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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].

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

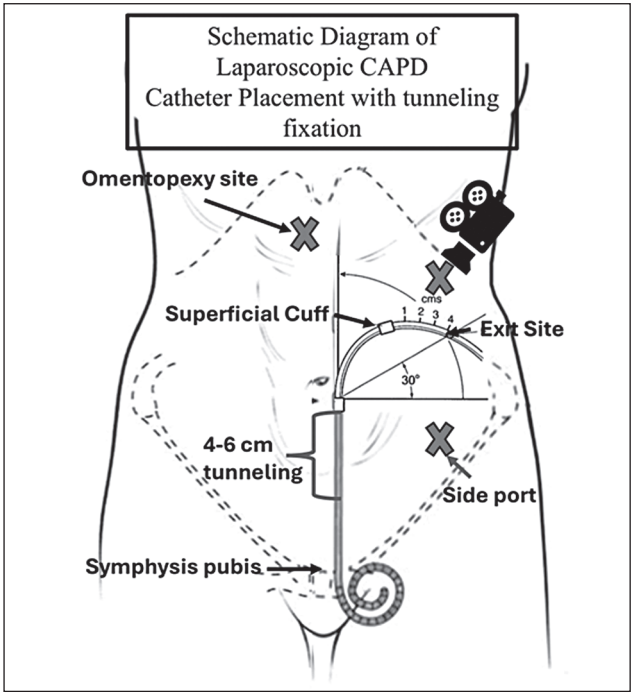


Figure 1. Sharba method

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).

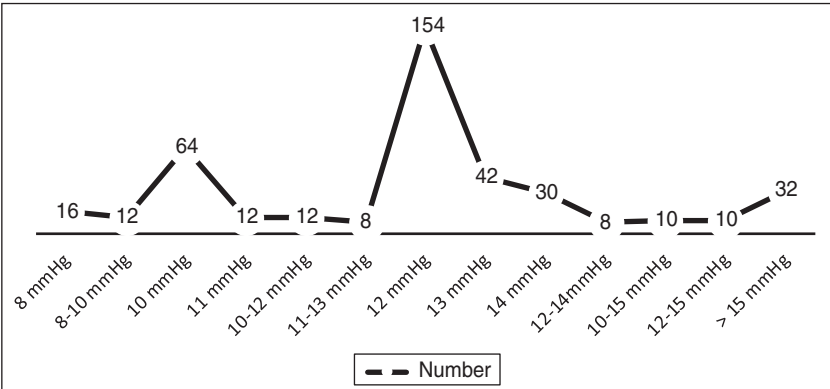


Figure 2. Intraoperative pressure used in our laparoscopic technique

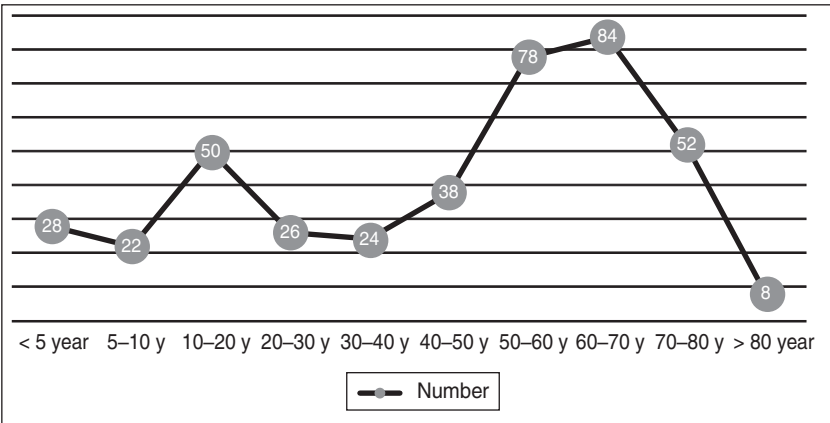


Figure 2. Age distribution in our patients that underwent the one-sided technique

