The variety of plant chemicals it contains, together with its powerful antioxidant capabilities, contribute to its great efficacy and the significant protective impact it has on human health [53].

One research found that shilajit, whether taken either orally or rectally, could lessen the severity of liver damage caused by ulcerative colitis [54]. This was accomplished by raising serum albumin levels, decreasing concentrations of direct and total bilirubin, and decreasing levels of liver enzymes (SGPT, SGOT, ALP). By bringing the control group's liver enzyme levels back to near-normal levels, oral gavage of shilajit proved to be more protective than rectal administration. This might be because shilajit's active components are better absorbed, because it acts directly on the liver before systemic effects manifest, or because it acts indirectly by regulating gastrointestinal secretions.

Keep in mind that this medication does come with a few unwanted side effects. Fulvic acid and dibenzo-alpha-pyrone are the main components of shilajit extract that are responsible for its antioxidant activities [55]. The shilajit aqueous extract showed a DPPH free radical scavenging capability of 11.9 µg/ml [56], as per an additional investigation. Additionally, shilajit's ability to scavenge free radicals was assessed by means of a rat liver culture model of oxidative stress caused by carbon tetrachloride (CCl₄), with lipid peroxidation serving as the marker for this kind of stress. The findings demonstrated that shilajit enhanced the rat model of antioxidant enzyme activity [57]. Another research looked at the effects of shilajit on liver and kidney tissue after bone cancer (osteosarcoma) spread in a rat model, and how it may work in conjunction with chemotherapy treatments to lessen those detrimental effects. The effects of two shilajit dosages (low and high) on biomarkers including bilirubin, ALT, ALP, and AST were assessed. Albumin and total protein levels were found to have increased significantly. When it came to bringing biomarker levels back to normal, the high dosage of shilajit worked better than the low dose. Similarly, uric acid, creatinine, and urea levels caused by bone cancer were significantly reduced when shilajit was used with chemotherapy procedures (CMF cocktail). Researchers observed that the lower dosage of shilajit had less impact on kidney function markers than the larger dose [12]. Many different active chemicals are responsible for shilajit's efficiency. These include aromatic carboxylic acids, terpenes, gum, sterols, phenolic compounds, polyphenols, gum, albumin, latex, and an extra active substance [58]. In addition to minerals, vitamins, fulvic and humic acids, trace elements, carbs, and plant components, shilajit also includes a number of other useful substances. Shilajit is a good herbal treatment because its pharmacological effects are enhanced by its integrated makeup. Its active plant components have a number of pharmaceutical uses, including immune system regulation, antiviral effects, protection against oxidative stress, and inflammation reduction. Any researcher with an interest in shilajit would do well to consult this scientific review. Investigating potential synergistic effects with other herbs could lead to useful nano-formulations, and the food and nutritional supplement industries could use it to create bioactive supplement products that promote health [53].

Limitation

The chemical components analysis performed to identify humic acids, fulvic acids, or mineral content were not identified in this study, but were based on previous studies. The positive effect of each component will be analyzed and studied separately. The current study did not include a histological study, but we thank you for this scientific proposal, and it will be studied in future research.

Conclusions

Conversely, the study found that shilajit aqueous extract effectively protected against MSG toxicity, leading to improved biomarkers and lower levels of liver and kidney enzymes, particularly at higher doses (200 mg/kg). Thanks to its antioxidant components, trace minerals, and fulvic acids,

shilajit has this effect. Based on these findings, shilajit shows promise as an adjuvant to mitigate the harmful effects of some industrial food additives, such as MSG. To validate these results in human and animal models and to comprehend the exact molecular pathways of its impact, more research is suggested.

Ethical approval and study participation

This study was approved by the Ethical Committee of Thi-Qar University under Protocol No. 8795438.

Availability of data and materials

The data used or analyzed during this study are available from the corresponding author upon formal request.

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Роль шиладжиту у зменшенні токсичного впливу глутамату натрію на рівень ферментів печінки та функцію нирок у білих мишей

Резюме. Актуальність. Глутамат натрію (MSG) широко використовується в харчовій промисловості як підсилювач смаку. Хоча в багатьох дослідженнях підтверджено, що тривале споживання MSG може спричинити оксидативний стрес у тварин, особливо в печінці та нирках, у цій роботі оцінювали біохімічні ефекти, пов'язані з гепатитом і запаленням нирок, спричиненими різними дозами MSG, а також вивчали захисну дію водного екстракту шиладжиту в білих мишей. **Мета:** оцінити біохімічну токсичність різних доз глутамату натрію щодо функціонального стану нирок і печінки в білих мишей. **Матеріали та методи.** П'ятдесят дорослих мишей були випадково поділені на 5 груп (десять тварин у кожній). Контрольна група (G1) отримувала чисту воду, тоді як експериментальна група (G2) — MSG у дозі 2 г/кг маси тіла. Третій групі (G3) давали таку саму дозу MSG плюс 100 мг/кг екстракту шиладжиту. Четверта група (G4) отримувала підвищену дозу MSG (4 г/кг), а п'ята (G5) — підвищену дозу MSG разом із 200 мг/кг шиладжиту. Лікування

здійснювалося перорально щодня протягом 14 діб. На 15-й день тварин умертвили для біохімічного аналізу зразків, включно з показниками функції нирок (сечовина, креатинін) і рівнями ферментів печінки (AST, ALT, ALP, GGT). **Результати.** У групах G2 і G4, які отримували лише MSG, спостерігалася значне ($P \leq 0,05$) підвищення вмісту ферментів печінки (ALP, AST, ALT), що свідчить про її істотне ураження. Натомість екстракт шиладжиту сприяв значному зниженню цих показників, демонструючи потенційну захисну дію проти токсичності MSG. **Висновки.** Високі дози глутамату натрію викликають порушення функцій печінки та нирок, що проявляються збільшенням рівня ферментів печінки і показників функції нирок, які свідчать про тканинні й функціональні ушкодження на тлі оксидативного стресу. Шиладжит може відігравати захисну роль при таких станах.

Ключові слова: глутамат натрію; шиладжит; печінка; нирки; ферменти печінки; функція нирок

DOI: <https://doi.org/10.22141/2307-1257.14.3.2025.533>Doaa Hazem Mohammed^{1,2}, Meethaq Sattar Abood², Ali Naeem Salman²¹Department of Pharmaceutical Sciences, College of Pharmacy, University of Thi-Qar, Thi-Qar, Iraq²Department of Biology, College of Education for Pure Science, University of Thi-Qar, Thi-Qar, Iraq

Immune response on interferon-gamma in rats infected with *C.albicans*

Abstract. Background. *Candida albicans* is the most frequent etiologic agent that causes opportunistic fungal infection called candidiasis, a disease whose systemic manifestation could prove fatal and whose incidence is increasing as a result of an expanding immunocompromised population. Here we review the role of interferon-gamma (IFN- γ) in host protection against invasive candidiasis. This study investigates the time- and sex-dependent variations in IFN- γ levels in *C.albicans*-infected rats, offering insights into the function of this cytokine in fungal immunity. **Materials and methods.** This study involved 100 rats, with 50 in the experimental group and 50 in the control group, each consisting of 25 males and 25 females. The experimental group received cyclosporine A (10 mg) 24 hours prior to the infection to suppress the immune response and facilitate *C.albicans* growth, whereas the control group was administered distilled water instead of *C.albicans* suspension. Following four days of infection, group 1 was anesthetized, and a blood sample was collected to measure IFN- γ levels. Group 2 was assessed at 8 days, group 3 at 12 days, group 4 at 16 days, and group 5 at 20 days, alongside the control group. **Results.** The present study demonstrated a significant increase ($p < 0.05$) in IL-10 concentration in both male and female rats infected with *C.albicans* compared to the control group. There was a significant increase in IFN- γ concentration in *C.albicans*-infected rats of both sexes, with a p value < 0.05 , with progression of disease; the highest concentration was reached on the 12th day of the experiment and then decline. In contrast, no significant changes were observed in the control group over the same period. **Conclusions.** The research highlights the essential function of IFN- γ in the immune response to *Candida albicans* infections, observing that the absence of notable differences between male and female rats suggests that additional factors affect IFN- γ regulation. The necessity for additional research on the interactions between sex hormones and cytokines is underscored, offering insights into the sex- and time-dependent regulation of IFN- γ during infections, while also highlighting the need for clarification of observed discrepancies and their underlying mechanisms.

Keywords: IFN- γ ; *C.albicans*; candidiasis; immune response; rats

Introduction

Interferon-gamma (IFN- γ) is a critical cytokine in the host immune response to fungal infections, including those caused by *Candida albicans* [1]. As a key mediator of the Th1 immune response, IFN- γ enhances the anti-fungal activity of immune cells such as macrophages and neutrophils by promoting phagocytosis, the production of reactive oxygen species (ROS), and the expression of pro-inflammatory cytokines [2]. In rodent models, IFN- γ has been shown to play a pivotal role in controlling *C.albicans*

infections, particularly during the acute phase of the immune response. However, the temporal dynamics of IFN- γ production during the early stages of infection remain an area of active investigation, as the immune response to *C.albicans* is highly regulated and varies depending on the stage of infection [3, 4].

Recent studies have demonstrated that IFN- γ levels do not significantly increase during the first four days of *C.albicans* infection in rats compared to control groups, suggesting a delayed or subdued early immune response [5].

This initial phase may reflect the time required for the immune system to recognize the pathogen and mount an effective Th1 response. The lack of a significant increase in IFN- γ during this period could also be attributed to the immunosuppressive strategies employed by *C.albicans*, such as the modulation of host immune receptors and the production of virulence factors that dampen early pro-inflammatory responses [6]. Additionally, the early immune response to *C.albicans* is often characterized by the activation of innate immune mechanisms, such as the recruitment of neutrophils and the production of other cytokines like IL-6 and TNF- α , which may precede the upregulation of IFN- γ [7]. Understanding the delayed IFN- γ response in the early stages of *C.albicans* infection is crucial for elucidating the mechanisms of immune evasion employed by the fungus and for developing targeted immunotherapies [8, 9]. This study aims to explore the temporal dynamics of IFN- γ production in *C.albicans*-infected rats, with a focus on the first four days of infection, to provide insights into the early immune response and its implications for disease progression and control.

Materials and methods

Candida collection and identification

One hundred twenty stool samples were collected from children with diarrhea at Mohammed Al-Moussawi Teaching Hospital between October 2023 and February 2024. The samples were subsequently transferred daily to the Microbiology Laboratory in the Department of Life Sciences, College of Education for Pure Sciences. Diarrhea samples were cultured on Sabouraud dextrose agar with chloramphenicol and incubated at 37 °C for 24 to 48 hours. Following growth, the positive samples were re-cultured on chromogenic agar to differentiate between *Candida* species and were assessed for germ-tube formation.

Animal groups

Adult male and female albino rats (*Rattus norvegicus*) were obtained from the Laboratory Animal Breeding Center in Babylon Governorate, as outlined in the accompanying manual that facilitates the process. The animals were housed in the Animal House of Thi-Qar University College of Education for Pure Science, with weights ranging from 180 to 200 grams and an age of 8 weeks.

Fungal infection procedure in rats

In the study, a total of 100 mice were utilized, comprising 25 male and 25 female subjects injected with a fungal suspension, alongside a control group of 50 mice, also divided into 25 males and 25 females. These were organized into five groups, with each group consisting of five mice. The duration of the experiment was twenty days. Five rats per group were administered the immunosuppressant cyclosporin A (10 mg) once, 24 hours prior to the commencement of the experiment. The mice were administered a *C.albicans* fungal suspension (3×10^8 cells/ml) in a volume of 100 μ l, equivalent to a concentration of 1 ml, orally using a specialized syringe, in a single instance, as a comparison to the

standard McFarland solution. The distribution of animals was as follows:

- group 1. On the 4th day, the animals were anesthetized and blood was drawn directly from the heart;
- group 2. On the 8th day, the animals were anesthetized and blood was drawn directly from the heart;
- group 3. On the 12th day, the animals were anesthetized and blood was drawn directly from the heart;
- group 4. On the 16th day, the animals were anesthetized and blood was drawn directly from the heart;
- group 5. On the 20th day, the animals were anesthetized and blood was drawn directly from the heart.

Evaluation of IFN- γ

The IFN- γ was evaluated in serum of rats by using third generation ELISA technique.

Statistical analysis

The data of this study was statistically analyzed by using SPSS version 26, based in using one-way ANOVA, two-way ANOVA for mean variation, LSD, and Chi-square at p value < 0.05 [10]. The LSD value is used for determining the significant differences between means in the ANOVA test, where we subtract any two means from the table and compare the result of the subtraction with the LSD value. If the value of the subtraction is equal to or higher than the LSD value, it indicates a significant difference, while if it is less, it indicates that there is non-significant difference.

Results

Identification of *Candida* spp. in patients with diarrhea

The current study was showed the highest isolated *Candida* spp. in stool samples of patient was *C.albicans* 57 (78.1 %), then *C.glabrata* 11 (15.1 %), while the lowest isolated *Candida* spp. was *C.parapsilosis* 5 (6.8 %), also in control group showed highest isolated *Candida* spp. in stool samples of was *C.albicans* 8 (44.44 %), then *C.glabrata* 7 (38.89 %), while the lowest isolated *Candida* spp. was *C.parapsilosis* 2 (11.11 %), in addition the only one isolate of *C.tropicalis* in control group 1 (5.56 %) the study also noted a significant difference at p value < 0.05 between patients and control group, as in Fig. 1.

Distribution of *Candida* spp. according to sex

The current study was showed the highest isolated *C.albicans* in patient group was in the male group 39 (78.0 %), while the lowest in male group was *C.parapsilosis* 3 (6.00 %), in addition not *C.tropicalis* detected in patient group 0 (0.0 %), with regard control group noted the highest isolated *C.albicans* in female group and *C.glabrata* in male group 5 (50.0 %), while the lowest species was *C.tropicalis* in both female 1 (10.0 %), the study also noted a non-significant difference at p value < 0.05 in patients group and significant in control group, furthermore, a significant difference between patient and control group, as in Table 1.

Distribution of *Candida* spp. according to age groups

The current study was showed the highest isolated *Candida* spp. was in the first age groups *C.albicans* 32 (72.73 %), *C.glabrata* 8 (18.18 %), and *C.parapsilosis* 4 (9.09 %) while the lowest in male group 23 (31.51 %), while the lowest isolated *C.parapsilosis* in fourth age group 1 (12.5 %), while in control group the highest isolated was *C.albicans* in first age group 3 (75.0 %), then in third age group 3 (60.0 %), while the lowest isolated were *C.albicans* and *C.parapsilosis* in second age group 2 (28.57 %), the study also noted a significant difference within patient and within control, and between patient and control groups at p value < 0.05, as in Table 2.

Distribution of *Candida* spp. according to residency

The current study was showed the highest isolated *C.albicans* was in the countryside residence 48 (84.21 %), and in city residence 9 (56.25 %), while the lowest isolated

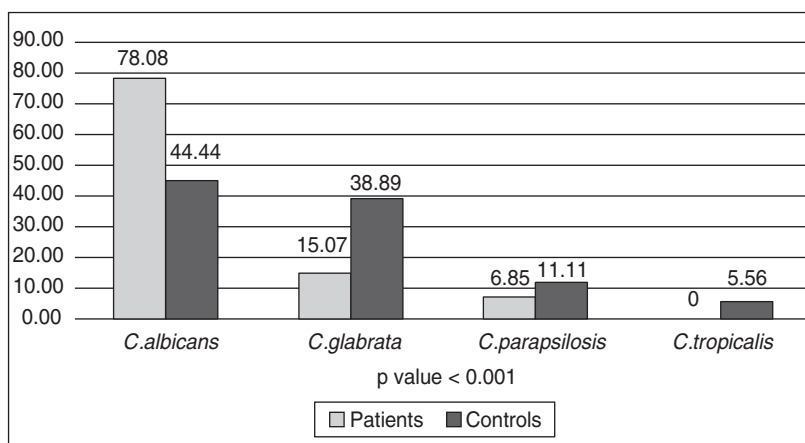


Figure 1. Identification of *Candida* spp. in patients with diarrhea and control group

Candida spp. was *C.parapsilosis* in both city and countryside residence 2 (12.5 %) and 3 (5.26 %), respectively, whereas in control group the study noted the highest isolated was *C.albicans* in countryside 4 (57.14 %), then *C.glabrata* in countryside 3 (42.86 %), while the lowest isolated was *C.tropicalis* 1 (9.09 %) in city residence, the study showed a significant difference at p value < 0.05 within patient

Table 1. Distribution of *Candida* spp. according to sex

| Groups | | Male | | Female | | p value |
|----------|-----------------------|------|-------|--------|-------|---------|
| | | N | % | N | % | |
| Patients | <i>C.albicans</i> | 39 | 78.00 | 18 | 78.26 | 0.634 |
| | <i>C.glabrata</i> | 8 | 16.00 | 3 | 13.04 | |
| | <i>C.parapsilosis</i> | 3 | 6.00 | 2 | 8.70 | |
| | <i>C.tropicalis</i> | 0 | 0.00 | 0 | 0.00 | |
| Controls | <i>C.albicans</i> | 3 | 37.50 | 5 | 50.00 | < 0.001 |
| | <i>C.glabrata</i> | 5 | 62.50 | 2 | 20.00 | |
| | <i>C.parapsilosis</i> | 0 | 0.00 | 2 | 20.00 | |
| | <i>C.tropicalis</i> | 0 | 0.00 | 1 | 10.00 | |

Notes: $CalX^2 = 69.5$; $TabX^2 = 12.59$; $DF = 6$; p value < 0.001.

Table 2. Distribution of *Candida* spp. according to age groups

| Age groups, years | | <i>C.albicans</i> | | <i>C.glabrata</i> | | <i>C.parapsilosis</i> | | <i>C.tropicalis</i> | | p value |
|-------------------|-------|-------------------|-------|-------------------|-------|-----------------------|-------|---------------------|-------|---------|
| | | N | % | N | % | N | % | N | % | |
| Patients | < 1–2 | 32 | 72.73 | 8 | 18.18 | 4 | 9.09 | 0 | 0.00 | < 0.001 |
| | 3–5 | 8 | 88.89 | 1 | 11.11 | 0 | 0.00 | 0 | 0.00 | |
| | 6–8 | 10 | 83.33 | 2 | 16.67 | 0 | 0.00 | 0 | 0.00 | |
| | ≥ 9 | 7 | 87.50 | 0 | 0.00 | 1 | 12.50 | 0 | 0.00 | |
| Controls | < 1–2 | 3 | 75.00 | 1 | 25.00 | 0 | 0.00 | 0 | 0.00 | < 0.001 |
| | 3–5 | 2 | 28.57 | 3 | 42.86 | 2 | 28.57 | 0 | 0.00 | |
| | 6–8 | 3 | 60.00 | 2 | 40.00 | 0 | 0.00 | 0 | 0.00 | |
| | ≥ 9 | 0 | 0.00 | 1 | 50.00 | 0 | 0.00 | 1 | 50.00 | |

Notes: $CalX^2 = 302.6$; $TabX^2 = 19.68$; $DF = 11$; p value < 0.001.

Table 3. Distribution of *Candida* spp. according to residency

| Groups | | City | | Countryside | | p value |
|----------|-----------------------|------|-------|-------------|-------|---------|
| | | N | % | N | % | |
| Patients | <i>C.albicans</i> | 9 | 56.25 | 48 | 84.21 | < 0.001 |
| | <i>C.glabrata</i> | 5 | 31.25 | 6 | 10.53 | |
| | <i>C.parapsilosis</i> | 2 | 12.50 | 3 | 5.26 | |
| | <i>C.tropicalis</i> | 0 | 0.00 | 0 | 0.00 | |
| Controls | <i>C.albicans</i> | 4 | 36.36 | 4 | 57.14 | < 0.001 |
| | <i>C.glabrata</i> | 4 | 36.36 | 3 | 42.86 | |
| | <i>C.parapsilosis</i> | 2 | 18.18 | 0 | 0.00 | |
| | <i>C.tropicalis</i> | 1 | 9.09 | 0 | 0.00 | |

Notes: $CalX^2 = 43.6$; $TabX^2 = 12.59$; $DF = 6$; p value < 0.001.

and within control and between patient and control, as in Table 3.