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 m², 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 post-operative 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 контралатерально 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 схилиються 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 oligoasthenoteratozoospermia 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 asthenoteratozoospermia, 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

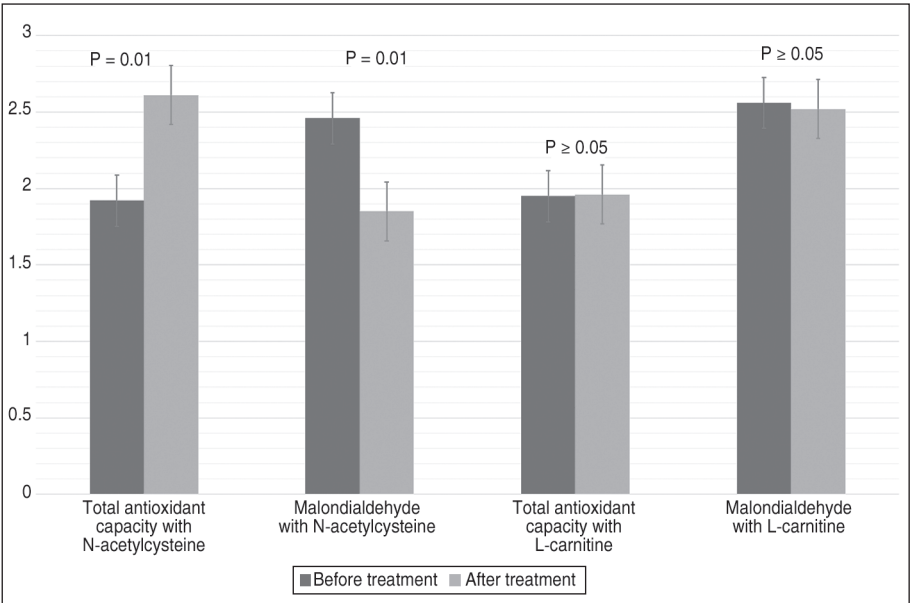


Figure 1. Malondialdehyde and total antioxidant capacity before and after treatment

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 щонайменше 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 decreased 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 metalothionein (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: * — $p \leq 0.01$.

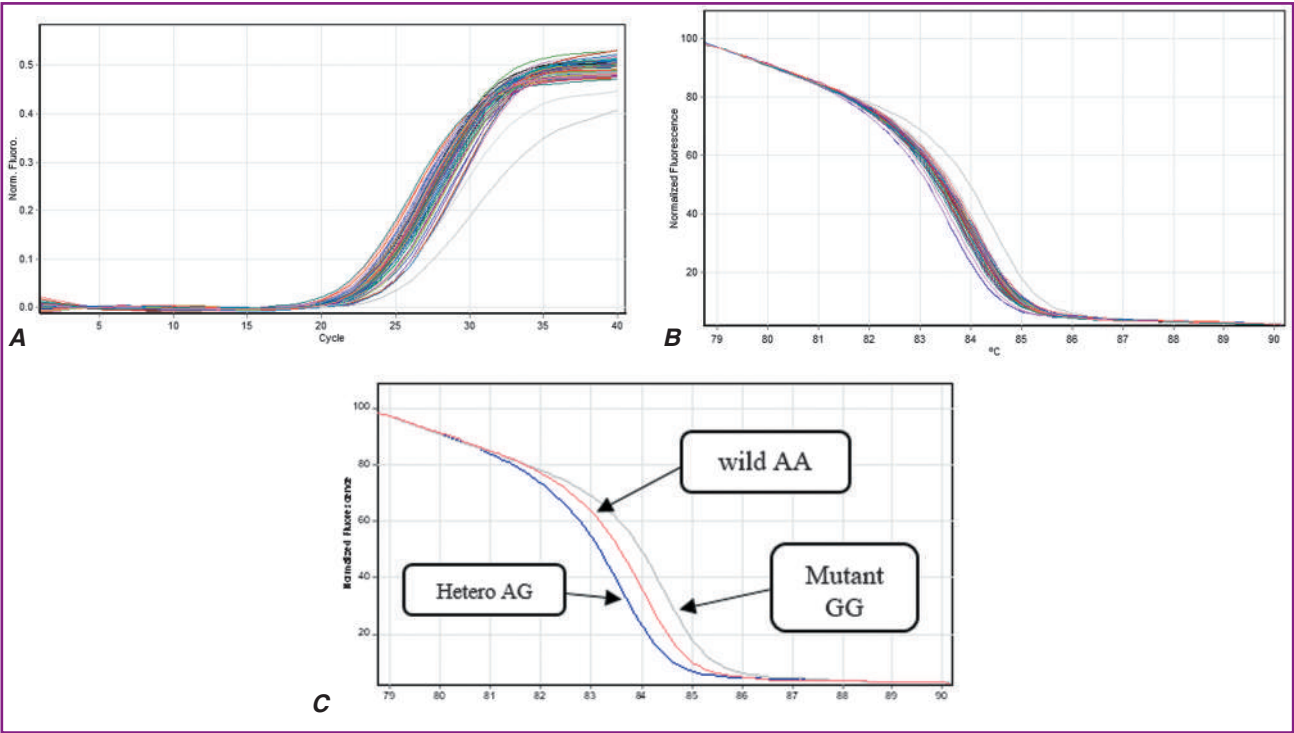


Figure 2. The genotype result for SNP rs28366003: A, B — the HRM result, C — the wild, heterozygous and mutant genotypes

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 life-style (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 $\pm$ SE, ppm	
		Zn	Pb
Patients	AA	65.16 $\pm$ 1.45	22.19 $\pm$ 1.21 ^a
	AG	63.30 $\pm$ 2.04	17.85 $\pm$ 0.81 ^b
	GG	61.55 $\pm$ 1.62	19.67 $\pm$ 1.20 ^{ab}
	L.S.D. (p-value)	5.839 NS (0.4549)	3.948 (0.0261)*
Controls	AA	96.94 $\pm$ 2.17	18.05 $\pm$ 0.55
	AG	99.62 $\pm$ 3.23	17.67 $\pm$ 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 подум'яної атомно-абсорбційної спектrophотометрії. 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 A 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

DOI: <https://doi.org/10.22141/2307-1257.14.3.2025.534>Emad Mahmoud Eltayef¹, Zainab Hudhi Farhood², Zaman Subhi Madloof²¹Faculty of Science, Mustansiriyah University, Iraq²Faculty of Education for Pure Science, University of Thi-Qar, Thi-Qar, Iraq

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

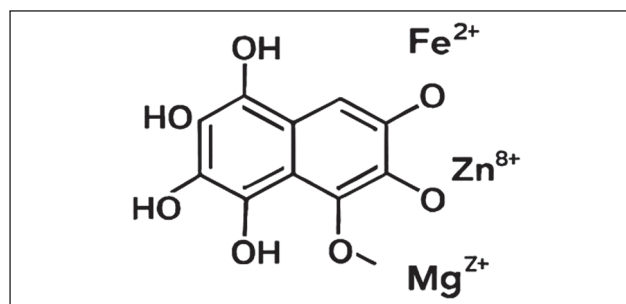


Figure 1. Chemical structure of shilajit

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 ± 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 ± SE)

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

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

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

Groups	N	Urea	Creatinine
G1 (control — distilled water)	10	25.51 ± 1.08	0.390 ± 0.009
G2 (MSG 2 g/kg)	10	31.29 ± 1.32	0.590 ± 0.020
G3 (MSG 2 g + Shi 100 mg)	10	32.63 ± 2.18	0.490 ± 0.012
G4 (MSG 4 g/kg)	10	28.25 ± 1.16	0.520 ± 0.018
G5 (MSG 4 g + Shi 200 mg)	10	23.62 ± 0.89	0.490 ± 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 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 uszkodження на тлі оксидативного стресу. Шиладжит може відігравати захисну роль при таких станах.