Evaluation of IFN- γ in rats according to sex

The analysis of serum IFN- γ levels revealed significantly elevated concentrations in the diseased group compared to the control group ($p < 0.01$). Male patients exhibited a mean IFN- γ level of 85.50 ± 21.10 pg/mL, while female patients had a slightly higher mean of 92.03 ± 18.60 pg/mL. In contrast, control males and females showed considerably lower levels, with means of 59.18 ± 4.77 pg/mL and 65.24 ± 7.32 pg/mL, respectively. Although both male and female patients demonstrated increased IFN- γ levels, there was no statistically significant difference between sexes within each group, as indicated by shared superscript letters. The least significant difference (LSD) value of 8.33 confirms the significance of differences observed between diseased and control groups. These findings suggest a potential role of elevated IFN- γ in the pathophysiology of the disease under investigation, as in Table 4.

Interaction between sex and time and its effect of IFN- γ level

Longitudinal analysis of IFN- γ levels in patients and controls over a 20-day period revealed significant temporal and group-specific variations ($p < 0.01$). In both male and

Table 4. Evaluation of IFN- γ (mean \pm SD) in rats according to sex

| Groups | | IFN- γ |
|----------|--------|---------------------|
| Patients | Male | 85.50 ± 21.10^a |
| | Female | 92.03 ± 18.60^a |
| Controls | Male | 59.18 ± 4.77^b |
| | Female | 65.24 ± 7.32^b |
| p value | | < 0.01 |
| LSD | | 8.33 |

Notes: ^a — the highest concentration, ^b — the second concentration.

female patients, IFN- γ concentrations increased progressively from day 4 to day 12, peaking at 119.50 ± 5.35 pg/mL and 125.00 ± 3.58 pg/mL, respectively. This marked elevation was significantly higher than in the corresponding controls, where IFN- γ levels remained relatively stable throughout the study period. While patient values dropped slightly by day 20, they remained elevated compared to controls. Significant differences were observed between male and female patients at several time points, particularly on days 4 and 20, as indicated by the least significant difference (LSD) values. In contrast, no statistically significant differences were found between male and female controls at any time point ($p = 0.276$ and $p = 0.792$, respectively). The two-way ANOVA confirmed a significant interaction between time and group ($p < 0.01$, $LSD = 9.03$), emphasizing the dynamic and disease-specific modulation of IFN- γ . These findings suggest that IFN- γ could serve as a potential biomarker for disease progression and inflammatory status in affected patients, as in Table 5.

Discussion

This finding of the present study was in line with previous studies, the study of Shankar et al. [11], that demonstrated the critical role of IFN- γ in the immune response to fungal infections, including candidiasis, and study of Glennon-Alty et al. [12], also recorded the IFN- γ is a key pro-inflammatory cytokine produced primarily by T helper 1 (Th1) cells and natural killer (NK) cells, and it plays a pivotal role in enhancing the antifungal activity of macrophages and neutrophils by promoting phagocytosis and the production of reactive oxygen species (ROS). The significant increase in IFN- γ levels in infected rats reflects the activation of a robust Th1-mediated immune response, which is essential for controlling *C.albicans* infections [13].

A non-significant difference in IFN- γ levels between male and female rats, both in the infected and control groups, is consistent with study of Abedini et al. [14], that had reported minimal sex-based differences in the production of IFN- γ during fungal infections. This suggests that the immune response to *C.albicans*, particularly the Th1

Table 5. Interaction between sex and time and its effect of IFN- γ level, mean \pm SD

| Days | Patients | | Controls | | LSD |
|---------|--------------------------------|--------------------------------|------------------|------------------|----------------|
| | Male | Female | Male | Female | |
| 4 | 67.42 \pm 8.85 ^c | 75.03 \pm 7.86 ^c | 55.72 \pm 3.76 | 64.47 \pm 8.58 | 10.1 |
| 8 | 81.43 \pm 8.21 ^b | 80.79 \pm 4.97 ^c | 58.40 \pm 4.58 | 61.83 \pm 9.02 | 9.34 |
| 12 | 119.50 \pm 5.35 ^a | 125.00 \pm 3.58 ^a | 62.17 \pm 6.92 | 67.37 \pm 8.30 | 8.43 |
| 16 | 93.48 \pm 14.10 ^b | 91.08 \pm 6.00 ^b | 60.62 \pm 3.17 | 67.00 \pm 6.07 | 11.2 |
| 20 | 70.61 \pm 8.58 ^c | 88.15 \pm 6.48 ^b | 58.99 \pm 3.70 | 65.55 \pm 5.98 | 8.61 |
| p value | < 0.01 | < 0.01 | 0.276 | 0.792 | p value < 0.01 |
| LSD | 12.4 | 7.86 | Non-sig | Non-sig | |

Notes: p value (LSD) < 0.01 (9.03); ^a — the highest concentration, ^b — the second concentration, and so on for the rest of the letters; also, the column that does not contain small letters did not record a significant difference.

response, may not be strongly influenced by sex hormones such as estrogen or testosterone. However, this finding contrasts with study of Harding and Heaton [15] that reported sex-based differences in cytokine production, with females often exhibiting stronger Th1 responses due to the immunomodulatory effects of estrogen. The discrepancy may be attributed to differences in experimental models, fungal load, or the timing of cytokine measurement, as the immune response can vary depending on the stage of infection [7].

The non-significant difference in IFN- γ levels between male and female rats in the control group further supports the idea that baseline levels of this cytokine are generally similar in the absence of infection. This is results was consistent with study of Dunn et al. [16] showing that sex-based differences in cytokine production are often more pronounced during active immune responses rather than at rest. However, previous study had reported subtle differences in baseline immune parameters between males and females, which could be influenced by genetic or environmental factors as reported by Bake et al. [17].

The results lie in the central role of IFN- γ in coordinating the immune response to *C.albicans*. The significant increase in IFN- γ in infected rats reflects the activation of a protective Th1 response, which is crucial for controlling fungal infections. The absence of sex-based differences in IFN- γ levels may be due to the dominant role of pathogen-associated molecular patterns (PAMPs) in driving the immune response, overshadowing the effects of sex hormones. Additionally, the timing of cytokine measurement in this study may have captured a phase of the immune response where sex-based differences are less pronounced.

The observed temporal pattern of IFN- γ levels in *Candida*-infected rats, characterized by an insignificant increase on the fourth day, a significant rise by the eighth day, a peak on the twelfth day, and a gradual decline by the sixteenth and twentieth days, reflects the dynamic nature of the immune response to fungal infections. IFN- γ , a critical Th1 cytokine, plays a pivotal role in activating macrophages and enhancing their antifungal activity through

mechanisms such as phagocytosis and the production of reactive oxygen species as recorded by study of Ye et al. [18]. A similar study done by Pawar et al. [19], the initial insignificant increase on the fourth day likely represents the early phase of infection, where the immune system is still initiating its response, while the significant rise by the eighth day and peak on the twelfth day indicate the activation of a robust Th1-mediated immune response, essential for controlling *Candida* proliferation. The subsequent decline in IFN- γ levels by the sixteenth and twentieth days suggests a transition to immune regulation to prevent excessive inflammation and tissue damage, consistent with the resolution phase of the immune response as observed by study of Noori et al. [20]. Recent study performed by Wang et al. [21] had demonstrated that IFN- γ levels correlate with the severity and progression of fungal infections, with peak production occurring during the acute phase of infection. The gradual decrease in IFN- γ levels may also reflect the establishment of immune homeostasis or the suppression of Th1 responses by regulatory mechanisms, such as the induction of anti-inflammatory cytokines like IL-10 [22]. These findings underscore the importance of IFN- γ in the host defense against *Candida* and highlight the dynamic nature of the immune response over the course of infection. The value of chronobiological aspects in the functioning of kidneys and the expediency of continuing scientific and practical research in this direction of medical science were emphasized [23].

Conclusions

This study reveals a consistent temporal pattern of IFN- γ production in both mice and rats infected with *Candida albicans*, characterized by an insignificant increase on the fourth day, a significant rise by the eighth day, a peak on the twelfth day, and a gradual decline by the sixteenth and twentieth days of infection. This pattern reflects the dynamic immune response to *C.albicans*, beginning with a subdued IFN- γ response during early infection, followed by a robust Th1-mediated response to control fungal proliferation, and concluding with immune regulation to prevent excessive inflammation. These findings emphasize the critical

role of IFN- γ in host defense against *C. albicans* and provide insights into the temporal dynamics of cytokine production during fungal infections. Further research is needed to explore the mechanisms behind these changes and potential sex-based differences, which could guide the development of targeted immunotherapies for candidiasis.

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Імунна відповідь на інтерферон-гамма у щурів, які були інфіковані *C. albicans*

Резюме. Актуальність. *Candida albicans* є найпоширенішим етіологічним агентом, що викликає опортуністичну грибкову інфекцію — кандидоз, системний перебіг якого може бути летальним. З огляду на збільшення кількості імунокомпрометованих осіб захворюваність на кандидоз зростає. У цьому дослідженні розглядається роль інтерферону-гамма (IFN- γ) у захисті організму від інвазивного кандидозу. **Мета:** вивчити часові та статевозалежні зміни рівня IFN- γ у щурів, інфікованих *C. albicans*, щоб оцінити функціональну роль цього цитокіну в протигрибковому імунітеті. **Матеріали та методи.** У дослідження було залучено 100 щурів, розділених на експериментальну ($n = 50$) і контрольну ($n = 50$) групи, кожна з яких складалася з 25 самців та 25 самок. Тваринам експериментальної групи вводили циклоспорин А (10 мг) за 24 години до інфікування з метою пригнічення імунної відповіді та полегшення росту *C. albicans*. Контрольна група отримувала дистильовану воду. Вимірювання рівня IFN- γ проводили у

п'яти часових точках: на 4-ту, 8-му, 12, 16 та 20-ту добу після інфікування. **Результати.** Отримані дані засвідчили вірогідне зростання концентрації IFN- γ ($p < 0,05$) у самців і самок щурів, інфікованих *C. albicans*, із максимальною концентрацією на 12-й день експерименту, після чого рівень цитокіну знижувався. У контрольній групі суттєвих змін IFN- γ протягом дослідження не зафіксовано. При цьому відмінностей в інтенсивності відповіді між статями не виявлено. **Висновки.** Отримані результати підтверджують важливу роль IFN- γ у протигрибковій імунній відповіді при інфікуванні *Candida albicans*. Відсутність виражених статевих відмінностей указує на можливу участь інших регуляторних факторів, зокрема гормональних. Необхідні подальші дослідження для з'ясування механізмів регуляції IFN- γ та його взаємодії зі статевими гормонами в контексті грибкових інфекцій.

Ключові слова: IFN- γ ; *C. albicans*; кандидоз; імунна відповідь; щури

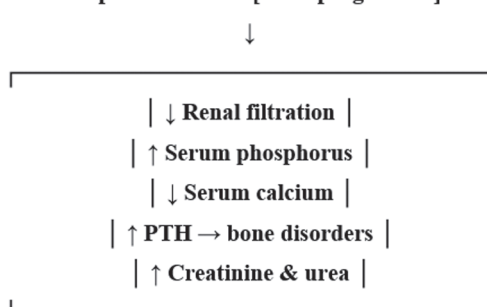
DOI: <https://doi.org/10.22141/2307-1257.14.3.2025.532>Noora Q. Al-Khafaji¹, Hanan B. Saadon¹, Sarah Jassim Abed²¹College of Sciences, University of Thi-Qar, Thi-Qar, Iraq²College of Health and Medical Techniques, National University of Science and Technology, Thi-Qar, Iraq

Calcium and phosphorus imbalances as biochemical markers in chronic kidney disease: a case-control study

Abstract. Background. Progressive loss of renal function is the hallmark of chronic kidney disease (CKD), which is often linked to biochemical abnormalities, especially in the calcium and phosphate metabolism. In the pathophysiology of secondary hyperparathyroidism, vascular calcification, and bone mineral abnormalities, these disruptions are crucial. The purpose of this research was to examine the blood calcium and phosphorus profiles of hemodialysis patients with CKD, to clarify if these profiles correlate with indices of renal function and compare results to those of healthy people in order to determine whether these profiles are diagnostically or prognostically relevant. **Materials and methods.** A comparative, cross-sectional research was carried out at Al-Hussain Hospital between December 2024 and June 2025, comprising 60 patients with end-stage renal disease who were between the ages of 25 and 72 years and were receiving long-term hemodialysis. At the same time, 30 healthy age- and gender-matched controls were included in the study. The amounts of serum urea, creatinine, calcium, and phosphorus were measured and analyzed using the conventional biochemical techniques. An examination of statistical data was carried out using SPSS version 26, with the level of significance set at $p < 0.05$. To analyze the connections between the parameters, Pearson's correlation was used. **Results.** In comparison with healthy controls, patients with chronic kidney disease showed substantially higher levels of serum phosphorus (5.37 ± 0.47 mg/dL) and creatinine (7.46 ± 1.15 mg/dL), as well as lower calcium levels (5.54 ± 0.41 mg/dL) ($p < 0.0001$ for all). The link between creatinine and phosphorus was somewhat positive ($r = 0.54$); however, calcium and phosphorus levels were negatively associated ($r = -0.30$). **Conclusions.** The presence of hyperphosphatemia and hypocalcemia is quite common in individuals with advanced CKD, and both conditions are directly linked to reduced renal function. Phosphorus levels may be used as a surrogate measure for renal impairment, as shown by the modest association that exists between phosphorus and creatinine. Monitoring calcium and phosphorus on a regular basis, in addition to traditional indicators, is essential for the early diagnosis of changes in mineral metabolism and the prompt treatment of problems connected to CKD.

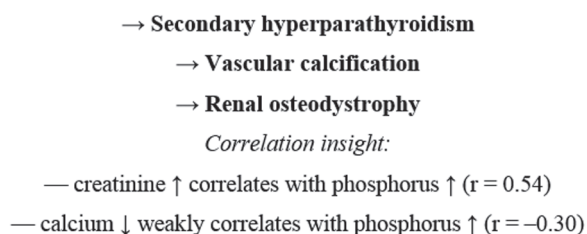
Keywords: chronic kidney disease; biomarkers; hyperphosphatemia; hypocalcemia; hemodialysis; bone mineral disorder; creatinine; phosphate-calcium axis

Graphical abstract [CKD progression]



↓

[Clinical complications]



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Introduction

Damage to the kidneys over time causes a major upset in the body's biochemical equilibrium, particularly with regard to calcium and phosphorus levels; this disorder is known as chronic kidney disease (CKD). In addition to being major factors in skeletal and cardiovascular problems, these two minerals are essential for evaluating the metabolic status of CKD patients [1]. Research has shown that hyperphosphatemia develops when the kidneys aren't working properly, and that calcium levels drop when vitamin D activation is low and absorption in the intestines is poor [2]. The secretion of parathyroid hormone (PTH) is stimulated by these disturbances, resulting in secondary hyperparathyroidism. Vascular calcification is promoted and bone disease (renal osteodystrophy) is exacerbated by this situation [3, 4]. These mineral metabolism problems continue to be a leading cause of mortality in CKD patients and have been significantly associated with increased cardiovascular risk [5].

The current research investigates the ways in which changes in calcium and phosphorus levels might serve as early indicators of difficulties connected to CKD. In addition to this, it highlights the clinical significance of these mineral abnormalities in terms of early identification and preventative therapy, as well as looks into the underlying processes that are responsible for their impact on patient health. The condition known as hyperphosphatemia is brought on by chronic renal disease, which causes phosphate excretion to be hindered. The disruption of calcium and phosphate balance that results from this condition, in conjunction with the reduced synthesis of calcitriol, is a contributing factor in the development of hypocalcemia and secondary hyperparathyroidism (SHPT) [6, 7]. The conversion of 25-hydroxyvitamin D to its active form, calcitriol, is diminished in patients with CKD due to the decreased activity of renal 1α -hydroxylase. Because of this decline, intestinal calcium absorption is impaired, which ultimately results in hypocalcemia. Furthermore, the retention of phosphate hinders the production of calcitriol, which only serves to exacerbate the calcium imbalance [8]. The parathyroid glands are stimulated to release PTH when hypocalcemia is present, which ultimately results in SHPT. Bone resorption is increased in order to release calcium when PTH levels are elevated; nevertheless, this compensatory process leads to bone abnormalities in patients with chronic kidney disease [9]. Systemic disruptions in mineral metabolism are included in chronic kidney disease-mineral and bone disorder (CKD-MBD). These disturbances include anomalies in calcium, phosphate, PTH, and vitamin D levels, which ultimately result in bone pathology and vascular calcification [1].

Materials and methods

Concerning data of samples

The research was conducted on 60 patients, both male and female, with end-stage renal disease (ESRD) who were receiving long-term hemodialysis treatment at the hemodialysis unit at Al-Hussain Hospital. The patients' ages ranged from 25 to 70 years old. It was from December 2024 to June 2025 that the research was conducted. In order to serve as a control group, 30 healthy individuals, both men

and females, were selected. Through the use of a specialized questionnaire, the personal information of each participant, both those who were ill and those who were well, was collected.

Study design is presented in Fig. 1.

Conditions for exclusion

Exclusion criteria for patients with hepatitis B, recent hospitalization, current phosphate binder therapy, cancer, inherited or acquired blood disorders, acute or chronic inflammation, recent hemorrhage, and any other condition that could affect their hematological parameters were outlined in the study.

Collection of samples

Before each hemodialysis session, four milliliters of blood were drawn from patients with CKD. The blood sample was then placed in a test tube, and after that, it was separated in order to produce blood serum. The samples were analyzed for levels of creatinine, blood urea, calcium, and phosphorus in order to determine the severity of the kidney function impairment. Additionally, all of the tubes were transported via an icebox until they arrived at the laboratory.

Statistical analysis

The data collected in this research was subjected to statistical analysis using SPSS version 26, with the independent sample t test being used to determine variance and the person coefficient being used to determine correlation at a p-value of less than 0.05 [10].

Results

Distribution of patients according to sex

As shown in Table 1, the current research demonstrated a statistically significant difference at a p-value of less than 0.05. The high number of patients in the male group was recorded at 40 (66.67 %), while the female group had 20 (33.33 %).

Distribution of patients according to age

Table 2 presents an illustration of the age distribution of patients who have been diagnosed with chronic renal failure.

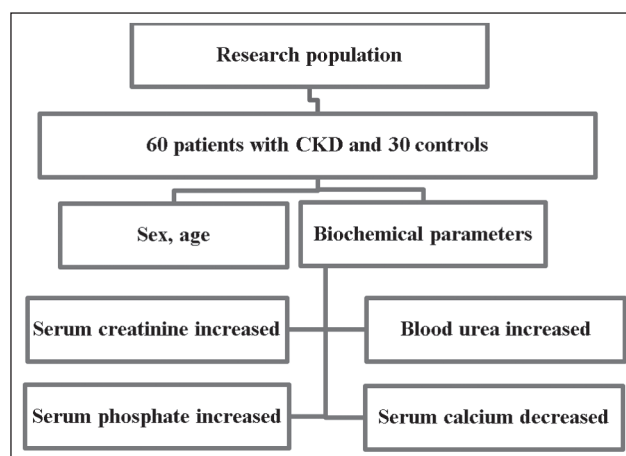


Figure 1. Flow chart of study design

It reveals that the largest proportion of patients, which accounts for 45 %, are within the age group of 57–72 years, followed by 35 % of patients aged 41–56 years, and only 20 % of patients aged 25–40 years. The study also demonstrated a significant difference between the age groups.