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

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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 high-est 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

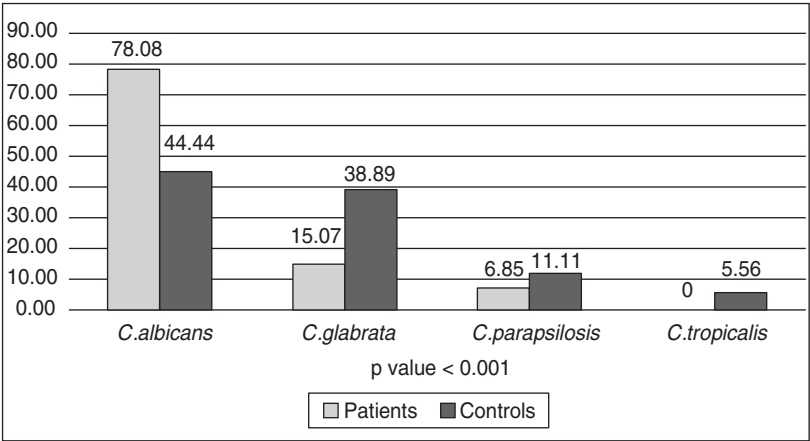


Figure 1. Identification of *Candida* spp. in patients with diarrhea and control group

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

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 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.

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

Days	Patients		Controls		LSD
	Male	Female	Male	Female	
4	67.42 ± 8.85 ^c	75.03 ± 7.86 ^c	55.72 ± 3.76	64.47 ± 8.58	10.1
8	81.43 ± 8.21 ^b	80.79 ± 4.97 ^c	58.40 ± 4.58	61.83 ± 9.02	9.34
12	119.50 ± 5.35 ^a	125.00 ± 3.58 ^a	62.17 ± 6.92	67.37 ± 8.30	8.43
16	93.48 ± 14.10 ^b	91.08 ± 6.00 ^b	60.62 ± 3.17	67.00 ± 6.07	11.2
20	70.61 ± 8.58 ^c	88.15 ± 6.48 ^b	58.99 ± 3.70	65.55 ± 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

О. СІДЕЛКОВСЬКИЙ

ЮРИДИЧНА ГІГІЄНА МЕДИЧНИХ ПРАЦІВНИКІВ

ПРОЧИТАЙ
ТА ПРАЦЮЙ
СПОКІЙНО

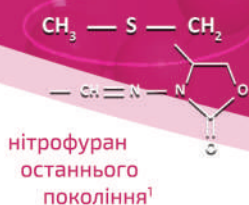
КЛІНІКА СУЧАСНОЇ НЕВРОЛОГІЇ "АКСІМЕД"

Посібник містить вкрай необхідну інформацію для кожного медичного працівника незалежно від напрямку його діяльності. Книга буде надійним помічником у разі виникнення конфліктних або непередбачуваних ситуацій у щоденній практичній діяльності.

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- Мінімальний рівень резистентності патогенів²⁻⁴
- Добре проникає в тканину передміхурової залози³⁻⁴