Evaluation of renal function test and electrolyte in renal patient and control group

The research obtained a statistically significant difference with a p-value of less than 0.05. In this investigation, the biochemical parameters (Table 3) that were evaluated revealed substantial differences in the levels of urea, creatinine, calcium, and phosphorus among patients who were diagnosed with CKD in comparison to healthy controls.

Table 1. Distribution of CKD patients according to sex

| Sex | N | % |
|---------------|----|-------|
| Males | 40 | 66.67 |
| Females | 20 | 33.33 |
| Total | 60 | 100 |
| p-value 0.046 | | |

Table 2. Distribution of CKD patients according to age

| Age, years | N | % |
|---------------|----|------|
| 25–40 | 12 | 20.0 |
| 41–56 | 21 | 35.0 |
| 57–72 | 27 | 45.0 |
| Total | 60 | 100 |
| p-value 0.009 | | |

According to Table 3, all four biochemical markers showed statistically highly significant differences between patients and controls ($p < 0.0001$). Urea and creatinine levels are markedly elevated in CKD patients, indicating renal impairment. Serum calcium is significantly lower in CKD patients, reflecting disturbances in mineral metabolism. Phosphorous levels are significantly higher, consistent with hyperphosphatemia common in CKD due to reduced excretion.

Person correlation between biochemical parameters

Table 4 shows that there is a modest positive association ($r = 0.54$) between serum creatinine and phosphorus levels, suggesting that both parameters tend to increase together when renal function deteriorates. Weak or negligible correlations were found between other parameters.

Discussion

Chronic kidney disease patients are 80 % male and 20 % female. Males are more likely to require renal replacement therapy such transplants or dialysis to achieve ESRD faster than females [11]. Biological and behavioral factors explain the gender disparity. Estrogen preserves kidney function in women, but testosterone may promote renal damage and fibrosis in males [12]. Men are also more likely to smoke, eat more protein, and be exposed to occupational nephrotoxins, which raise CKD risk [13].

Health system factors may apply. According to research, men are more likely to be sent to nephrology services early, undergo dialysis, and get kidney transplants than women [11]. Due to access and treatment disparities, CKD groups may have varied demographics. Thus, the finding that males made up a bigger fraction of the sample may indicate biological differences in disease onset and healthcare access and delivery [14]. Recognizing and addressing these issues is essential to gender-equitable CKD treatment.

Table 3. Biochemical parameters of kidney function in study samples, mg/dL (mean ± SD)

| Parameter | Patients | Controls | p-value |
|-------------|---------------|--------------|----------|
| Urea | 135.90 ± 2.53 | 30.78 ± 2.19 | < 0.0001 |
| Creatinine | 7.46 ± 1.15 | 1.05 ± 0.13 | < 0.0001 |
| Calcium | 5.54 ± 0.41 | 9.34 ± 0.14 | < 0.0001 |
| Phosphorous | 5.37 ± 0.47 | 3.34 ± 0.14 | < 0.0001 |

Table 4. The correlation among the study parameters of patients

| Parameters | Urea | Creatinine | Calcium | Phosphorous |
|-------------|-------|------------|---------|-------------|
| Urea | 1.00 | -0.31 | -0.03 | 0.17 |
| Creatinine | -0.31 | 1.00 | -0.01 | 0.54 |
| Calcium | -0.03 | -0.01 | 1.00 | -0.30 |
| Phosphorous | 0.17 | 0.54 | -0.30 | 1.00 |

Global epidemiological study shows that CKD prevalence rises with age, and this distribution matches that trend. Due to structural and functional kidney degradation, such as nephron loss, glomerulosclerosis, and vascular stiffness, GFR normally declines with age [15]. Physiological changes in elderly people raise the likelihood of chronic kidney disease even without other health issues.

The main risk factors for CKD, hypertension and type 2 diabetes, are also more frequent in older people. These co-occurring diseases gradually damage renal function [16]. This study largely comprised patients aged 57–72, consistent with the Chronic Kidney Disease Prognosis Consortium's findings that CKD incidence and severity grow dramatically after 50 [17].

Younger people may have better renal reserve and reduced chronic disease risk, which may explain why 20 % of patients are in this age range. Hereditary nephropathies, autoimmune diseases, and congenital anomalies cause CKD in this age group [18]. While uncommon, early-onset CKD has a longer disease course and a higher risk of lifelong complications, making it important to detect and treat. This aging chronic kidney disease burden emphasizes the need for preventive nephrology therapy and age-specific screening programs to reduce the risk of ESRD and its healthcare costs [19].

Compared to healthy controls, CKD patients have significant biochemical alterations in urea, creatinine, calcium, and phosphorus. Chronic renal illness causes kidney function decrease and mineral metabolism disturbances, which these changes reflect. Compared to controls, CKD patients exhibited substantially higher urea and creatinine levels (135.90 ± 2.53 mg/dL and 7.46 ± 1.15 mg/dL, respectively) with a p -value < 0.0001 . Our findings support serum urea and creatinine as primary markers of GFR and total renal function. Kidney failure reduces excretion, causing blood nitrogenous waste accumulation [20].

Additionally, CKD patients had significantly lower blood calcium levels (5.54 ± 0.41 mg/dL) compared to controls (9.34 ± 0.14 mg/dL). Reduced renal conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D may impede intestinal calcium absorption in chronic kidney illness, causing hypocalcemia [21]. Hyperphosphatemia and PTH resistance in renal and bone tissues aggravate secondary hyperparathyroidism [22].

Patients with CKD exhibited significantly higher blood phosphorus levels (5.37 ± 0.47 mg/dL) compared to controls (3.34 ± 0.14 mg/dL) (p -value < 0.0001). Severe CKD induces hyperphosphatemia due to renal phosphate excretion reduction. Arterial calcification, increased phosphorus levels, and cardiovascular morbidity and mortality have been associated to chronic renal disease [23, 24]. This study shows that serum calcium and phosphorus are negatively correlated in CKD patients, supporting mineral metabolism dysregulation. Low calcium and high phosphorus, creatinine, and urea values indicate renal excretory dysfunction and bone-mineral metabolism issues. Preventing cardiovascular events and bone disorders in CKD patients requires early detection and treatment of these abnormalities.

Table 4 shows a modest positive correlation ($r = 0.54$) between blood creatinine and phosphorus levels rising concurrently as renal function declines. This is pathophysiologically feasible since CKD decreases renal excretion of inorganic phosphate and creatinine, a nitrogenous waste product [21]. Hyperphosphatemia causes arterial calcification, cardiovascular disease, and increased mortality, particularly in advanced chronic renal disease [25]. Phosphorus and calcium had a moderate negative correlation ($r = -0.30$) supporting their adverse physiological relationship. In chronic kidney disease, poor renal phosphate clearance raises blood phosphorus. This increases PTH and FGF-23, which limit calcitriol synthesis and calcium absorption. This relationship affects hypocalcemia and secondary hyperparathyroidism in CKD-MBD [26].

A weak negative connection with creatinine ($r = -0.31$) and calcium ($r = -0.03$) and a minor positive correlation with phosphorus ($r = 0.17$) were the only meaningful relationships. Urea is a sensitive indication of nitrogen retention, although protein ingestion, catabolism, and hydration status affect its variability, as these modest associations suggest [1]. The counterintuitive inverse association between urea and creatinine may be owing to individual differences in CKD patients' creatinine production or tubular secretion, particularly in muscular wasting or malnutrition. Since creatinine and calcium show no significant relationship ($r = -0.01$), hormonal and gastrointestinal factors may have a higher influence on calcium levels in CKD than glomerular filtration alone. Our data show that vitamin D, phosphate, and parathyroid hormone maintain calcium homeostasis.

To conclude, the correlation analysis shows that multiple pathophysiological pathways cause CKD. When creatinine and phosphorus are considerably connected, both minerals are reliant on renal excretion. When calcium and phosphorus are negatively related, mineral balance is upset. Our data show that chronic renal disease therapy requires extensive biochemical monitoring to decrease systemic risks and improve patient outcomes.

Conclusions

Biochemical abnormalities including hypocalcemia and hyperphosphatemia are related with chronic kidney disease, which decreases renal function. These anomalies induce secondary hyperparathyroidism, CKD-MBD, cardiovascular disease, and mineral and bone disorders. High blood creatinine and phosphorus levels show how mineral metabolism and renal clearance are linked. Preventing CKD complications requires early detection and treatment. Biochemical markers must be consistently monitored in chronic kidney disease treatment to improve patient outcomes and quality of life. These indicators include calcium, phosphorus, PTH, and creatinine.

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Authors' contributions. Noora Q. Al-Khafaji — conceptualization, data curation, investigation, methodology, project administration, resources, software, original draft, review & editing; Hanan B. Saadon, Sarah Jassim Abed — conceptualization, data curation, investigation, methodology, project administration, original draft, review & editing.

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**Порушення рівнів кальцію та фосфору як біохімічні маркери при хронічній хворобі нирок:
дослідження типу «випадок — контроль»**

Резюме. Актуальність. Прогресуюча втрата функції нирок є ознакою хронічної хвороби нирок (ХХН), що часто супроводжується біохімічними порушеннями, зокрема обміну кальцію та фосфатів. Ці розлади відіграють ключову роль у патофізіології вторинного гіперпаратиреозу, судинної кальцифікації і порушень мінерального обміну кісткової тканини. **Мета:** вивчити профілі кальцію та фосфору в крові пацієнтів із ХХН, які перебувають на гемодіалізі, з'ясувати, чи корелюють вони з показниками функції нирок, та порівняти результати з даними здорових осіб для визначення діагностичної або прогностичної значущості. **Матеріали та методи.** Порівняльне поперечне дослідження було проведено в лікарні Аль-Хуссейн з грудня 2024 року по червень 2025 року. У ньому взяли участь 60 пацієнтів з термінальною стадією ХХН віком від 25 до 72 років, які тривалий час отримували гемодіаліз. Також у дослідження було включено 30 здорових осіб контрольної групи, порівняних за віком та статтю. Рівні сечовини, креатиніну, кальцію та фосфору в сироватці крові вимірювали та аналізували за допомогою стандартних біохімічних методів. Статистичний аналіз проводили за допомогою програми SPSS версії 26 із рівнем значущості $p < 0,05$. Для аналізу

зв'язків між параметрами використано коефіцієнт кореляції Пірсона. **Результати.** Порівняно зі здоровими особами контрольної групи пацієнти із хронічною хворобою нирок мали значно вищі сироваткові рівні фосфору ($5,37 \pm 0,47$ мг/дл) та креатиніну ($7,46 \pm 1,15$ мг/дл), а також нижчий уміст кальцію ($5,54 \pm 0,41$ мг/дл) ($p < 0,0001$ для всіх). Зв'язок між креатиніном та фосфором був дещо позитивним ($r = 0,54$), однак рівні кальцію та фосфору мали негативну кореляцію ($r = -0,30$). **Висновки.** Гіперфосфатемія та гіпокальціємія є досить поширеними явищами в осіб із прогресуючою ХХН, і обидва стани безпосередньо пов'язані зі зниженою функцією нирок. Рівень фосфору може бути використаний як сурогатний показник ниркової недостатності, що підтверджується його незначною кореляцією з умістом креатиніну. Регулярний моніторинг рівнів кальцію та фосфору на додаток до традиційних показників є важливим для раннього виявлення порушень мінерального обміну та своєчасного лікування ускладнень, пов'язаних із ХХН.

Ключові слова: хронічна хвороба нирок; біомаркери; гіперфосфатемія; гіпокальціємія; гемодіаліз; порушення мінерального обміну; креатинін; фосфатно-кальцієва вісь

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Gender disparities in kidney transplant access and outcomes: a population-based analysis

Abstract. Background. Kidney transplantation is seen as a better treatment option in end-stage kidney disease (ESKD) because it offers greater longevity as well as quality of life. Access to kidney transplantation and post-transplantation outcomes is generally hindered by gender disparities. Women are underrepresented as kidney recipients but overrepresented as living donors. Gender disparities in kidney transplantation exist as a result of various factors, such as biological and sociocultural one, including sensitization from prior pregnancies, biological differences in the immune system, or gendered patterns of health care access or referral. Women receive fewer transplant referrals, face delays in evaluations, and obtaining encouragement for transplant compared with men. **Materials and methods.** This population-based study uses the Early Steps to Transplant Access Registry data and focuses on ESKD patients from Georgia, North and South Carolina. It examines gender differences across the full transplant care continuum, from referral to post-transplant outcomes. We statistically adjusted for clinical prognostic variables and demographic factors to evaluate the independent contribution of gender on transplant access and outcomes. **Results.** Our examination shows that women with ESKD are significantly less likely to be listed or receive a kidney transplant compared to men, even after controlling for other variables. The requirements persist throughout the transplant process, and the inequities are evident at every stage. While biologically related considerations of risk (graft rejection) and differences in metabolism of immunosuppressive medications likely partially explain some variance in outcomes, gender-related social determinants (e.g., provider bias) and systemic inequities generally explain more variance in the access gap for women, by comparison. It is also important to note new emerging challenges of great concern, such as the under-researched healthcare needs of transgender people, and the impact of COVID-19 on transplant recipients that compound gender inequities related to access to transplantation. **Conclusions.** This study highlights the pressing need to identify and address gender equity considerations in kidney transplantation decisions. Although there may be clinical justification for gender inequities, these issues emanate predominantly from modifiable sociocultural and institutional factors. Transplantation systems have not officially considered sex- and gender-sensitive means to eliminate barriers to access. Immediate policy intervention, education, and support are needed to ensure equitable access and equity of outcomes for all people, especially those from diverse gender identities.

Keywords: kidney transplantation; gender disparities; access to care; sex-based outcomes; transplant equity

1. Introduction

Kidney transplantation (KT) is the most effective kidney replacement therapy, clearly maintaining a patient's life, providing an improved quality of life while reducing long-term costs in comparison to dialysis [1]. Clinical and economic advantages of KT are well established,

yet there remains a persisting gap in the utilization of KT based on gender, sex, and socio-economic status. This gap is not only restricted to the absence of resources, health care finances, or availability of the organs; rather, it extends to biological inequalities and socio-cultural inequities.

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Biology refers to sex with the genetic and anatomical traits, along with hormonal aspects that may determine the progression of disease, the person's immunologic compatibility, as well as the immunoregulatory pharmacokinetics of medications given to suppress immune system functioning. Gender, on the other hand, is a social, psychological, and cultural identity that defines the norms, actions, and relationships a person takes part in regarding health care [2]. Sex and gender are often interchanged in usage. Still, in reality, these concepts should be analyzed separately as they relate to transplant medicine to fully comprehend all aspects of inequities.

Even though women currently outnumber male living organ donors, they remain underrepresented as recipients of kidney transplants [3]. A considerable amount of literature suggests that women are less likely to be referred for transplantation, undergo appropriate evaluations, and subsequently be placed on the waiting list relative to men, and this persists even when clinical factors are accounted for [4, 5]. This disparity exists even when evidence suggests that women, on average, have similar or better postoperative outcomes [6]. Perceptions of increased frailty, pregnancy-related heightened sensitization, autoimmune diseases, and greater psychosocial barriers to care amongst women contribute to this paradox [7].

In the United States, the US Renal Data System (USRDS) and the Early Steps to Transplant Access Registry (E-STAR) provided insights into these pathways, revealing stark differences between males and females at each stage of the transplant process from referral to evaluation and finally to transplantation [5]. The most striking disparities of these related to comorbidities and organizational bias are found among older women and those with diabetes-related end-stage kidney disease (ESKD). Furthermore, in India and many other low- and middle-income countries, economic hardship, poor health literacy, lack of personal agency, and caregiving responsibilities in these societies greatly reduce women's access to KT [8].

The inequities in pediatric transplantation systems show cultural bias when girls are less likely than boys to receive preemptive transplants or get placed on a waitlist. Even in high-income countries, there is a lower socioeconomic women's KT rate, which shows that there is a dominant exposed pattern of inequality and oppression in women's access to transplants globally [9].

The study area includes the southeastern United States, specifically Georgia, North Carolina, and South Carolina, which are classified under End Stage Renal Disease Network 6. These states were chosen because of the high burden of chronic kidney disease (CKD) in these areas, coupled with their unique demographic profile — higher proportions of African Americans and low-income communities and their disproportionate susceptibility to advanced stages of kidney failure. The region has an intersection of socio-economically disadvantaged populations with healthcare access barriers, limited availability of the healthcare specialty services, healthcare disparities, and their lack of access to tertiary healthcare facilities makes it an ideal region to study the intersection of gender, race, and socioeconomic factors

on the access and outcomes of kidney transplantation. The study uses data from all nine adult transplant centers within the states which are linked through the E-STAR, allowing for a thorough population-based study of disparities across the entire continuum of kidney transplant care. This focus allows the investigation and documentation of systemic as well as community level barriers which can inform targeted policy and clinical interventions.

This study aims to examine population-based disparities in access to and outcomes of kidney transplantation, with a particular focus on all processes involved in the transplantation continuum. Using multicenter registry data with attributed causes of ESKD, this study aims to determine whether such disparities are consistent across demographic and clinical subgroups. It aims to address gaps in policies, actions, or clinical standards designed to enhance equity and intersectional considerations in the access and outcomes of transplants for all genders.

2. Literature review

Research over the past twenty years demonstrates the continued presence of gender inequities in terms of access to, and outcomes associated with, kidney transplantation. Harding et al. (2023) performed a retrospective cohort study using over 2.3 million adults from the USRDS conducted a longitudinal analysis over the years 1997–2020 capturing points of sex inequity in transcending transplant avenues such as waitlists, living donor kidney transplantation (LDKT), and deceased donor kidney transplantation. The findings showed that despite the evolution of replacement therapies, inequity for women has been maintained and, in some cases, even widened [10].

Of particular interest, in the study, it was noted that generally women starting on kidney replacement therapy are more likely to be older, from a lower socio-economic status, with a greater prevalence of comorbidities such as diabetes, and obesity. These dimensions reflect systemic inequities at each stage of the transplant continuum. Furthermore, concerning the adjusted hazard ratio (HR) for women receiving LDKT showed a downward trend, for example, 0.89 from 1997–2000 to 0.79 from 2017–2020. This is suggestive of worsening access between men and women accessing LDKT [11].

Complementary studies support these findings. Women with type 2 diabetes mellitus and CKD were found to have much lower rates of receiving deceased donor kidney transplants than women without diabetes, sometimes presenting in less severe degrees of cardiovascular comorbidity than men. Ahearn et al. (2020) alluded to their findings to indicate that non-clinical or structural determinants of health factors like bias and socioeconomic status, physician-centered, clinically motivated frameworks may trump clinical determinants of health in providing determinations of eligibility for transplant.

Biological sensitization, such as pregnancy, is one of the other contributing factors of the disparity that is biological sensitization. Sensitization leads to the formation of anti-human leukocyte antigen antibodies, and because of this, it can result in obstacles when matching for living donors such

as a child, spouse, or parent. Of all the sensitizing factors, such as blood transfusions, transplant surgeries, and other sensitizing events, pregnancy appeared to be the most significant factor on women's sensitization [13, 4].

The situation is exacerbated by a lack of gender representation in referrals and evaluations related to the early transplant processes. One study conducted in the Southeastern United States focused on the E-STAR, which compiled data from all adult transplant centers in Georgia, North Carolina, and South Carolina. Findings showed that women were consistently less likely to be referred for further evaluation and, when referred, were less likely to survive long enough to be listed compared to men. This reinforces the idea that inequities are embedded in the case before any surgery occurs [12].

Additionally, women's relatively higher percentages of body fat — compared to height and weight — may heighten risk during surgery; therefore, the clinician may hedge on the decision to eliminate some, if not entire publications were ultimately eliminating women from factors that would impact elimination, illustrating obvious biases in the assessment of these risks. Cultural factors and cultural roles contribute to the issue as well; women are more likely to be kidney donors, which potentially stems from the traditional caregiver role, but are less likely to be recipients. This represents a profoundly entrenched socio-cultural paradox.

In conclusion, while kidney transplantation is still the best option for ESKD, there is a need for systemic change addressing the persistent and growing gender inequities at all levels of the process. These include policy changes, particular initiatives at the very start of the transplant process, and within knowledge care located within the specific biological and socio-cultural context of women.

3. Materials and methods

3.1. Study design and data source

This study overall works with two datasets, the USRDS and the E-STAR, which are fused together. The study population was patients aged between 18 and 79 years diagnosed with ESKD initiating dialysis on or after January 1, 2015, and before January 31, 2020. This study was limited to three

southeastern states: Georgia, North Carolina, and South Carolina. This region was of special consideration due to the high prevalence of chronic kidney disease and the unique demographics, particularly African Americans.

To achieve complete and accurate tracking through all phases of the transplant pathway, patient-level data from the USRDS was linked to all nine-adult transplant center referral and evaluation forms from the specified regions. This connection allowed for complete capture of transplant referral and evaluation data for the entire region, offering detailed insights into the initial phases of the kidney transplantation process.

The implementation of the E-STAR proved vital for capturing undocumented steps, including initial referrals for transplant, evaluation commencement and completion, and eligibility assessments. These steps are crucial for elucidating gaps in healthcare equity and identifying the scope of inequitable care distribution among various demographic groups, even though they are often absent from national surveillance datasets. It is also important to note that individuals listed as “medically unsuitable”, “psychologically unfit”, or too old for transplant (based on the Centers for Medicare & Medicaid Services (CMS) Form 2728) were excluded. This allows the study population to be more representative of individuals with realistic pathways toward receiving kidney transplantation.

In any case, the absence of these transplants has enabled a methodologically sound analysis of the early stages of transplant access across a diverse and medically underserved population, thereby strengthening the examination of sex and gender differences in the referral and evaluation processes for kidney transplantation.

The data presented in Table 1 highlights significant gender disparities throughout the kidney transplantation process for patients with ESKD, particularly in the Southern United States. Although women comprise 42.1 % of the ESKD population, they are consistently underrepresented in key stages of the transplant pathway. Referral rates reveal a 10 % gap between genders, with only 60 % of eligible women being referred for transplant compared to 70 % of men. This early-stage inequality may contribute to poorer

Table 1. Kidney transplant gender disparity

| Category | Value, % | Source/Comment |
|--|----------|--|
| Proportion of female ESKD patients (South US) | 42.1 | E-STAR (2015–2019) |
| Referral rate for women | 60 | Estimated from referral stage data |
| Referral rate for men | 70 | Estimated from referral stage data |
| Survival rate (5 years post-ESKD) | 48 | Approx. survival trend from population-based studies |
| Dialysis dependency rate | 82 | Estimated from dialysis continuation post-ESKD |
| Quality of life score (1–10 scale) | 4.5 | Subjective quality assessments in related studies |
| Women receiving pre-ESKD nephrology consultation | 35 | Derived from evaluation data in underserved areas |
| Men receiving pre-ESKD nephrology consultation | 52 | Derived from evaluation data in underserved areas |

outcomes for women, as shown by a lower five-year survival rate of 48 % and a higher dialysis dependency rate of 82 %. Furthermore, women report a lower average quality of life score (4.5 out of 10), underscoring the impact of delayed or missed transplant opportunities.

Table 1 also illustrates that only 35 % of women receive pre-ESKD nephrology consultations, compared to 52 % of men, indicating missed chances for early intervention and timely referrals. These disparities are rooted in both clinical and social determinants of health, including provider bias, caregiving roles, and limited access to specialized care. Collectively, these figures underscore the urgent need for gender-sensitive strategies in nephrology care and transplant evaluation to close the gap in kidney transplant access and outcomes.

3.1.1. Tracking referrals and qualitative aspects

In the E-STAR study, systematic retrieval of referrals was done through standardized referral and evaluation forms obtained from all nine adult transplant centers in Georgia, North Carolina, and South Carolina. Through the USRDS, these forms were also merged with patient-level data, which facilitated tracking of the entire continuum from dialysis initiation to transplant milestones. In this case, referral was defined as any noted suggestion for evaluation within a year of commencing dialysis and evaluation was labeled as the appointment occurring within six months post referral. This integration allowed the assessment of measurable provision toward accessing transplantation within a predefined time-frame.

While the E-STAR had extensive clinical and demographic information, it lacked qualitative variables like physician referral rationale, clinical summaries, or subjective evaluations documented in an open-text format. Therefore, no qualitative physician motivations or reasoning were recorded alongside the dataset. Nonetheless, the study incorporated system and organizational level variables, such as the for-profit status of the facility and social worker to patient ratios, as proxy indicators of provider resources and organizational practices. Such contextual indicators provide institutional insight and motivations and potential biases within the referral patterns. Although the report provided the conclusions based on the unaddressed structural inequities and underlying provider assumptions, those elements were neither coded nor analyzed qualitatively.

3.2. Inclusion and exclusion criteria

Eligibility criteria were defined for a cohort with a liver transplantation eligibility assessment to ensure the findings of the study are precise and meaningful. Only adults with diagnosed ESKD were included if they could clinically and demographically qualify for a transplant. On the contrary, participants were excluded if they were considered medically, psychologically, or age-wise unsuitable on the CMS Form 2728 — essentially a transplant evaluation paperwork used to document important patient attributes at the onset of kidney replacement therapy.

The study also did not include participants with incomplete data sets, specifically those without race/ethnicity in-

formation or the primary attributed cause of their ESKD. These fields are essential for analyzing inequities in the distribution of transplant opportunities relative to race or diagnosis and are essential to the credibility of analysis that relies on stratified population comparisons. Such analyses require the presence of critical information to be accurate and reliable.

After applying these exclusion criteria, the final analytic cohort included 43,548 adult patients who started dialysis from January 1, 2015, to December 31, 2019, in the southeastern region of the United States. This curated cohort served as the foundation for all subsequent analyses, allowing the research team to evaluate sex/gender differences in transplant referral and evaluation with ample statistical power and demographic representation. The thorough exclusion process helped improve the accuracy of the study's findings and strengthened the validity of the conclusions drawn concerning the population most likely to benefit from enhanced policies concerning equitable transplant access.

3.3. Exposure and outcomes

The primary exposure variable in this study was sex/gender recorded on the CMS Form 2728. By standardized clinical reporting within the USRDS, patients were classified in a mutually exclusive manner as either male or female based on clinician-reported gender. While this binary classification does not encapsulate the full breadth of gender diversity, including transgender and non-binary identities, it conforms to historical CMS documentation practices and was important for uniformity across the expansive dataset analyzed.

The study focused on three primary outcome measures. Each of these milestones is critical in the progression of kidney transplantation. The outcomes are relevant to gaps or inequities associated with sex/gender disparities within the continuum of care for patients with ESKD.

This was defined as whether a patient was actively referred for transplant evaluation within one year of starting dialysis treatment. Referral is a vital preliminary step within the greater transplant process, and omissions or significant delays at this stage severely limit future opportunities. The study assessed the referrals within a bounded time window to assess punctual access to referral, and thus, autonomy, which is essential for clinical outcomes. This outcome was captured if a patient commenced evaluation within six months after the referral date. Evaluation consists of thorough clinical and psychosocial tests and determines a patient's overall candidacy for transplantation. Timely initiation of evaluation is critical. Without prompt evaluation initiation, there is the likelihood of delay, which could suggest inefficiencies or obstacles, maybe due to demographics, socioeconomic class, or provider-level bias. The last primary outcome was whether or not a patient was added to the transplant list preemptively or after evaluation. Being placed on the list is a key milestone that enhances the likelihood of receiving a kidney transplant, whether from a living or deceased donor. This specific outcome sheds light on how sex and gender differences may shape the culmination of early transplant processes and participation in the national waiting list.

In combination, all three of these outcomes provided an opportunity for the researchers to analyze inequities at different, incremental stages in a kidney transplant pipeline — from referral to listing — thereby providing a systemic evaluation of where and how gender-based inequities in access to kidney transplantation manifest.

3.4. Covariates and patient characteristics

The investigation added a wide range of patient-level, facility-level, and neighborhood-level variables to capture the clinical, demographic, and socioeconomic factors that may impact the accessibility of a kidney transplant and the potential confounding factors of sex/gender on surgical outcomes.

3.4.1. Patient-level covariates

These factors offered important information regarding the situation of each patient that could influence their eligibility for a kidney transplant.

A. Age. As a participant's age is recorded at the initiation of dialysis, it becomes relevant both from a medical eligibility and provider perception standpoint of suitability. Additionally, older patients are more likely to encounter age-related comorbidities, which may hinder their referral and evaluation.

B. Race/ethnicity. Respondents' racial and ethnic identities are recorded to capture inequities across gender. It is documented that non-Hispanic Black and Hispanic populations face greater barriers to access due to structural racism and healthcare inequities.

C. Body mass index (BMI). As an indicator of obesity and body composition, BMI also indicates possible risks to surgical intervention. During referral and waitlisting phases, the medical community appears to be concerned about postoperative complications, particularly in women with higher BMI.

D. Primary cause of ESKD. Documented were the conditions identified that cause kidney failure, which include: type 2 diabetes, hypertension, glomerulonephritis, and polycystic kidney disease. These causes may affect eligibility for transplantation and progression, especially in diabetic and hypertensive individuals who tend to face greater barriers to access.

E. Comorbidities. Diagnosing other diseases, such as cardiovascular disease, cancer, and cerebrovascular disorders, was analyzed through diagnostic coding. These comorbidities may disqualify patients or delay their evaluation for transplantation.

F. Pre-ESKD nephrology care. It was noted whether or not the patient received nephrology care before kidney failure. This care improves chances of better outcomes and referrals, leading to higher rates of successful engagements with specialists and improved preparation for transplantation.

G. Transplant education. Participating in formal courses dedicated to enhancing knowledge about transplants was included, as these programs improve patient self-advocacy. Women and underserved populations disproportionately lack education on these topics.

H. Insurance type. To better capture the context of each patient, we recorded their insurance type (ex., Medicaid, Medicare, or private insurance). Insurance type affects one's access to evaluation appointments, needed medications, and selection of facilities. It also serves as a proxy for socioeconomic status.

3.4.2. Facility-level covariates

The dialysis and transplant centers' characteristics were incorporated to account for institutional differences in the delivery of care.

— For-profit status. These facilities were classified based on their for-profit or non-profit designation. Patient outcomes may be influenced by for-profit centers due to differences in resource allocation, staffing models, referrals, and overall center operations.

— Patient-to-social worker ratio. Referring to the workload of social workers that educate, coordinate, and assess transplant patients psychosocially, this metric captures a social worker's workload and resources relative to patients. Limited resources available per patient suggest a higher ratio and may result in diminished support and slowed referrals.

3.4.3. Neighborhood-level covariates

These are sociodemographic variables relative to patients' residential ZIP codes and refer to the immediate environment and community context as determinants of health. Census-derived local poverty rates: These served as a proxy for neighborhood-level socioeconomic status. High-poverty areas are associated with limited access to healthcare, educational resources, and negative health outcomes. The study sought to address multiple potential confounding factors by incorporating an extensive socio-demographic framework, thus providing a more nuanced and contextualized analysis of the continuum of kidney transplant sex/gender disparities. Such thorough adjustment enabled the differentiation of genuine disparities from those influenced by demographic, clinical, or structural inequities.

3.5. Assessing systemic and provider-level factors

While the E-STAR does not include qualitative provider narratives or direct assessments of individual bias, this study utilized proxy variables on multiple levels to assess systemic and provider-related factors contributing to the gender gap within kidney transplantation. These factors spanned patient and facility level datasets to capture administrative, organizational, and clinical decision-making frameworks within the system.

The facility level included:

— for-profit status, serving as a marker for prioritization of resources and patient throughput which may impact referrals;

— patient-to-social worker ratio, capturing the burden on care coordination. A higher ratio may postpone transplant education and psychosocial assessment disproportionately affecting women and other marginalized groups.

At the patient level, several indirect measures of provider contact were analysed.

Pre-ESKD nephrology consultation rates as an indicator of specialist access and leadership regarding the provision of appropriate guidance along the transplant pathway.

Attendance in transplant education programs, as an indicator of availability and provider motivation towards transplantation.

These variables acted as quantifiable proxies for assessing systemic inequities alongside possible biases from providers. Although they do not capture individual acts of bias or discrimination, they reflect access aversion patterns which may stem from unvoiced biases or standardized norms within practices. With integrated qualitative techniques such as interviewing providers and analysing narratives in electronic health records, precision in identifying biases could be sharpened greatly in future research.

3.6. Statistical analysis

Descriptive statistics reported the baseline characteristics. Cumulative incidence curves stratified by sex/gender were used to estimate the time to each event: referral, evaluation start, and waitlisting. The primary inferential method applied was the Cox proportional hazards model to estimate HR for each transplant step with relevant covariate adjustment. Also, logistic regression was conducted to explore differences in odds for important binary outcomes.

Fig. 1 illustrates the gender-wise distribution across key transplant process steps — referral, evaluation start, and waitlisting. It shows that at each stage, women consistently have lower percentages compared to men, with the disparity becoming most pronounced at the waitlisting stage. This visualization supports the statistical findings and underscores gender-based inequities in transplant access.

Cox proportional hazards model

$$h(t | X) = h_0(t) \cdot \exp(\beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n), \quad (1)$$

where $h(t|X)$ is the hazard function at time t given covariates X , and β_i are the model coefficients.

Logistic regression for binary outcomes

$$\log \left(\frac{p}{1-p} \right) \propto \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_n X_n,$$

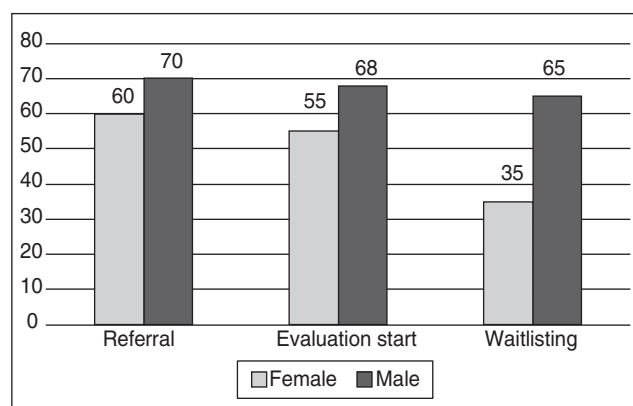


Figure 1. Gender-wise distribution across transplant steps, %

where p is the probability of an outcome (e.g., waitlisting), and X_i are explanatory variables.

3.6.1. Subgroup and sensitivity analyses

Subgroup analyses were stratified by age, race, and BMI to assess interaction effects of sex/gender with regard to referrals, evaluations, and waitlisting processes. Sensitivity analyses included competing risk models (fine-gray) and adjustments for pre-ESKD treatment and year of treatment.

3.7. Ethical considerations

This investigation abides by the STROBE guidelines for observational studies as well as the Declaration of Helsinki. Ethical review was obtained from Emory University's IRB (IRB00113572).

Fig. 2 shows the stepwise approach used in the investigation of a population-based study on gender differences in access to and outcomes of kidney transplants. The study starts with the identification of adult patients with ESKD who commenced dialysis between January 1, 2015, and December 31, 2019. These individuals were located within the boundaries of the USRDS and were limited to End Stage Renal Disease Network 6, which covers the states of Georgia, North Carolina, and South Carolina. Patients who were missing information on race or cause of ESKD, those who were medically or psychologically unfit for a transplant, and those who met other diverse criteria were excluded. The final cohort included 43,548 patients who could be analyzed.

From this final cohort, the first outcome assessed was whether the patient was referred for an evaluation for a kidney transplant within 12 months of starting dialysis. For those referred, the subsequent step assessed whether the patient completed the evaluation for the transplant within six months of the referral. After undergoing evaluation, the

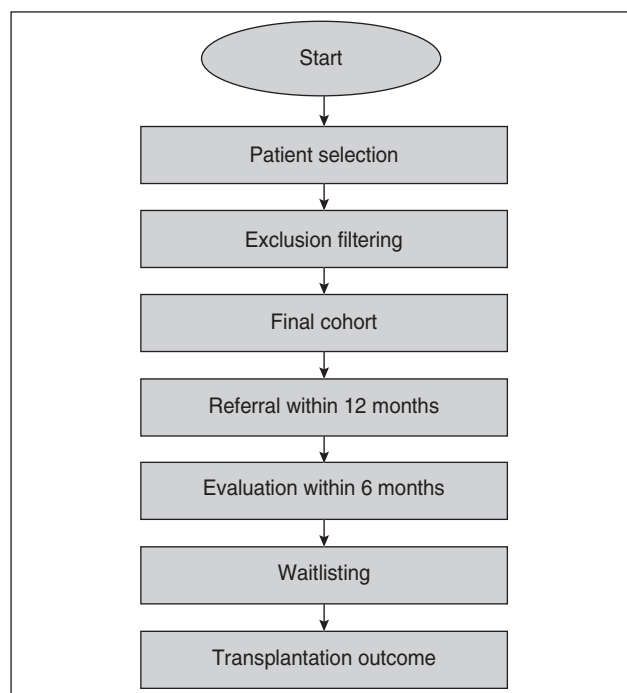


Figure 2. Methodological flowchart

methodology determined whether patients were able to be waitlisted for a kidney transplant, and finally, whether they received a kidney transplant.

Each step in the sequence — referral, evaluation, waitlisting, and transplantation — highlights important moments in the transplant care process that may diverge by sex and gender differences. In this way, the flowchart is pertinent to the study’s aims as it illustrates patient progression through each stage and indicates points where gender comparisons were made about access and outcomes.

4. Results

Table 2 illustrates that the study of 43,548 patients who started dialysis between 2015 and 2019 showed a concerning gender imbalance throughout the kidney transplantation process. The study population consisted of 42.1 % women and 57.9 % men. Notably, women accounted for over 60 % of living kidney donors but participated as less than 35 % of the recipients. This unequal ratio was observed in all phases of the transplant process: referral, evaluation, waitlisting, and transplant. Within a year of starting dialysis, women were 10 % less likely to be referred to a transplant surgeon (HR: 0.90, 95% confidence interval (CI): 0.88–0.93) and 7 % less likely to start the evaluation phase within 6 months after referral (HR: 0.93, 95% CI: 0.89–0.96). After evaluation, the chances of being waitlisted were roughly the same (HR: 0.98, 95% CI: 0.93–1.03) as were the chances of receiving a transplant (HR: 1.02, 95% CI: 0.95–1.10). Though these ratios appear balanced, the inequity at earlier stages limited overall effectiveness and increased the likelihood of lower transplant rates.

Table 2. Gender distribution of kidney transplant recipients

| Transplant process step | Women (%) | Men (%) |
|-------------------------|-----------|---------|
| Started dialysis | 42.1 | 57.9 |
| Referred | 38 | 62 |
| Evaluated | 35 | 65 |
| Waitlisted | 35 | 65 |
| Transplanted | 34 | 66 |
| Living donors | 60 | 40 |

As noted in Table 3, women aged 60–79 and those with diabetes or obesity demonstrated significantly lower odds of being evaluated and referred for transplant. For example, women in the class iii obesity category had a 24 % lower likelihood of being referred compared to men of similar BMI (HR: 0.76, 95% CI: 0.70–0.82). These inequities were further exacerbated by other factors such as race and socioeconomic status. Non-Hispanic Black women and those living in areas of concentrated poverty face even worse delays, or a complete lack of referrals.

4.1. Transgender and gender-diverse individuals: emerging observations

The primary dataset used (CMS Form 2728) considers sex to be a binary attribute and does not accommodate formal analysis of transgender individuals, but clinical reports and parallel studies suggest that transgender (TGD) and gender-diverse individuals encounter specific barriers to kidney transplantation, such as systemic biases and blatant discrimination in medical settings, the need for extensive legal documentation, and hormone therapy or prior surgical interventions that may complicate donor matching and immune compatibility about transplant. Delay due to prejudice associated with record-keeping or evaluation referral for TGD individuals compounds institutional biases. These concerns are increasing the need for systematic exclusion of gender identity from national transplant registries, which would render comprehensive data for TGD populations beyond inequities in care.