Коротка характеристика лікарського засобу МАКМІРОР, таблетки, вкриті оболонкою. Р/С № UA/5045/01/01, наказ МОЗ України № 07 від 05.01.2017 р. **Склад:** 1 таблетка містить ніфуратель 200 мг. **Лікарська форма:** таблетки, вкриті оболонкою. **Показання:** вульвовагінальні інфекції, викликані чутливими до препарату збудниками (патогенними мікроорганізмами, трихомонадами, грибами, дріжджами, хламідіями, грибами роду *Candida*). Заворовання сечостатевої системи (цистит, уретрит, пієлонефрит, пієліт). **Протипоказання:** відома індивідуальна підвищена чутливість до діючої речовини або до інших компонентів препарату. **Спосіб застосування та дози.** Вульвовагінальні інфекції. **Дорослі:** 1 таблетка 3 рази на добу після їжі для лікування жінки і її партнера при можливості. Для місцевого лікування використовують Макмірор комплекс, капсули вагінальні м'які або Макмірор комплекс, крем вагінальний. **Важливо:** пацієнтам, які використовують для лікування тільки таблетки, необхідно збільшити дозу до 4 таблеток на добу. Необхідно утримуватися від сексуальних контактів під час лікування, в іншому випадку необхідно використовувати Макмірор комплекс, крем вагінальний перед кожним статевим актом. **Діти від 10 років і старші:** рекомендована доза становить 10 мг на 1 кг на добу, розділена на 2 прийми. Приймають препарат після їжі. **Тривалість лікування становить в середньому 10 днів. Інфекції сечовивідних шляхів. Дорослі:** рекомендована доза препарату залежить від тяжкості захворювання і становить 3-6 таблеток на добу (тобто по 200-400 мг) на прийом 3 рази на день після їжі. Курс лікування становить в середньому 1-2 тижні. **Діти від 6 років і старші:** рекомендована доза становить 10-20 мг на 1 кг маси тіла на добу, розділена на 2 прийми. Приймають після їжі. Таблетки Макмірор при необхідності можна застосовувати для продовження курсу лікування або повторного циклу лікування інфекцій сечовивідних шляхів. **Побічні реакції.** Порушення з боку травного тракту: рідко (<1/10 000): нудота, гірота в роті, діарея; дуже рідко (<1/10 000): блювання, диспепсія. Порушення з боку шкіри і підшкірної клітковини. Можливі алергічні реакції: дуже рідко (<1/10 000): висипання на шкірі, кропив'янка, свербіж. Порушення з боку нервової системи: периферичні нейропатії. **Взаємодія з іншими лікарськими засобами та інші види взаємодій.** Клінічно значуща взаємодія препарату з іншими лікарськими засобами не встановлена. **Фармакологічні властивості. Фармакокінетика.** Діючою речовиною Макмірор є похідне нітрофурану — ніфуратель. Дослідження *in vivo* / *in vitro* продемонстрували широкий спектр дії проти мікроорганізмів, що викликають інфекції сечостатевої системи, також властива антипротозойна і протигрибкова активність. Ніфуратель є протибактеріальним засобом для грамнегативних і грампозитивних аеробних і анаеробних бактерій. Ніфуратель не діє на *Lactobacillus* spp. Ніфуратель не викликає перехресну резистентність мікроорганізмів до інших препаратів. За 30 років не виявлено жодного випадку резистентності до ніфурателю. **Фармакокінетика.** Ніфуратель швидко метаболізується практично у всіх тканинах організму. Період напіврозпаду становить $2,75 \pm 0,8$ години. Приблизно 0,5 % ніфурателю виводиться з сечею в незміненому вигляді. Інша частина виводиться у вигляді метаболітів. Ніфуратель не виявлений у внутрішньочеревній циркуляції. Для отримання детальної інформації ознайомтесь з інструкцією для медичного застосування лікарського засобу. Інформація для фахівців охорони здоров'я і поширення під час семінарів, конференцій, симпозіумів та інших наукових заходів з медичної тематики.

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Схема лікування хронічного простатиту^{3, 4}

Лікування	1-7-й день лікування	Наступні 10 днів лікування
Левовфлоксацин	0,5 г 1 раз в день	
Орнідазол	0,5 г 3 рази в день	
Ніфуратель (Макмірор)		2 табл. 3 рази в день



Інформація про лікарський засіб. Інформація для фахівців охорони здоров'я для використання в професійній діяльності.

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