5. Discussion

Table 4 illustrates that research adds to the accumulating body of literature demonstrating that the inequalities among the genders about kidney transplant procedures begin well before the actual allocation of the transplant; they begin at the referral and evaluation stages. In particular, women are disadvantaged at almost all levels when suffering from ESKD due to type 2 diabetes and hypertension, which are the leading causes of ESKD, in the Southern region of the United States. These inequities arise from critical factors, which include worsened survival rates, increased reliance on dialysis, and a reduction in life quality among female patients. The results emphasize the need for the incorporation of gender-sensitive approaches in clinical nephrology and in the protocols for referral to other specialists for kidney

Table 3. Disparities in access and clinical indicators

| Subgroup | Disparity description | HR | 95% CI |
|------------------------------|---|---------------|---------------|
| Women aged 60–79 | Lower odds of evaluation and referral | Not specified | Not specified |
| Women with diabetes | Lower likelihood of evaluation and referral | Not specified | Not specified |
| Women with class III obesity | 24 % lower likelihood of being referred compared to similar BMI men | 0.76 | 0.70–0.82 |
| Non-Hispanic Black women | Worse delays or lack of referrals | Not specified | Not specified |
| Women in high poverty areas | Worse delays or lack of referrals | Not specified | Not specified |

transplant evaluation. Addressing these inequities would improve access to kidney transplants and, therefore, improve outcomes for women.

5.1. Possible explanations for disparities

These disparities may stem from several underlying issues. To begin with, women tend to have lower referral and waitlisting rates than their male counterparts with diabetes- and hypertension-attributed ESKD. This indicates a possible providers' bias stemming from the perception of frailty of women, particularly older and obese women. Moreover, due to higher caregiving burdens, women may deprioritize their own healthcare needs, which further decreases the likelihood of receiving a referral. This, along with lower income, education, and greater systemic inequities, serve to disproportionately affect women as social determinants of health. In addition, the risk of sensitization related to pregnancy increases donor incompatibility and complicates surgical planning for women. Together, these integrate biological and sociocultural aspects to limit women's access to transplantation.

5.1.1. Restrictions on gender classification

An essential restriction of this study is that gender identity was captured as binary (male and female) in both the E-STAR and the CMS Form 2728. This means that transgender, non-binary, and gender-diverse individuals are excluded from the dataset. This narrows the scope of analysis to compared to a cisgender-affirming population and hinders understanding concerning how systemic inequities would differently impact gender-diverse populations. The absence of gender identity data disables inclusive research and highlights the need to expand criteria in consideration of registries' data collection policies.

5.1.2. Impact of the COVID-19 pandemic

Even though this investigation was conducted using data between 2015 and 2019, it can be reasonably assumed that the COVID-19 pandemic period worsened the existing gender disparities pertaining to access for kidney transplants. There is evidence detailing the impact on women during the COVID-19 period that includes but is not limited to, disproportionately augmented caregiving responsibilities, limited access to clinics, and economic hardships. A combination

of these factors likely diminished their chances of being referred or evaluated for a transplant. Moreover, hospitals had limited resources, which disproportionately affected provision of services aimed at women with complex health issues. Furthermore, due to the absence of gender identity tracking in national datasets, the impact on transgender and non-binary persons remains unassessed. Further studies are required to analyze the impact of the pandemic on all gendered groups in relation to access for transplantation.

5.2. Recommendations for addressing gender disparities in kidney transplants

The transplant community concentrates their efforts on a proactive strategy for managing women with ESKD resulting from diabetes and hypertension far earlier in the disease process. Clinical strategies include pre-ESKD nephrology consultation, appropriate and on time referrals, as well as educational sessions about the transplant process geared towards women. Training also has modules addressing the caregiving bias to weak and obese women. On the other hand, systematic policies like national monitoring of data on referral with evaluation may assist in tracking and mitigating gaps of data inequities. In addition, compliance with scheduled antihypertensive and antihyperglycemic medications tailored to these risks would qualify more women for transplants. Other essential components include multicenter studies with adequate geographical representation from low middle-income countries and transgender people. These studies analyze the sociocultural, economic, and mental health components of the gender disparity. Closing these gaps makes it possible to design coherent equitable frameworks and policies for organ transplants for all genders and diverse communities.

Alongside quantitative results, future registry frameworks should integrate qualitative elements like provider comments, referral rationales, and patient interviews to achieve a greater understanding of the disparities that exist in the kidney transplantation procedure for different genders. Understanding the context and rationale behind referrals can shed light on healthcare practitioner-level biases and socio-cultural barriers that motivate caregivers which are often masked by structured datasets. With more comprehensive frameworks, future studies will be able to assess the balance between the clinical and psychosocial determinants of the accessibility of transplants. Such an approach

Table 4. Implications of gender disparities in kidney transplant access and outcomes

| Issue/Observation | Description |
|--|--|
| Early-stage inequities | Gender disparities begin at the referral and evaluation stages before transplant allocation |
| Regional impact | Women with ESKD due to diabetes/hypertension in the Southern U.S. are especially disadvantaged |
| Contributing factors | Worsened survival rates, dialysis reliance, and reduced life quality for women |
| Need for gender-sensitive approaches | Call for gender-sensitive protocols in clinical nephrology and specialist referrals |
| Expected impact of addressing inequities | Improved transplant access and outcomes for women through equitable practices |

will augment the richness of the data as well as the design of the specialized corrective measures.

Moreover, transplant registries like the E-STAR and the USRDS should advance to incorporate data fields capturing gender identity beyond the binary framework. By including transgender and non-binary options, researchers would be able to expose inequities that remain concealed due to current reporting practices. Including gender-diverse individuals in transplant registries is vital for advancing equitable health care and ensuring that all population-based interventions are appropriate and effective.

5.3. Expanded recommendations with specific policy interventions

To eliminate the gaps in the disparity of gender-based differences in kidney transplantation, several targeted policy actions are needed. Transplant centers along with dialysis centers should be set up to enforce systematic bias training on communication and referral workshops on scrub nursing for touches and providers on gender-sensitive healthcare. Also, both federal and regional healthcare authorities need to amend all national transplant registries, including the USRDS and the E-STAR, to incorporate non-binary and transgender genders so that there will be better monitoring of the neglected groups. Furthermore, policy initiatives should be introduced which add equity-based evaluation and referral in given performance indicators based on evaluation of care and referral as an equity-based gap driving metrics which the institution incentivizes monitoring woman and care disparity drive gaps. In addition, gender-sensitive nephrology education prior to ESKD should be universal among cultures, particularly to those with low health literacy or high caregiving burdens. Finally, women in these regions of high poverty, especially the Southern US, need to be adequately educated and supported throughout the transplant process by community-based programs and patient navigators that are directly funded for these purposes.

6. Conclusions

In the Southeastern United States, women have an even longer history of the struggle for equal access to the healthcare they need. This region of the country frequently has a higher incidence of type 2 diabetes and hypertension, as they account for 75 % cases. Women with ESKD experience challenges in not being referred and evaluated for a transplant compared to men. Women with equal survival rates are less likely to be referred and evaluated for transplants, and the likelihood diverges based on age, race, and obesity. These trends imply that there would need to be correct gender interventions and remedying social biases that result in disparity in the care women receive must be considered.

Social factors including caregiving burdens and systemic biases — stack the deck against equity and influence the experiences of women. These barriers are much heavier in countries where resources were already limited, making life hard for women. In these contexts, financing is constrained, and social barriers create a perfect storm. In these situations, we have to use a systematic approach that seeks to eliminate

bias while implementing fair referral systems and strong systems with a patient focus, ensuring women and marginalized populations have equitable access. Policies need to be made at the national and international levels to review data with gender distinctions, establish systems that allow for fair allocation, develop finance programs that are low-cost for marginalized populations, and build on the transnational framework in transplantation.

Moreover, the study benefited greatly from the E-STAR with regards to monitoring early-stage transplant processes like referral, evaluation, and waitlisting, which many national datasets overlook. Incorporating data from all nine adult transplant centers from Georgia, North Carolina, and South Carolina gave practical illumination on the precise points and manners where gender-based disparities emerge.

Lastly, future research should clarify differences between sex (biological) and gender (sociocultural) variables in their relationship with transplant outcomes. Ultimately, equalized transplant systems will resurrect the evolution from reactive, evidence-based policy to a proactive approach that ensures timely and life-saving kidney transplants for everyone, regardless of sex, gender identity, and/or socioeconomic status.

Ethics approval. This study was conducted in accordance with the ethical standards of the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board (IRB) at Emory University (IRB00113572), covering the use of patient-level data from the USRDS and the E-STAR. All personal identifiers were removed before analysis to ensure confidentiality.

Data availability. The data that support the findings of this study are available from the USRDS and the E-STAR but restrictions apply to the availability of these data, which were used under license for the current study and are not publicly available.

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Гендерні відмінності в доступі до трансплантації нирок та її результатах: популяційний аналіз

Резюме. Актуальність. Трансплантація нирок вважається кращим методом лікування при термінальній стадії ниркової недостатності (ТСНН), оскільки забезпечує більшу тривалість життя та поліпшення його якості. Проте доступ до трансплантації та результати після неї часто обмежуються через гендерні відмінності. Жінки рідко бувають реципієнтами нирок — більшою мірою вони є живими донорами. Гендерна нерівність у сфері трансплантації зумовлена низкою біологічних і соціокультурних чинників, зокрема сенсibiliзацією після вагітності, біологічними відмінностями в імунній системі, а також гендерними особливостями доступу до медичної допомоги або направлення. Жінки отримують менше направлень на трансплантацію, стикаються із затримками в обстеженнях і рідше порівняно з чоловіками отримують підтримку в питаннях трансплантації. **Матеріали та методи.** Це популяційне дослідження базується на даних реєстру the Early Steps to Transplant Access Registry і охоплює пацієнтів із ТСНН із Джорджії, Північної та Південної Кароліни. У ньому розглянуто гендерні відмінності на всіх етапах — від направлення до результатів після трансплантації. Проведено статистичну корекцію щодо клінічних прогностичних змінних і демографічних чинників для оцінки незалежного впливу статі на доступ до трансплантації та її результати. **Результати.** Аналіз показує, що жінки із ТСНН значно рідше, ніж чоловіки, потрапляють до листа очікування або отримують трансплантат, навіть після врахування інших факторів. Ці відмінності зберігаються протягом усього

процесу трансплантації, і нерівність очевидна на кожному етапі. Хоча певні біологічні аспекти, як-от ризик відторгнення трансплантата чи відмінності в метаболізмі імуносупресивних препаратів, частково пояснюють деяку варіацію в результатах, гендерно обумовлені соціальні детермінанти (наприклад, упередженість постачальників медичних послуг) та системна нерівність загалом пояснюють більшу складність у доступі для жінок. Також слід звернути увагу на нові перешкоди, що викликають серйозне занепокоєння, зокрема недостатньо досліджені медичні потреби трансгендерних людей та вплив пандемії COVID-19 на реципієнтів трансплантатів, що посилює гендерну нерівність у доступі до трансплантації. **Висновки.** Це дослідження підкреслює нагальну необхідність виявлення та врахування питань гендерної рівності при прийнятті рішень щодо трансплантації нирки. Незважаючи на можливі клінічні обґрунтування гендерної нерівності, такі проблеми виникають переважно через модифіковані соціокультурні та інституційні фактори. У системах трансплантації досі офіційно не враховано гендерну чутливість при усуненні бар'єрів до доступу. Необхідне термінове втручання на рівні політики, просвітницька робота й підтримка, аби забезпечити справедливий доступ і рівність результатів для всіх людей, особливо тих, хто має різну гендерну ідентичність.

Ключові слова: трансплантація нирки; гендерна нерівність; доступ до медичної допомоги; результати за статевою ознакою; трансплантаційна рівність

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Analysis of the use of artificial intelligence systems for the development of physical exercise programs during rehabilitation of nephrology patients

Abstract. Background. Artificial intelligence (AI) is a direction of mathematical computer modeling based on the abstract essence of mathematical thinking. Chronic kidney disease (CKD) is a nosological unit, its final stage (end-stage renal disease) has seen an exponential increase over the past decade and is considered by the World Health Organization as a global problem by cause of death. The global healthcare industry is one of the main planes for practical application of modern developments in the field of AI thanks to machine learning algorithms that provide new opportunities for solving the most complex problems of medicine and pharmacy. The purpose was to analyze the possibility of using physical exercise complexes (PECs) created by AI system in patients with CKD undergoing renal replacement therapy and to compare PECs created by AI with the list of PECs used in clinical practice (systematic reviews and meta-analyses) for rehabilitation care in nephrology. **Materials and methods.** Scientometric analysis of professional literature from electronic databases PubMed, Embase, Scopus and Web of Science, Cochrane CENTRAL was conducted. According to the purpose of the study, the following methods were used: bibliosemantic, systematic approach, descriptive modeling using AI systems — Gemini and ChatGPT. **Results.** AI systems (Gemini and ChatGPT) proposed exercise programs for patients with CKD that take into account different stages of rehabilitation (respiratory, aerobic, strength, stretching and relaxation). At the time of the descriptive modeling, the database used by Gemini and ChatGPT is sufficient for their routine use in the development of exercise therapy complexes for the rehabilitation of nephrological patients with different nosologies. **Conclusions.** Artificial intelligence is a tool in the hands of a physician to provide medical care; the quality of this tool will depend on the qualifications of the physician who will teach (machine learning) AI to use their knowledge and competencies to optimize the process of creating rehabilitation complexes for patients with kidney disease from the standpoint of evidence-based medicine.

Keywords: physical exercises; chronic kidney disease; rehabilitation aid; artificial intelligence

Introduction

Artificial intelligence (AI) is a field of mathematical computer modeling based on the abstract essence of mathematical thinking. The history of the formation and development of AI, a term officially coined in 1956 by John McCarthy [1], is rather ambiguous, distinguishing between

the forerunners of formation, the stage of inception, the stages of rapid development — boom and the stages of decline — “winters” [2–4].

Chronic kidney disease (CKD) is a nosological unit of kidney disease that has seen an exponential increase in patients with end-stage renal disease over the past decade

and is considered by the World Health Organization to be a global problem by cause of death [5–7].

The global healthcare industry is one of the main “planes” of practical application of modern AI developments. At the initial stages of AI development, the focus was on the development of machine learning algorithms. Thanks to them, new opportunities were created to solve the most complex problems in medicine [8–14] and pharmacy [15, 16]. Recently, Chatbot’s have been widely used in medicine to automate various types of tasks, from making an appointment with a doctor to receiving basic first aid recommendations and even psychological assistance. Currently, the most advanced AI Chatbot’s are two competing neural networks — Gemini by Google and ChatGPT by OpenAI. They are capable of generating texts in various subject areas (including medical), translating, creating creative content, and answering user questions in an informative manner.

The purpose. To analyze the use of physical exercise complexes (PECs) created by the AI system for use in patients with CKD undergoing renal replacement therapy for rehabilitation care and to compare these PECs with the list of PECs used in clinical practice according to the scientometric search of research results (systematic reviews and meta-analyses) on rehabilitation care in nephrology practice.

Materials and methods

The study was performed within the framework of the scientific theme of the Department of Pediatrics, Neo-

natology and Perinatal Medicine of Bukovinian State Medical University on the subject “Chronobiological and adaptive aspects and peculiarities of vegetative regulation with pathological conditions in children of various age groups”. The state registration No. 0122U002245, the deadline: 01.01.2022 — 31.12.2026. Scientometric analysis of professional literature of electronic databases PubMed, Embase, Scopus and Web of Science, Cochrane CENTRAL. According to the purpose of the study, the following methods were used: bibliosemantic, systematic approach, descriptive modeling using AI systems: Gemini and ChatGPT.

Results

These days, AI, through the use of mathematical methods to predict or classify various types of medical data, structured or unstructured, has made significant changes in a number of professional areas in the medical field [8–14], and nephrology is no exception [17, 18].

According to the objective, the study consisted of two stages. At the first stage of our study, we tasked two AI systems, Gemini and ChatGPT, with suggesting a set of exercises that would be best for patients with CKD on hemodialysis. Both programs emphasized the importance of consulting a doctor before starting exercises and having a physiotherapist monitor the exercises. The exercise sets suggested by the AI included aerobic exercises, strength exercises, and exercises.

Gemini AI suggested the following exercises (Fig. 1):

1. Aerobic exercises (walking, cycling, swimming) 20–30 minutes, 3–5 times a week to improve cardiovascular function, increase endurance, improve mood and sleep quality.

2. Strength exercises (chair lifting, arm bending with dumbbells or water bottles, leg extensions, exercises with an elastic band) 2–3 times a week to strengthen muscles, improve metabolism, and maintain bone density.

3. Flexibility exercises (stretching of neck, shoulder, back, and leg muscles) daily to improve joint mobility, reduce the risk of injury, and relieve muscle tension.

ChatGPT AI suggested the following set of exercises (Fig. 2, 3):

1. Breathing exercises (daily, 5–10 minutes) to improve blood oxygenation and reduce stress.

2. Warm-up (slow walking on the spot, circular movements of arms, shoulders, feet) 5–7 minutes.

3. Aerobic exercises (walking on the spot or on a treadmill, exercise bike, soft stepping) for 10–15 mi-

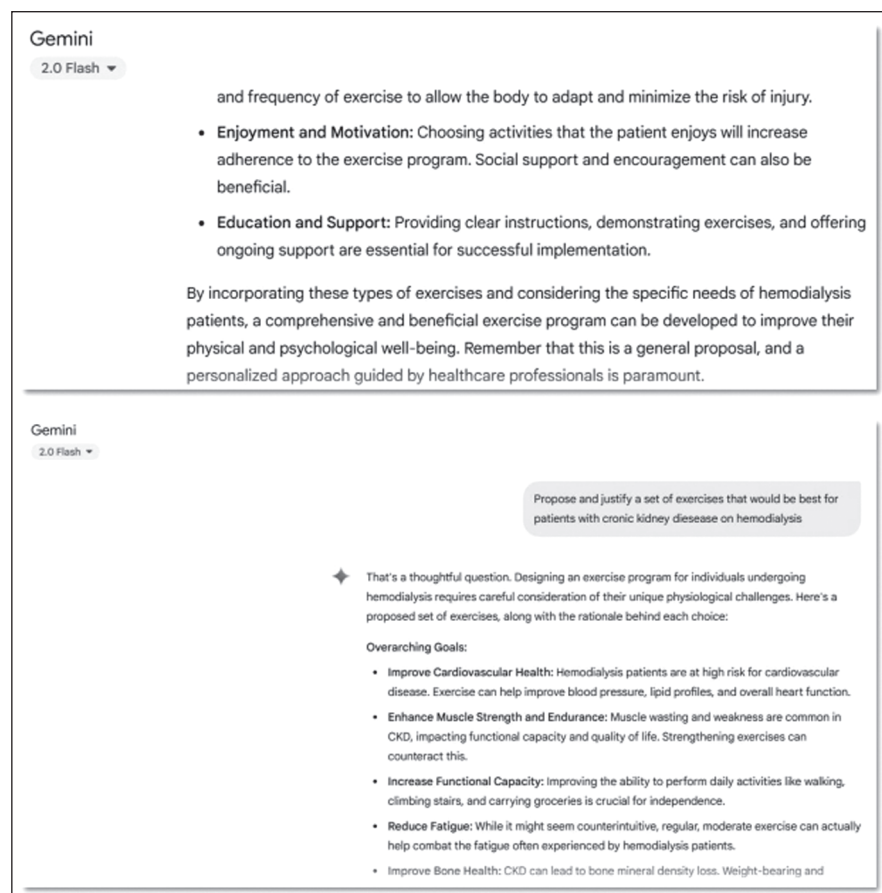


Figure 1. Gemini AI suggested the following exercises

minutes to improve cardiovascular function.

4. Exercises for muscle strength (squatting to a chair, extending and bending the legs in a sitting position, lifting the arms with a small weight, elbow plank) for 10–15 minutes to prevent muscle atrophy.

5. Stretching and relaxation (stretching calf muscles, stretching back, relaxation) 5–10 minutes to improve flexibility, reduce the risk of muscle spasms and improve psycho-emotional state.

At the second stage of the study, during a systematic review and scientometric analysis of the professional literature of the electronic databases PubMed, Embase, Scopus, Web of Science, Cochrane CENTRAL, we selected and summarized the results of 3 meta-analyses, which included 131 scientific studies evaluating the effect of physical activity on the condition of patients with CKD on renal replacement therapy (5780 patients with CKD) [19, 20, 22].

The authors of meta-analyses have found that among the possible options for physical activity, aerobic and strength exercises, as well as their combinations, give the best results. To evaluate the

ChatGPT ▾

Suggest a set of exercises that would be best for patients with chronic kidney disease on hemodialysis

Exercise can be highly beneficial for patients with **chronic kidney disease (CKD) on hemodialysis**, improving cardiovascular health, muscle strength, and quality of life. However, the exercise plan must be tailored to their health status, dialysis schedule, fatigue levels, and any comorbidities (e.g., diabetes, hypertension, heart disease). Always consult a healthcare provider before starting a new routine.

Here's a **safe and effective set of exercises** for hemodialysis patients, split into **three main categories**:

ChatGPT ▾

Propose and justify a set of exercises that would be best for patients with chronic kidney disease on hemodialysis

For patients with **chronic kidney disease (CKD) on hemodialysis**, the best exercise regimen should focus on **improving cardiovascular health, muscle strength, endurance, and overall quality of life**, while being **safe, low-impact, and adaptable** to their fluctuating energy levels. Here's a proposed set of exercises, categorized and justified:

ChatGPT ▾

Conclusion

The best exercise set for hemodialysis patients combines **aerobic, resistance, flexibility, and balance training**, with an emphasis on **intradialytic exercise** to increase compliance and health outcomes. This holistic approach supports **physical function**, reduces **CKD complications**, and enhances **mental and emotional well-being**.

Figure 2. ChatGPT AI suggested the following set of exercises

ChatGPT ▾

31 Weekly Sample Exercise Routine for Hemodialysis Patients

| Day | Type of Exercise | Duration | Details & Focus |
|-----------|---|-----------------|--|
| Monday | Intradialytic Aerobic + Resistance Training | 20–30 mins | - Pedal a mini-cycle or do seated leg lifts during dialysis - Light dumbbell curls or band exercises for arms - Avoid fistula arm |
| Tuesday | Walking + Stretching + Balance | 30 mins | - 15–20 min walk (indoors or outdoors) - 10 min stretching (hamstrings, calves, shoulders) - 5 min balance (heel-to-toe, single-leg stand) |
| Wednesday | Intradialytic Resistance + Flexibility | 20–30 mins | - Seated resistance band exercises (legs, arms) - Gentle yoga/stretching post-exercise |
| Thursday | Restorative Activity + Flexibility | 15–20 mins | - Light yoga or tai chi session - Full-body stretching - Deep breathing exercises |
| Friday | Intradialytic Aerobic + Light | ↓ 20–30 mins | - Pedal or march in place |

Figure 3. ChatGPT AI suggested the following set of exercises for patients with CKD on hemodialysis

results, the authors of scientific studies most often used the 6-minute walk test and quality of life, and it should be noted that physical activity did not show a significant improvement in quality of life in patients with CKD. In most studies, aerobic and strength training alone and together (both interdialytic and intradialytic) significantly improved the results of the 6-minute walk test [19, 20, 22].

Using Gemini AI, we summarized the physical exercises proposed by the authors of the articles included in the meta-analyses [19, 20, 22]. The exercises were used both individually and in combination. They differed in duration (from 10 minutes to an hour), frequency (from daily to 2–3 times a week), and intensity (which was regulated by the Borg scale or maximum heart rate and gradually increased).

A set of exercises for patients with CKD on hemodialysis:

1. Aerobic:
 - cycling ergometry;
 - walking;
 - jogging;
 - aerobic movements with range of motion: wrist rotation, ankle flexion and extension;
 - exercises without resistance;
 - pedaling. Strength exercises;
 - exercises for the upper extremities: chest press, biceps curls, triceps extensions, dumbbell press, incline deadlift, overhead dumbbell press;
 - exercises for the lower extremities: squats, knee extension and flexion, leg press, plantar flexion of the foot, hip abduction and adduction, hip flexion, lower leg raises, lower extremity raises;
 - exercises for the core muscles: abdominal exercises, pelvic lifting;
 - specialized exercises: exercises with an elastic ball;
 - stretching: exercises to stretch the lower extremities.
2. Combined training:
 - aerobic + strength. Aerobic exercises are represented by cycling, walking, step aerobics, circuit training. Strength exercises include exercises with weights, elastic bands, bodyweight exercises, dynamic exercises;
 - aerobic + stretch/flexibility. Combine cycling ergometry with stretching and flexibility exercises;
 - strength + stretching/flexibility. Combine strength exercises with stretching and flexibility exercises;
 - aerobic + strength + stretching/flexibility + balance exercises. The most comprehensive combination that includes all the main components of physical fitness.

Summarizing the results of the second stage of our study, we can conclude that the set of physical activities proposed by AI corresponds to the physical exercises provided by the authors of meta-analyses that included 131 scientific studies assessing the effect of physical activity on the condition of patients with CKD undergoing renal replacement therapy [19, 20, 22].

Discussion

Both AI systems proposed PT complexes for CKD patients requiring renal replacement therapy that take into account different stages of rehabilitation (respiratory, aerobic,

strength, stretching, and relaxation). Thus, the knowledge base inherent in Gemini AI and ChatGPT AI is sufficient to be used in the development of physical activity programs for the rehabilitation of patients with different diagnoses [17, 18, 23–29].

Currently, the issue of the legal framework for the use of artificial intelligence in medical practice remains unresolved in Ukrainian legislation, while there is hope that this issue will be resolved with the availability of international experience and recommendations [30, 31].

Prospects for further research. Clinical analysis of physical rehabilitation methods for patients with CKD created using AI.

Conclusions

Artificial intelligence is a tool in the hands of a doctor to provide medical care, the quality of this tool will also depend on the qualifications of the doctor who will teach (machine learning) AI to use the doctor's knowledge and competencies to optimize the process of creating complexes of exercise therapy for rehabilitation purposes for patients with kidney disease.

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Аналіз використання систем штучного інтелекту для розробки програм фізичних вправ під час реабілітації нефрологічних пацієнтів

Резюме. Актуальність. Штучний інтелект (ШІ) — це напрям математичного комп'ютерного моделювання, що базується на абстрактній сутності математичного мислення. Хронічна хвороба нирок (ХХН) є нозологічною одиницею, кількість пацієнтів з термінальною стадією якої за останнє десятиріччя експоненційно зросла, тому Всесвітня організація охорони здоров'я визнає її глобальною проблемою за показником смертності. Світова індустрія охорони здоров'я є одним із основних напрямів практичного застосування сучасних розробок у сфері ШІ завдяки алгоритмам машинного навчання, які відкривають нові можливості для вирішення найскладніших задач медицини та фармації. **Мета:** проаналізувати можливість використання комплексів фізичних вправ (КФВ), створених системами ШІ, у пацієнтів із ХХН, які проходять замісну ниркову терапію, та порівняти КФВ, запропоновані ШІ, зі списком КФВ, що застосовуються в клінічній практиці (систематичні огляди та метааналізи) для реабілітаційної допомоги в нефрології. **Матеріали та методи.** Було проведено наукометричний аналіз професійної літератури з електронних баз даних PubMed, Embase, Scopus

та Web of Science, Cochrane CENTRAL. Відповідно до мети дослідження використано такі методи: бібліосемантичний, системний підхід, описове моделювання з використанням систем ШІ — Gemini та ChatGPT. **Результати.** Системи ШІ (Gemini та ChatGPT) запропонували програми вправ для пацієнтів із ХХН, що враховують різні етапи реабілітації (дихальні, аеробні, силові, розтяжка та релаксація). На момент описового моделювання база даних, що використовується Gemini та ChatGPT, є достатньою для їхнього рутинного використання при розробці комплексів фізичних вправ для реабілітації нефрологічних пацієнтів із різними нозологіями. **Висновки.** Штучний інтелект є інструментом у руках лікаря для надання медичної допомоги; якість цього інструменту залежатиме від кваліфікації лікаря, який навчатиме (машинне навчання) ШІ використовувати свої знання та компетенції для оптимізації процесу створення реабілітаційних комплексів для пацієнтів із захворюваннями нирок з позиції доказової медицини.

Ключові слова: фізичні вправи; хронічна хвороба нирок; реабілітаційна допомога; штучний інтелект

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Роль сечовини при патологічних станах

Резюме. Сучасні дослідження демонструють значну роль сечовини та її продуктів в розвитку низки захворювань. В основі її патологічних впливів лежить дисфункція ферментів циклу сечовини вродженого чи набутого характеру, що реалізується через активацію оксидативного стресу, апоптозу, карбамілювання, гормональну дизрегуляцію. Вивчення механізмів впливу цієї органічної сполуки на патофізіологічні процеси відкриває широкі перспективи в застосуванні нових фармакологічних підходів для лікування захворювань нирок, печінки, серцево-судинної та нервової систем, онкопатології. З іншого боку, ізольоване визначення рівня сечовини в крові або її співвідношення з креатиніном та альбуміном є надійними прогностичними маркерами тяжкості та смертності при різноманітній патології.

Ключові слова: сечовина; патологія; апоптоз; карбамілювання

Сечовина — це органічна сполука з невеликою масою, яка є основним продуктом азотистого обміну внаслідок катаболізму білків та амінокислот. Основними органами, які беруть участь в підтриманні балансу сечовини в організмі, є печінка та нирки. Аналіз літератури, який був поданий у попередньому огляді, показав, що сечовина є не тільки кінцевим продуктом обміну, але і фізіологічно активною речовиною. Традиційно вважається, що сечовина бере участь у процесі обміну білків та осморегуляції. Але спектр її дії значно ширший. Це регуляція швидкості клубочкової фільтрації (ШКФ), артеріального тиску, репродуктивної функції, апоптозу, окиснювального стресу, запалення та фіброзу [1–4]. Підтвердженням важливої фізіологічної ролі сечовини є відкриття позапечінкового шляху утворення сечовини, при якому не відбувається нейтралізації аміаку. Це може вказувати на важливу роль сечовини в регуляторних процесах та прагнення організму зберегти її на постійному рівні в межах від 2,5 до 8 ммоль/л. Її рівень може змінюватись при фізіологічних і патологічних станах, які включають гостру та хронічну патологію.

Причинами підвищення концентрації рівня сечовини в крові можуть бути зневоднення внаслідок зменшення вживання рідини або її втрати під час діареї, блювання, потовиділення; дисфункція нирок внаслідок їх гострого пошкодження (ГПН) або хронічної

хвороби нирок (ХХН) шляхом порушення фільтрації та реабсорбції сечовини, що має значення, зокрема, для пацієнтів з серцевою недостатністю (СН) внаслідок зменшення кровотоку в нирках. Стани, які супроводжуються підвищенням катаболізму білків (травми, оперативні втручання, опіки, запальні процеси в м'язах, системне запалення) та метаболічною дизрегуляцією при ендокринних захворюваннях (цукровий діабет (ЦД), захворювання щитоподібної залози, наднирників), призводять до надмірного накопичення сечовини в крові. Цьому сприяє підвищене всмоктування продуктів розпаду білкових компонентів крові при шлунково-кишкових кровотечах. Підвищена концентрація сечовини в крові притаманна патологічній вагітності при патології розвитку плода та дисфункції печінки або нирок у вагітної. Необхідно враховувати також ятрогенні впливи на зростання концентрації сечовини при призначенні діуретиків, антибіотиків, стероїдних гормонів [5].

Зниження рівня сечовини спостерігається в меншій кількості випадків і здебільшого обмежується захворюваннями печінки (стеатоз, гепатит, цироз печінки (ЦП)) [6]. Досить рідко причиною зниження утворення сечовини можуть бути генетичні порушення циклу сечовини (ЦС), що призводять до гострої або хронічної інтоксикації амонієм [7].

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Метою нашого огляду є розгляд ролі сечовини та її діагностичного та прогностичного значення при деяких патологічних станах.

Класичною моделлю для вивчення механізмів дії сечовини є пацієнти з ХХН та термінальною стадією ниркової недостатності (ТХНН). При цих станах концентрація сечовини в крові може перевищувати в 5–6 разів показники у здорових осіб. Раніше вважалося, що ці рівні сечовини можуть суттєво не впливати на організм. Більш пізні дослідження вказують на прямі та непрямі побічні ефекти сечовини на різні органи, хоча її патогенність досі піддається сумніву, оскільки дослідження, що оцінюють її побічні ефекти, не мають однорідності [8–10].

Підвищений рівень сечовини в концентраціях, типових для пацієнтів з уремією, викликає руйнування кишкового епітеліального бар'єра, що призводить до транслокації бактеріальних токсинів у кровотік та системного запалення. Механізм цього процесу був з'ясований в експерименті. Накопичення сечовини у внутрішньо- та позаклітинній рідині у пацієнтів та тварин з прогресуючою ХХН призводить до її значного надходження до шлунково-кишкового тракту (ШКТ) шляхом пасивної дифузії та включення до залозистого секрету. У просвіті кишечника сечовина гідролізується спонтанно мікробною уреазою, утворюючи велику кількість аміаку, який перетворюється на гідроксид амонію. Останній є їдкою основою, здатною викликати цитотоксичність та пошкодження тканин, що сприяє різкому порушенню бар'єрної функції та руйнуванню ключового білка TJ (щільного з'єднання): кляудину-1, оклюдину та ZO1 [11].

Існують поширені непрямі наслідки підвищеного рівня сечовини в результаті реакцій карбамілювання, де продукт катаболізму сечовини — ізоціанова кислота — реагує з тіолами та аміногрупами білків, амінокислот, цукрів тощо. Ізоціанова кислота також виробляється в організмі в результаті метаболізму тиоціанату за участю мієлопероксидази [12, 13]. Вказаний процес суттєво впливає на структурні та функціональні властивості білків і призводить до прискореного процесу їх старіння. Карбамілювання також впливає на такі макромолекули, як гемоглобін, ліпопротеїни плазми, альбумін, мембранні білки та еритропоетин у пацієнтів із ХХН, сприяючи розвитку анемії, нирковому фіброзу, атеросклерозу [13]. Так, у пацієнтів із ХХН спостерігалися зміни у структурі гемоглобіну та негемових білків еритроцитів [14]. *In vitro* після обробки еритроцитів сечовиною відмічається збільшення плинності ліпідної мембрани та зміни цитоскелета мембрани еритроцитів [15]. Карбамілювання та окиснення *in vitro* призводять до апоптозу в лімфоцитах [16]; викликають зміни у вторинній та третинній структурі білків, впливаючи на доступність активного центру білка для ферментів. Цей процес також призводить до змін у білок-білкових та/або білок-ліпідних взаємодіях. Так, карбамілювання колагену I типу призводить до порушень у структурі потрійної спіралі, що знижує полімеризаційну здатність нормальних волокон [17]. Карбамілювані білки можуть активувати мезангіальні клітини до профібро-

генної форми, що може сприяти розвитку ниркової недостатності [18].

У свою чергу, карбамілювані ліпопротеїни низької щільності (ЛПНЩ) впливають на атеросклеротичний процес через участь в утворенні пінистих клітин, індукції апоптозу ендотеліальних клітин та проліферації гладком'язових клітин. У цьому процесі бере участь мієлопероксидаза [12, 19, 20]. Сечовина індуктує вироблення активних форм кисню (АФК) в ендотеліальних клітинах аорти людини, що призводить до активації прозапальних шляхів та інактивації антиатеросклерозного ферменту PGI₂-синтази в ендотеліальних клітинах артерій людини, викликаючи зміни в мітохондріальних білках і в експресії запальних маркерів [20, 21]. Крім того, у клітинах гладких м'язів аорти людини сечовина впливає на експресію VAD (промотора смерті, пов'язаного з В-клітинною лімфою 2 (BCL2), проапоптозного члена родини BCL2) [22]. Це явище може сприяти посиленню апоптозу, що спостерігається в артеріальній стінці пацієнтів із ХХН, і може сприяти медіальній кальцифікації судин [23]. При дослідженні впливу фізіологічних і патологічних концентрацій сечовини у пацієнтів з ХХН на лінію ендотеліальних клітин людини з мікроциркуляції (Human Microvascular Endothelial Cells-1, HMEC-1) було встановлено, що сечовина (5 г/л) спричиняє зниження швидкості проліферації та стимулює перехід ендотелію в мезенхіму (EndMT), сприяє перебудові актинових ниток, значно збільшує експресію матриксних металопротеїназ 2 (MMP-2) та змінює рівень інших біомаркерів EndMT (кератину, фібриліну-2 та колагену IV). Після впливу сечовини на HMEC-1 виявилась суттєво порушеною експресія білків диметиларгініндиметиламіногідролази і вазорину, які безпосередньо пов'язані з серцево-судинними захворюваннями (ССЗ) [24].

Крім того, сечовина стимулює оксидативний стрес та дисфункцію в адипоцитах, що призводить до інсулінорезистентності. Було показано, що обробка адипоцитів 3T3-L1 сечовиною в концентраціях, подібних до тих, що є у пацієнтів з ХХН, призводить до продукування активних форм кисню (АФК), викликає інсулінорезистентність, збільшує експресію адипокінів, ретинол-зв'язуючого білка 4 та резистину, а також збільшує рівень модифікованих О-зв'язаним N-ацетилглюкозаміном сигнальних молекул інсуліну [25]. При ХХН часто спостерігається порушення гомеостазу глюкози, що призводить до підвищеної смертності пацієнтів. Це, ймовірно, пов'язано з порушенням секреції інсуліну, що може бути результатом прямої дії сечовини на β-клітини підшлункової залози [26].

Цікавим є той факт, що тимчасовий вплив сечовини на клітини викликає стійке вироблення мітохондріальних АФК та ендотеліальну дисфункцію навіть після гемодіалізу. Стійке збільшення рівня АФК після того, як клітини тривало не піддаються впливу сечовини, може відігравати важливу роль у подальшому пошкодженні нирок та їх функціональному зниженні, незважаючи на зниження рівня сечовини після діалізу. Результати дослідження пропонують молекулярну основу для ро-

зуміння уремічної пам'яті, яка може постійно спричинити розвиток судинних пошкоджень, викликаних уремією, навіть коли уремічний стан знижений або нормалізований. Уремічна пам'ять може пояснити нездатність періодичного діалізу зменшити частоту ССЗ у пацієнтів з ТХНН [27].

Останніми роками все більше досліджень показують, що рівень азоту сечовини (BUN) не лише є відображенням функції нирок, а й може бути пов'язаний із запальним станом організму. Системний імунозапальний індекс (SII) — це комплексний індекс, який враховує кількість тромбоцитів, нейтрофілів і лімфоцитів і вважається ефективним для відображення імунного статусу організму та запальної реакції. Дослідження, які були проведені у популяції підлітків у США, виявили значну негативну кореляцію між рівнями SII та BUN, причому рівні BUN зменшувалися, коли рівні SII зростали (BUN як залежна змінна та SII як змінна результату) [28].

Здатність до синтезу сечовини пов'язана з функціональною масою печінки, тому вона знижується у пацієнтів з ЦП або порушенням функції печінки з інших причин [3, 4]. Зниження здатності до синтезу сечовини погіршує здатність пацієнта виводити потенційно токсичні рівні азотистих речовин, і, зрештою, це призводить до підвищеного ризику печінкової енцефалопатії [29].

Виражений дефіцит або повна відсутність активності ферментів ЦС — карбамілфосфатсинтази (CPS1), орнітинтранскарбамоїлази (ОТС), аргініносукцинатсинтази (ASS1), аргініносукцинатліази (ASL), аргінази (ARG) або продуцента кофактора N-ацетилглютаматсинтази — призводить до накопичення аміаку та інших метаболітів попередників упродовж перших днів життя. Невдовзі після народження розвивається набряк головного мозку з ураженням ЦНС (печінкова енцефалопатія, неврологічні та рухові порушення). При менш тяжкому дефіциті цих ферментів і при недостатності ARG пусковим моментом гіперамоніємії можуть бути захворювання або стресові ситуації у будь-якому віці [7]. Механізмів, залучених у патофізіологію порушення циклу сечовини (ПЦС), багато, але є дані, що підсилений оксидативний стрес та запальний процес відповідальні за частину пошкоджень клітин мозку. Так, при обстеженні пацієнтів з різними дефіцитами основних ферментів ЦС встановлено підвищений вміст у крові продуктів окиснення ліпідів (похідних тіобарбітурової кислоти), білків (карбонілу) та збільшення прозапальних (IL-6, IL-8, TNF- α) та протизапальних цитокінів (IL-10) [30].

Цікавим є вивчення ЦС у пацієнтів із захворюваннями печінки різної етіології. Так, у пацієнтів з алкогольним гепатитом (АГ) порушується синтетична функція та присутній запальний компонент, але як ці протилежні ефекти збалансовані, залишається незрозумілим. Встановлено, що введення низьких доз алкоголю різко знижує синтез сечовини у здорових добровольців, тимчасово сприяючи збереженню азоту, і цей ефект, ймовірно, не залежить від гормональної регуляції [31].

Результати дослідження показали, що АГ значно знижує здатність до синтезу сечовини, і це відбувається

до рівня, який раніше спостерігався лише при гострій печінковій недостатності. Крім того, зниження здатності до синтезу сечовини пов'язане зі збільшенням тяжкості клінічного захворювання. Метаболічна недостатність при АГ призводить до нездатності печінки адекватно сприяти метаболічній регуляції, що спостерігається при інших стресових станах, пов'язаних з позапечінковим запаленням. Це може бути поганою прогностичною ознакою, оскільки ставить під загрозу здатність організму адаптуватися до гомеостатичних потреб запалення при АГ [32].

Порушення ЦС має прогностичне значення у пацієнтів з неалкогольним стеатогепатитом і може спричинятися епігенетичним пошкодженням генів ферментів ЦС та підвищенням старінням гепатоцитів. Це призводить до гіперамоніємії, активації зірчастих клітин та прогресування захворювання з розвитком фіброзу. Проблема може посилюватися паралельними змінами в глутаміновій/глутаматній системі. В експерименті було встановлено зниження експресії генів та білків ОТС та CPS1, а також активність ОТС, що пов'язано з процесами гіперметилування. Встановлено, що накопичення жиру в печінці має зворотний вплив на зниження функції цих ферментів. У свою чергу, накопичення аміаку в печінці призводить до запалення, активації зірчастих клітин та розвитку фіброзу. Це може бути важливим механізмом переходу легкого стеатозу в стеатогепатит і далі в ЦП та гепатоцелюлярну карциному (ГЦК) [33, 34].

Встановлено також U-подібний зв'язок між рівнем сечовини та тяжкістю захворювання печінки. У пацієнтів з низьким рівнем сечовини спостерігався підвищений ризик розвитку фіброзу печінки, ЦП та печінкової декомпенсації [35].

Здатність до синтезу сечовини прискорюється в стресових ситуаціях, таких як біль, нещодавня операція, неконтрольований діабет і запалення, що відбувається поза печінкою [36]. Так, експериментально індуковане запалення призводить до збільшення здатності до синтезу сечовини у щурів, що може сприяти втратам азоту в організмі та м'язової маси тіла, а отже, становити загрозу для цілісності організму і погіршити клінічний перебіг [37, 38].

Підвищений рівень сечовини крові був незалежно пов'язаний з порушенням функції периферичних нервів у пацієнтів з ЦД 2-го типу (ЦД2) [39].

У літературі є повідомлення, що пацієнти з активним запальним захворюванням кишечника мають негативний баланс азоту. Проведені дослідження з перевірки базального та амінокислотного стимульованого синтезу сечовини у пацієнтів з хворобою Крона та неспецифічним виразковим колітом вказують на підсилений її синтез при активному запальному процесі, при цьому рівень регуляторів синтезу сечовини (глюкагон, кортизол, IL-6, IL-1 α , IL-1 β , TNF- α) не відрізнявся у пацієнтів з неактивним запальним процесом. Це вказує на те, що прискорене перетворення амінокислотного азоту в печінці сприяє менш ефективній економії азоту у пацієнтів з активними запальними захворюваннями кишечника [40].

Після неускладненої хірургічної травми у людини було встановлено, що амінокислоти видаляються з крові печінкою шляхом подвоєння печінкової ефективності синтезу сечовини. При будь-якій концентрації амінокислот у плазмі виводиться вдвічі більше аміноазоту, ніж азоту сечовини, і таким чином втрачається можливість для синтезу білка. Ця стресова реакція печінки триває протягом одного тижня після операції. Збільшення синтезу сечовини саме по собі пояснює близько 50 % післяопераційних втрат азоту. Виявлення патофізіологічних змін після хірургічної травми, ймовірно, є вирішальним для зусиль щодо покращення післяопераційної захворюваності та смертності [41].

Дизрегуляція сечовини, ймовірно, є спільним порушенням у мозку пацієнтів з нейродегенеративними розладами — хворобою Альцгеймера (ХА), хворобою Гантінгтона, Паркінсона та судинною деменцією — і може відігравати певну роль у патогенезі цих захворювань через руйнування гематоенцефалічного бар'єра, оксидативний стрес та карбамілювання, тому вона становить потенційну терапевтичну мішень [42]. Класичні ознаки патології ХА, які, як вважається, її спричиняють, включають бляшки амілоїду бета, а також нейрофібрилярні тау-клубки. Однак дослідження цих класичних ознак не можуть пояснити причинно-наслідкового зв'язку. Підвищений рівень сечовини та аміаку, виявлений у випадках ХА, вказує на дисфункціональний ЦС, задіяний в ХА. Ферменти ЦС, що становлять інтерес при патології ХА, включають ОТС, ізоформи NOS, ARG1, ARG2, орнітиндекарбоксилази (ODC) та є перспективними терапевтичними мішенями. Метаболіти сечовини при ХА мають різну концентрацію в різних ділянках мозку та різних типах клітин (нейрони, мікроглія, астроцити). Зрештою, роль UT-V як модулятора кліренсу робить цей білок ключовою мішенню для дослідження ролі ЦС в мозку хворих на ХА [43].

Важливим для клінічної практики є те, що метаболічні перепрограмування в ЦС мають вирішальне значення в прогресуванні пухлин різної локалізації.

Встановлено, що сечовина та її метаболіти негативно впливають на прогресування гліоми — найпоширенішої злоякісної пухлини ЦНС. При вивченні впливу p53 — білка-супресора пухлин — на прогресування гліоми шляхом регулювання ЦС було встановлено інгібуючий вплив p53 на експресію ферментів ЦС та генез сечовини в клітинах гліоми. Нокдаун CPS1, першого ключового ферменту ЦС, пригнічує проліферацію, міграцію та інвазію клітин гліоми. Рівень поліаміну, метаболіту ЦС, також регулювався p53 у клітинах гліоми шляхом пригнічення ЦС, що сприяє пригніченню прогресування гліоми [44].

ГЦК є одним із найбільш смертельних видів раку у світі. Встановлено, що CPS1 слабо експресується в тканинах ГЦК та циркулюючих пухлинних клітинах, що негативно корелює зі стадією та прогнозом ГЦК. Подальші дослідження показують, що CPS1 — це палиця з двома кінцями. З одного боку, він пригнічує активність фосфатидилхолін-специфічної фосфоліпази С, блокуючи біосинтез діацилгліцеролу (DAG), що призводить

до зниження регуляції шляху DAG/протеїнкінази С для пригнічення інвазії та метастазування ракових клітин. З іншого боку, CPS1 сприяє проліферації клітин, збільшуючи внутрішньоклітинний S-аденозилметіонін для посилення модифікації m6A мРНК члена 3 родини розчинених речовин 1 — ключового транспортера для споживання аспартату. Зрештою, надмірна експресія CPS1 аденосасційованого вірусу може гальмувати прогресування ГЦК. У сукупності це призводить до того, що CPS1 є перемикачем між проліферацією та метастазуванням ГЦК шляхом збільшення внутрішньоклітинного рівня аспартату [45].

Порушення регуляції ЦС було виявлено і при колоректальному раку (КРР). Однак вплив сечовини на розвиток КРР залишається незрозумілим. При обстеженні пацієнтів з потенційними факторами ризику (режим харчування, метаболічні фактори — ІМТ, ШКФ, ЦД2, генетичний профіль) виявлено, що нижчі рівні сечовини в сироватці крові були пов'язані з вищим ризиком розвитку КРР, причому більш виражений ефект спостерігався у осіб з ЦД2. Підтримка стабільного рівня сечовини в сироватці крові має важливі наслідки для профілактики КРР [46].

Якщо говорити в цілому про патологічні стани, які пов'язані з порушенням ЦС, то для повного розуміння патофізіологічних процесів необхідно нагадати про механізми регуляції синтезу сечовини.

Для повноцінного функціонування ЦС, окрім п'яти основних ферментів, також потрібні інші ферменти та мітохондріальні транспортери амінокислот. Повний ЦС експресується в печінці та невеликою мірою також в еритроцитах. Однак високорегульована експресія кількох ферментів, присутніх у ЦС, відбувається також у багатьох інших тканинах, де ці ферменти беруть участь у синтезі NO, поліамінів, проліну та глутамату [47]. Швидкість утворення сечовини в ЦС контролюється та обмежується ферментами першого та третього циклів — CPS1 та ASS1 відповідно. CPS1 є ферментом, що генерує потік, а ASS1 є ферментом, що лімітує швидкість, оскільки він має найнижчу максимальну швидкість серед ферментів циклу. Швидкість синтезу сечовини головним чином визначається концентрацією в крові її субстрату, амінного азоту, а співвідношення «субстрат — продукт» регулюється низкою гормонів та інтерлейкінів [48, 49]. Крім того, вироблення сечовини залежить від функціональної маси печінки [50].

Глюкагон, інсулін та глюкокортикоїди є основними регуляторами експресії ферментів ЦС у печінці. На противагу цьому ферменти циклу сечовини в непечінкових клітинах регулюються широким спектром про- та протизапальних цитокінів та інших агентів. Регуляція цих ферментів значною мірою транскрипційна практично у всіх типах клітин [47]. Глюкагон є найсильнішим відомим регулятором, який підвищує синтез сечовини як у базальному стані, так і під час стимуляції аланіном [51]. Водночас інсуліноподібний фактор росту-1 (IGF-1) є сильним знижувальним регулятором її синтезу [49]. Споживання білка в раціоні збільшує, а вуглеводів — зменшує концентрацію

амінокислот у крові, що відповідним чином впливає на синтез сечовини. Припускається, що споживання білка підвищує синтез сечовини не тільки за рахунок субстратного ефекту, а і за рахунок стимуляції ферментів. Показано, що зниження кліренсу азоту в печінці глюкозою залежить від гіперглікемії та досягається адитивним ефектом прямої гормонезалежної дії глюкози та опосередковано через пригнічення глюкагону. Інсулін не є прямим контролером кліренсу азоту в печінці, але все ж вважається важливим регулятором синтезу сечовини завдяки своєму знижувальному впливу на концентрацію амінокислот у крові [52]. Підвищення регуляції синтезу сечовини амінокислотами та споживанням харчового білка може бути поруше-

не у пацієнтів із хронічним панкреатитом (ХП) через знижену секрецію глюкагону [53].

Введення глюкокортикоїдів призводить до втрати азоту у вигляді сечовини частково через специфічний печінковий механізм, що підтверджується підвищеним функціональним кліренсом азоту в печінці. Глюкокортикоїдні рецептори печінки контролюють системну функцію та ЦС печінки шляхом транскрипційної регуляції експресії ARG1. Гормон росту має протилежний ефект, а також нейтралізує ефект глюкокортикоїдів при одночасному застосуванні з преднізолоном. Це доповнює розуміння розвитку та можливостей лікування катаболізму стероїдів [54].

Визначені зміни сечовини згруповані в табл. 1.

Таблиця 1. Причини змін рівня сечовини в крові

| Причини підвищення концентрації сечовини в крові (сечовина > 7,8 ммоль/л або > 22 мг/дл) | |
|---|---|
| Фізіологічні | |
| Збільшення вироблення сечовини | Підвищення білка в раціоні ↑ |
| Переважає катаболічних процесів, зниження ШКФ | Вікове збільшення рівня сечовини (похилий вік ↑) |
| Патологічні | |
| Захворювання нирок (порушення реабсорбції та фільтрації)/ниркова недостатність | ГПН ↑↑↑ ХХН ↑ — ↑↑, ХХН V ст. ↑↑↑ |
| Дегідратація | Зменшення вживання рідини ↑ Зневоднення внаслідок діареї ↑ — ↑↑, блювання ↑ — ↑↑, потовиділення ↑ |
| Зниження ниркової перфузії | Серцева недостатність ↑ (декомпенсована ↑↑) Гіповолемічний шок ↑↑ Тяжка гіпотензія ↑ — ↑↑ |
| Стани з підвищеним катаболізмом білків | Голодування ↑ Травми ↑ Опіки ↑ — ↑↑ Великі оперативні втручання ↑ Системне запалення ↑ Міозити ↑ |
| Підвищене надходження білка (protein breakdown) | Шлунково-кишкові кровотечі ↑ |
| Метаболічна дисрегуляція | Ендокринні захворювання (ЦД, патологія щитоподібної залози, наднирників) ↑ |
| Патологія вагітності | Патологія розвитку плода ↑ Дисфункція печінки або нирок у вагітних ↑ |
| Ятрогенні фактори | Стероїдні гормони, діуретики, антибіотики |
| Причини зниження концентрації сечовини в крові (сечовина < 2,5 ммоль/л або < 7,0 мг/дл) | |
| Фізіологічні | |
| Зниження синтезу | Дієта з низьким умістом білків ↓ |
| Зниження синтезу та підвищення виведення | Вагітність ↓ |
| Патологічні | |
| Генетичні | Порушення будь-якого з ферментів циклу сечовини (CSP1, OTC, ASS1, ASL, ARG) або продуцента кофакторів (NAGS): повний дефіцит ↓↓ — ↓↓↓, частковий ↓ |
| Гіпергідратація | Надмірне споживання або введення рідини ↓ Синдром неадекватного діуретичного гормону ↓ |
| Зниження синтезу | Захворювання печінки, які супроводжуються гострою печінковою недостатністю ↓↓, хронічною печінковою недостатністю (НАСГ ↓, хронічний гепатит ↓, алкогольний гепатит ↓ — ↓↓, цироз печінки ↓↓) |

В експерименті встановлено, що за умов гострого післяопераційного стресу катехоламіни, кортикостерон та глюкагон разом забезпечують близько 40 % збільшення синтезу сечовини *in vivo*. Простагландин E2 (PGE2) сам по собі не впливає на синтез сечовини, але прискорює дію гормонів [41]. PGE протягом тривалого часу вважалися катаболічними гормонами, але останні дані свідчать про те, що вони можуть секретуватися у критично хворих пацієнтів для протидії гормонам стресу, стимулюючи синтез білка. Їх використання для поліпшення мікроциркуляції печінки та як цитопротекторних засобів перебуває під пильною увагою. У пацієнтів з ЦП PGE1 знижує швидкість синтезу сечовини в печінці незалежно від гормонів та/або печінкового кровотоку, можливо, впливаючи на периферичному рівні на транспорт амінокислот, тим самим зменшуючи надходження амінокислот до печінки [55].

На обмін сечовини впливає і рівень гідратації організму. Так, встановлено, що гостре помірне зневоднення знижує як загальний синтез сечовини, так і її чутливість до глюкагону. Останнє було пов'язане з втратою внутрішньоклітинної води. Таким чином, зневоднення може призводити до економії азоту за рахунок внеску печінки в гомеостаз азоту. Механізм цього ефекту та зв'язок з потоками натрію та калію невідомі [56].

Дослідження впливу іонів натрію та калію на обмін сечовини показують таке.

Численні експерименти довели, що виснаження калію знижує експресію генів та синтез білка, тим самим знижуючи ріст, концентрацію та активність ферментів загалом. Рівень аміаку в плазмі значно зростає під час гіпокаліємії, що вказує на зниження функціональності ЦС, але інші механізми також можуть бути важливими [57]. Дослідження показали, що при експериментальній гіпокаліємії вміст калію в нирках та м'язовій тканині значно знизився, але залишився незмінним у тканині печінки. Експресія білків натрієво-калієвих насосів печінки залишилася незмінною. Гіпокаліємія знижувала здатність до синтезу сечовини шляхом зниження експресії ключових ферментів циклу сечовини ASS1, що призводило до вираженої гіперамоніємії. Але це явище є оборотним після поповнення калію [58].

Вплив натрію на обмін сечовини ще достатньо не вивчений. Однак доведено, що сечовина створює позитивний баланс натрію в плазмі й ефективна при синдромі неадекватної секреції антидіуретичного гормону [59] та для корекції натріємії у пацієнтів з гіперволемічною серцевою недостатністю [60]. Пероральна сечовина наразі рекомендується в Європі для лікування хронічної гіпонатріємії [61].

Істотна роль сечовини в патологічних процесах робить її цінним маркером в прогнозуванні перебігу захворювання та смертності. Класично рівень сечовини разом з креатиніном використовується для оцінки функції нирок, а ізольоване визначення в крові — як маркер порушення функції печінки. Низка клінічних досліджень показала діагностичне значення ізольованого визначення азоту сечовини при різних нозологіях.

Дослідження показали важливість визначення азоту сечовини в крові для раннього прогнозування смертності при гострому панкреатиті [62], СН [63]. Підвищений рівень сечовини тісно пов'язаний з 30-денною смертністю від усіх причин у пацієнтів, госпіталізованих з гострою патологією [64], та збільшенням смертності у критично хворих пацієнтів з рівнем креатиніну 0,8–1,3 мг/дл незалежно від рівня креатиніну в сироватці крові [65]. Серед пацієнтів з нестабільним коронарним синдромом та переважно нормальною або незначно зниженою ШКФ підвищений рівень сечовини в крові пов'язаний зі збільшенням смертності незалежно від оцінок ШКФ на основі креатиніну та інших біомаркерів [66].

У клінічній практиці більш ефективним для прогнозу захворювань вважається використання відносних величин. Так, велике прогностичне значення має співвідношення азоту сечовини (BUN)/креатиніну, що в першу чергу враховує вплив функціонального стану нирок на перебіг захворювання. Співвідношення BUN/креатиніну може відображати нейрогуморальну активацію (особливо підвищення рівня аргініну вазопресину), зміну ниркового кровотоку або інші патофізіологічні механізми, які не включені в звичайні прогностичні змінні.

Більш високе співвідношення BUN/креатинін асоціювалось з гіршими наслідками при гострій серцевій недостатності, у пацієнтів із хронічною серцевою недостатністю у всьому спектрі фракції викиду лівого шлуночка незалежно від ШКФ та NT-proBNP [67]; та мало значну прогностичну цінність для смертності від усіх причин для цієї категорії пацієнтів [68]. Підвищений рівень сечовини крові та співвідношення BUN/креатинін при госпіталізації є незалежними предикторами довгострокової смертності у пацієнтів з інфарктом міокарда з елевацією сегмента ST незалежно від ШКФ при госпіталізації [69]. Високий рівень BUN/креатиніну суттєво збільшує ризик розвитку ІХС у пацієнтів з ЦД 2-го типу [70]. Цей індекс має певну специфічність і пов'язаний з початком та смертністю від ГПН у пацієнтів з COVID-19 [71].

Інший прогностичний індекс — це співвідношення BUN/альбумін, яке деякі дослідники ідентифікують як новий показник запального і харчового статусу. Його значення як незалежного прогностичного маркера було доведено для розвитку контраст-індукованої нефропатії у пацієнтів, які проходять коронарографію або черезшкірне коронарне втручання [72]. Він є найкращим параметром для прогнозування смертності та перебування у відділенні інтенсивної терапії у пацієнтів з нехронічними захворюваннями нирок [73]. Підвищений коефіцієнт BUN/альбумін був сильним та незалежним предиктором внутрішньолікарняної та 90-денної смертності від усіх причин у пацієнтів з загостренням хронічного обструктивного захворювання легень [74].

Сучасні дослідження демонструють значну роль сечовини та її продуктів у розвитку низки захворювань. В основі її патологічних впливів лежить дисфункція ферментів циклу сечовини вродженого чи набутого характеру, що реалізується через активацію оксидативного стресу, апоптозу, карбамілювання, гормональну диз-

регуляцію. Вивчення механізмів впливу цієї органічної сполуки на патофізіологічні процеси відкриває широкі перспективи в застосуванні нових фармакологічних підходів для лікування захворювань нирок, печінки, серцево-судинної та нервової систем, онкопатології. З іншого боку, ізольоване визначення рівня сечовини в крові або її співвідношення з креатиніном та альбуміном є надійними прогностичними маркерами тяжкості та смертності при різноманітній патології.

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The role of urea in pathological conditions

Abstract. Modern research shows the significant role of the urea and its products in the development of a number of diseases. Its pathological effects are based on dysfunction of the urea cycle enzymes of congenital or acquired nature, realized through the activation of oxidative stress, apoptosis, carbamylation, hormonal dysregulation. Studying the mechanisms of action of this organic compound on pathophysiological processes opens up broad pros-

pects for the application of new pharmacological approaches to the treatment of kidney, liver, cardiovascular and nervous diseases, and oncopathology. On the other hand, isolated determination of the blood urea level or its ratio with creatinine and albumin are reliable prognostic markers of severity and mortality in various pathologies.

Keywords: urea; pathology; apoptosis; carbamylation

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Gamma-aminobutyric acid as an element of the mechanisms of cerebro-renal interactions

Abstract. *The presence of many common aspects in autoregulatory mechanisms and processes of ensuring the constancy of the internal environment determines the uniqueness of the cerebro-renal system. Gamma-aminobutyric acid (GABA), in addition to the key coordinating role in brain activity and its metabolism, has inherent regulatory effects in non-neuronal tissues. Given the fact that there is a relationship between GABA levels and the functional and metabolic state of other organs and systems, the aim of the work is to focus on scientific information regarding local GABAergic systems, the location of their components in the nephron and the renal effects of GABA under different conditions. In addition to the fact that GABA has therapeutic potential against acute kidney injury and chronic kidney disease, pharmacological modulators of GABA can provoke nephrotoxicity. The reasons for the diversity of renal responses under the influence of GABA and agents with agonist activity are multifactorial in nature, which should be taken into account, and within the framework of GABAergic strategies, effective and safe therapeutic approaches should be sought and applied.*

Keywords: *gamma-aminobutyric acid; cerebro-renal system; mechanisms of interrelationship*

The nervous system and the kidneys interact to maintain normal body homeostasis. In pathological processes, disruption of these relationships can lead to impaired renal function and sodium ion processing, resulting in fluid and electrolyte imbalance. Increasing evidence suggests the importance of interactions between the nervous system and the kidneys, given the high prevalence of acute kidney injury (AKI) and chronic kidney disease (CKD) in patients with cerebrovascular diseases [1]. The presence of many common anatomical and physiological aspects determines the uniqueness of the cerebro-renal system. In addition to the need for a stable and constantly high blood volume and local autoregulation of blood flow, there is a close connection between the brain and the kidneys both in normal and pathological conditions. Nerve impulses from the central nervous system (CNS) regulate renal blood flow, glomerular and tubular processes. The kidneys interact with the CNS through thinly myelinated and unmyelinated nerve fibers to regulate sodium ion processing [2].

Among neurohumoral regulators of homeostasis, one of the most common neurotransmitters in mammals is gamma-aminobutyric acid (GABA), which is distributed in most brain regions and in 40 % of inhibitory synapses in adult vertebrates [3]. Components of the GABAergic system have structural, molecular, and functional differences and are present in neuronal and non-neuronal tissues, modulate physiological processes, and participate in the pathogenesis of a number of diseases [4].

The aim of the work is to focus on scientific information regarding local GABAergic systems, in particular the location of its components in the nephron, and the renal effects of GABA under different conditions of renal function.

GABA is a natural amino acid that acts as the primary inhibitory neurotransmitter in the CNS. Performing its biological function at the interneuronal synapse, GABA binds to postsynaptic receptors that modulate ion channels, hyperpolarize the cell, and inhibit action potential transmission [5]. GABA signaling in the CNS has been extensively

studied. GABA, GABA_A, and GABA_B receptors, despite their differences in physiological, biochemical, functional, and pharmacological properties, are the main inhibitory receptors in the CNS and they regulate neuronal excitability when GABA is released into the postsynaptic nerve terminal [6]. GABA is functionally opposite to the major excitatory neurotransmitter glutamate (glutamic acid, Glu), which, like GABA, is the most abundant neurotransmitter in the CNS. GABA-mediated neuronal activity occurs due to the coordinated and dynamically regulated balance between inhibitory (mainly GABAergic) and excitatory (mainly glutamatergic) effects mediated by GABA receptors and Glu receptors (NMDA receptors). Proper GABA/Glu balance is essential for the normal functioning of most complex brain processes, and imbalance has been implicated in neurodevelopmental pathology, neurodegenerative/neurological, psychiatric diseases, and acute neurological disorders [7, 8].

The biological significance of GABA is not limited to maintaining excitatory/inhibitory balance. In addition to the fact that GABA is a key coordinator of brain activity and its metabolism, the GABA system has regulatory functions in other, non-neuronal tissues and organs. It is now known that GABA levels are interconnected with the physiological state of metabolic organs and the pathogenesis of metabolic diseases. GABA is synthesized in significant quantities in the islets of the pancreas [9]. GABA released from β -cells can have both autocrine and paracrine effects in human islets of Langerhans. The result of the action of GABA through GABA_A receptors on α -cells of the pancreas is the production of glucagon, on β -cells — insulin secretion [10].

Disturbances in GABA signaling have significant consequences in several physiological processes in the liver, as well as liver diseases. Today, there is a sufficient number of scientific reports that GABAergic innervation of the liver not only exists, but may also play an important role in the regulation of liver development and function. Activation of GABAergic processes can protect the liver from toxic damage to hepatocytes, and GABA production by hepatocytes plays a key role in the regulation of blood glucose and feeding behavior in obesity; therefore, reducing GABA in the liver improves insulin sensitivity [11].

GABA has been reported to affect cardiovascular regulation through central and peripheral GABAergic mechanisms. A systematic review and meta-analyses show that oral GABA reduces blood pressure in patients with high normal blood pressure and stage 1 hypertension [12]. GABA_A receptor activation/inhibition affects post-infarction ventricular remodeling by modulating monocyte/macrophage subsets [13]. Atrioventricular node pacemaker cells have been shown to have an intrinsic GABAergic system [14]. Along with GABAergic vesicles, GABA metabolic enzymes, receptors, and transporters have been identified in atrioventricular node pacemaker cells. In the same study, the authors suggest that the endogenous GABAergic system, by ensuring consistent atrioventricular contraction, plays a key role in the conduction of impulses from the atria to the ventricles.

AKI is known to modulate the CNS, and the end result is an increase in central sympathetic influences, which ex-

acerbates kidney damage [15]. In CKD, renal denervation modulates sympathetic outflow also through GABAergic mechanisms [16]. Stimulation of GABA_B receptors in the CNS mediates the preventive effect of GABA in AKI due to inhibition of increased renal sympathetic activity during renal ischemia/reperfusion [17]. Functional relationship between the kidneys and the CNS through GABA was confirmed by research [18], where it was established that afferent renal nerves are involved in GABAergic changes in the paraventricular nucleus of the hypothalamus. Renal afferents are known to play a crucial role in the regulation of renal function, being activated by changes in pressure, fluid composition and oxygen levels in the kidneys. It is suggested that an increase in GABAergic inputs to the paraventricular nucleus occurs to attenuate sympathetic excitation in renovascular hypertension in rats, carrying information from the kidneys to the CNS.

It is worth noting that sympathetic activation increases the expression of the renin-angiotensin system, the synthesis of its components and the release into the circulatory system [19, 20]. When the renin-angiotensin system, which controls blood pressure and sodium homeostasis, is overactivated, its sodium-retaining effect is mediated by intrarenal and extrarenal, including central, mechanisms [21]. Evidence for the involvement of GABA in the integrated effects of the renin-angiotensin system in the kidneys, cardiovascular system, and CNS comes from studies indicating the dependence of GABAergic synaptic inputs in the CNS on the activity of angiotensin II [22].

Thus, the role of the GABAergic system in renal function has been practically established. The hypothesis of the existence of a renal GABAergic system has received evidence from the study on the effects of GABA on the prevention of renal pathology and the identification of GABA components in the kidneys. Studies in rats with hypertension and CKD showed that GABA_B receptor expression in the CNS was increased, central GABA levels were reduced in the cerebrospinal fluid, and peripheral GABA levels were increased in serum. Renal denervation in CKD restored glutamate decarboxylase (GAD) activity, similar to the effect observed with baclofen (a GABA_B receptor agonist), and systemic administration of gabapentin (a GABA analog) reduced blood pressure. It has been shown that improving GABA system dysfunction prevents the development and reduces the severity of cardiorenal syndrome in rats with CKD [23].

Of particular note is the information about the specificity of the distribution of GABA components in the nephron. An understanding of the peculiarities of the localization of the renal components of the GABAergic system provides an understanding of the functional significance of this amino acid in renal processes, the mechanisms of interaction between the nervous system and the kidneys through the GABA system, which is of great importance for the progress of knowledge in the field of the theory of diseases based on the patterns of pathology development [24].

Studies on the expression of the GABAergic system in non-neuronal tissues have shown that each of its components is present in the kidneys. Analysis of the presence of

GABA-like immunoreactivity (GABA-LI) in the rat kidney using light and electron microscopy revealed GABA-positive structures in vibratome sections of the medulla and cortex [25]. The specific distribution of GABA in the tubular epithelium demonstrated the functional significance of this amino acid in the transport processes in the tubules. Thus, the distribution of GABA-LI was heterogeneous: the inner strip of the outer medulla was most strongly and almost uniformly labeled, while GABA-LI in the cortical substance was mostly limited to only a few tubules. GABA-positive structures included epithelial cells of the thin and thick ascending parts of the loop of Henle, connecting tubules and collecting tubules. In GABA-positive connecting tubules and collecting tubules, immunoreactivity was present in the cytoplasm of approximately half of the epithelial cells. As shown by electron microscopy in this study, the labeled cells in the collecting tubules were light (principal) cells.

Analysis of GABA-related RNA template molecules by RT-PCR revealed a unique set of GABA receptor subunits and subtypes in the kidneys of Wistar-Kyoto rats [26]. Thus, in the renal cortex, as in the cerebral cortex of Wistar-Kyoto rats, the expression of GABA_A receptor subunits, $\alpha 1$, $\beta 3$, δ , ϵ , π , was detected, mainly in the apical region of the cortical tubules. Immunofluorescence study of protein localization showed that the $\alpha 1$ subunit is widely distributed in the proximal tubules; $\beta 3$ subunits were observed in the proximal tubules, in particular in tall cells and cells with a structure similar to a brush border, as well as in the distal tubules. The staining of the π subunit was mainly in the distal tubules and to a lesser extent in the proximal tubules. At the same time, immunoblotting showed that the kidneys can express similar or higher amounts of $\beta 3$, $\rho 1$ subunits than the brain. Both subtypes of GABA_B receptors, R1 and R2, and the $\rho 1$ and $\rho 2$ subunits of the GABA_C receptor were also found in the rat kidney cortex. At the same time, GAD enzymes involved in the synthesis of GABA, GAD67 and GAD65, the GABA transporter, GAT2, and the GABA-transaminase enzyme that metabolizes GABA were expressed in the rat kidney, which, according to these scientists, suggests the existence of a local renal GABAergic system with an autocrine/paracrine mechanism.

It is worth noting that 90 % of the renal cortex is made up of renal tubules, which play an important role in homeostasis and are the structures with the greatest energy needs of the kidneys. Tubular segments (proximal and distal segments, nephron loop, collecting tubules) have unique reabsorption properties, most pronounced in the cells of the proximal convoluted tubules. Under normal conditions, all glucose, amino acids, 65 % of sodium ions and water are reabsorbed in the proximal segment; sodium, potassium and chloride ions are reabsorbed together through a symporter in the thick ascending limb of the loop of Henle; in the distal segment, as in the collecting tubules, primary active transport of sodium ions occurs on the basolateral membrane and secondary — on the apical membrane [27]. The energy supply of tubular reabsorption is carried out by Na⁺/K⁺-ATPase, which is able to modulate the sensitivity and expression of neurotransmitter receptors, in particular GABA and NMDA; therefore, it participates in the control of the

functions of membrane neurotransmitter receptors [28]. In turn, modulation of Na⁺/K⁺-ATPase activity by endo- and exogenous ligands alters transtubular transport in the kidneys, suggesting a relationship between this pump and the renal GABA system.

Given the presence of the GABAergic system in the kidneys, a study was conducted on the influence of the GABA/glutamate system on the vasoactive response from renal microvessels [29]. The results demonstrate for the first time that activation of endogenous GABA and NMDA receptors in the kidneys significantly alters microvascular diameter with important consequences for renal blood flow. The GABA- and Glu-mediated effects on renal capillaries revealed in this study were surprisingly similar to their central regulatory effects on the CNS capillaries. It was noted that since dysregulation of renal blood flow is associated with CKD, alterations in the GABA system may have a significant impact on long-term renal function.

The important role of GABA in renal physiology and pathology is evidenced by the results of studies on its renoprotective effects. GABA-enriched salt has a protective effect against the negative impact of high salt intake in patients with cisplatin-induced nephrotoxicity, which is characterized by suppression of hematological and biochemical toxicity, renal cell apoptosis, and renal inflammation [30]. GABA administration significantly improved the markedly elevated blood urea nitrogen and creatinine levels and decreased creatinine clearance in the progression of glycerol-induced renal failure, and fractional excretion of sodium ions was also reduced [31].

The first study to investigate the immunomodulatory mechanisms of GABA in renal failure demonstrated the effects of GABA on renal inflammation both *in vivo* and *in vitro*. GABA regulates renal inflammation by significantly reducing serum inflammatory markers, induction of monocyte migration, and the number and infiltration of macrophages, which are crucial for the initiation of renal inflammation. The results suggested that GABA has a protective effect against renal injury [32]. The use of a combination of amikacin and GABA without/with loading on chitosan nanoparticles confirmed the protective effects of GABA against amikacin nephrotoxicity, as it improves renal function, oxidative stress and demonstrates a significant homeostatic role mediated by the suppression of inflammatory cytokines of the Th1, Th2 and Th17 types [33].

Recent studies have revealed a novel role for GABA in combating oxidative stress under high glucose conditions. In Mongolian sheep kidney cells, GABA markedly increased cell viability and effectively mitigated oxidative damage induced by high glucose stress through upregulation of antioxidant genes and regulation of metabolic pathways, suggesting a potential mechanism for adaptation to extreme conditions [34]. The antioxidant effects of GABA are linked to its impact on mitochondria [35, 36]. The kidneys, especially the cells of the proximal tubules, are rich in mitochondria, so nephrotoxicity of drugs is manifested, in particular, by mitochondrial damage [37]. GABA type A receptor-associated protein is a protein that plays a role in stabilizing GABA_A receptors and is involved in mitophagy.

By removing dysfunctional mitochondria from renal tubular epithelial cells, reducing local inflammation and oxidative damage, activation of mitophagy is protective in AKI [38].

Of particular note are reports of nephrotropic effects of pharmacological neuromodulators that act through the GABAergic system. Piracetam, a nootropic drug, a cyclic derivative of GABA, improves markers of renal function such as urea and creatinine, reduces histological damage, the presence of inflammatory cells in the renal tubules, and inhibits apoptosis in cisplatin-induced nephrotoxicity [39]. At the same time, the use of GABA analogues for adequate control of postoperative pain was associated with a risk (higher for gabapentin compared to pregabalin) of decreased kidney function and the development of CKD [40]. Use of the tranquilizer diazepam, which increases GABA receptor sensitivity, is associated with an increased risk of AKI in children [41]. Valproic acid, whose anticonvulsant activity is provided by inhibition of the enzyme GABA-transaminase and GABA reuptake in brain tissues, can cause obvious damage to the renal tubules, which is associated with proximal tubular mitochondrial toxicity [42]. The renal effects of new-generation anticonvulsants with anxiolytic activity, neurosteroids (ganaxolone), which, like their endogenous analogues, activate extrasynaptic GABA_A receptors, have not yet been studied. However, their need for complex molecules to enhance biopharmaceutical properties such as β -cyclodextrin raises the risk of nephrotoxicity, especially in renal disease [43].

As noted above, GABA receptor subunits are widely represented in the renal tubules, which probably causes a similar damaging effect of other drugs with nephrotoxicity (antimicrobials, cytostatics, nonsteroidal anti-inflammatory drugs), mainly in the proximal segment of the nephron. The presence of GABA receptors in vascular smooth muscle receptors determines the vascular mechanisms of renal effects. The following should be noted here: not only components of the GABAergic system can be targets; the pharmacological action of GABA analogues, like any drugs, has a multifactorial dependence; the function of GABA receptors in the kidneys has not been definitively determined; the activity of GABA receptor subunits is specific and depends on their structure and function; the direct and indirect mechanisms of GABA in the norm and pathophysiology of the kidneys remain completely unknown. At the same time, taking into account the broad relationships of GABA in the functional and metabolic continuum of the body, its diverse reactions in non-neuronal tissues, and the spectrum of protective effects, research on the renal GABA system, the search and application of effective and safe approaches to pathogenetic therapy of kidney pathology remain relevant for GABAergic strategies.

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Гамма-аміномасляна кислота як ланка механізмів цереброренальних взаємозв'язків

Резюме. Наявність багатьох спільних аспектів в авторегуляторних механізмах і процесах забезпечення сталості внутрішнього середовища зумовлює унікальність цереброренальної системи. Гамма-аміномасляної кислоти (ГАМК), окрім ключової координаторної ролі в активності мозку і його метаболізмі, притаманні регуляторні впливи в нейрональних тканинах. Беручи до уваги те, що існує взаємозв'язок між рівнями ГАМК і функціональним та метаболічним станом інших органів і систем, метою роботи є зосередження уваги на наукових відомостях щодо локальних ГАМКергічних систем, розташування їхніх компонентів у нефроні та ренальних

впливів ГАМК за різних умов. Поруч із тим, що ГАМК має терапевтичний потенціал проти гострого пошкодження і хронічної хвороби нирок, фармакологічні модулятори ГАМК можуть спровокувати нефротоксичність. Причини різноманітності ниркових реакцій під впливом ГАМК і агентів з агоністичною активністю мають багатofакторну природу, що слід брати до уваги, а в межах ГАМКергічних стратегій потрібно шукати та застосовувати ефективні й безпечні терапевтичні підходи.

Ключові слова: гамма-аміномасляна кислота; цереброренальна система; механізми взаємозв'язків