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Evaluation of Some Immunological Markers in Patients with Acute Pyelonephritis Caused by *Proteus mirabilis*

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ABSTRACT

Background*: Acute pyelonephritis caused by Proteus mirabilis is a severe urinary tract infection considered by deep tissue inflammation; microRNAs and chemokine's play vital roles in regulating the host immune response. This study goals to evaluate the disease station and determine the diagnostic efficacy of the immune axis linking miR-370 and CCL2 in patient serum.* ***Methodology****: This study employed a case-control design involving a overall sample of 120 individuals, comprising 60 patients with P.mirabilis persuaded pyelonephritis and 60 healthy controls. Demographic data were recorded, and miR-370 gene expression levels were assessed using quantitative real-time PCR , while serum CCL2 concentrations were measured via enzyme-linked immunosorbent assay . Statistical analyses included Pearson correlation and receiver operating characteristic (ROC) curve analysis.* ***Results****: Clinical valuation showed that fever (75%) and flank pain (50%) were the greatest common symptoms. Immunological analysis revealed lesser miR-370 expression levels in patients (0.55 ± 0.01) compared to healthy controls (1.89 ± 0.02) at P < 0.001, while serum CCL2 concentrations were higher in patients (11.45 ± 0.011) vs. control (1.26 ± 0.017) pg/mL at P < 0.001. A strong negative correlation was experiential between the two markers (r = -0.645, P < 0.001). Combining the two indicators into a diagnostic panel reached high discriminative accurateness, with an area under the curve of 0.954, sensitivity of 92.0%, and 90.5%. of a specificity.* ***Conclusion****: The present study confirms that pyelonephritis causes a profound disturbance characterized by the loss of miR-370-mediated immune-regulatory suppression, leading to a marked increase in serum CCL2 levels. This dual-biomarker panel serves as an accurate, non-invasive tool for the early diagnosis of acute kidney injury.*

Keywords: Immune markers, Pyelonephritis, P. mirabilis.

Article Information

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INTRODUCTION

Urinary tract infections (UTIs) are among the greatest common bacterial infections in clinical practice. Acute pyelonephritis represents the most severe and destructive form of these infections; if left undiagnosed and untreated, it can lead to renal tissue damage, kidney failure, or urosepsis (1). *P.mirabilis* is a significant causal agent acting as a dangerous opportunistic pathogen particularly in complex infections and among patients with urinary catheters. This bacterium possesses several virulence factors, most notably the enzyme urease, which hydrolyzes urea, leading to increased urine alkalinity and the formation of kidney stones,the presence of flagella grants it superior motility, enabling it to ascend from the bladder to the kidney(2).

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Proteus mirabilis relies on the activation of immune mechanisms involving the stimulation of various inflammatory mediators. A key mediator among these is C-C motif chemokine ligand 2 (CCL2), which acts as a major chemoattractant by recruiting monocytes, dendritic cells, and T cells to the site of inflammation in the kidney to inhibit bacterial growth and progression Although this acute response is vital for eradicating the bacteria, persistently raised levels of CCL2 are strongly related with bigger severity of renal tissue harm and the development of fibrosis in circumstances of acute inflammation (3).

Research has focused on appreciative the precise molecular appliances governing this immune response, with microRNAs emergent as key controllers of post-transcriptional gene expression in renal tissues during infection (4). miR-370 stands out as a hopeful molecule related to the regulation of inflammatory pathways, apoptosis, and stress responses. Evidence point to that altered miR-370 levels play a essential role in modulating the cruelty of inflammation and immune related diseases by directing cytokine signaling pathways and regulating the construction of inflammatory cytokines and chemokines (5).

This research was assumed in response to an urgent epidemiological and health requirement; health institutes in Najaf Governorate are soundtrack persistently great rates of complex urinary tract infections and kidney stones. In Iraq, this epidemiological design is usually related to hot climatic conditions and the great salinity of water causes factors that rise the risk of dehydration and the creation of calculi, making an ideal environment for the propagation of *P.mirabilis* and the appearance of its virulence factors (6). Antibiotic resistance amongst medical isolates in hospitals attitudes a significant therapeutic encounter, necessitating the search for original immunological biomarkers to expedite the early forecast of kidney injury and avoid the deterioration of renal occupation (7)

Understanding the interplay between two immunological markers specifically, the level of the miR-370 and CCL2 levels in patients with *P.mirabilis* induced pyelonephritis in Najaf city offers an opportunity to discover cellular pathological indicators within a resident context. Assessing these biomarkers not only improves scientific understanding of the local immune response but too paves the system for the development of non-invasive diagnostic

METHODOLOGY

1. Clinical Study and Sampling

Patient Group: Collection of patients recently diagnosed by a physician with acute pyelonephritis caused wholly by *Proteus mirabilis* (established by urine culture and bacterial identification by VITEK 2) **Control Groups** are Healthy controls

2. Sample Size Determination

Based on medical statistical standards for relative research (specifically case-control studies), the sample size is intended as follows to confirm the study's robustness for periodical: Sample Size Calculation Formula (Charan and Biswas Formula): The sample size is indomitable based on earlier studies concerning microRNA or cytokines in renal inflammation, using a formula for comparing 2 groups with a statistical power of 80% and a significance level of $\alpha = 0.05$. proposed sample size distribution across Najaf Hospitals: To certify statistical efficiency, it is projected to collect a minimum of 120 samples.

Target Patients: 60 patients identified with *Proteus mirabilis* pyelonephritis (collected from the interior medicine and urology wards of Najaf hospitals, such as Al-Sadr Teaching Hospital and Al-Hakim Hospital).

Healthy control group: 60 healthy individuals, matched for sex and age, showing no signs of inflammation in the kidneys or elsewhere in the body.

3. Justification for Sample Size and Work Plan in Najaf:

Proteus mirabilis is the most common causative agent of urinary tract infections; therefore, the study requires the examination of 60 positive samples that meet the inclusion criteria.

4. Inclusion Criteria

The patient must exhibit symptoms of acute pyelonephritis as diagnosed by a physician and present with white blood cells (pyuria) in a general urine examination (GUE). A positive urine culture must show pure growth of *P.mirabilis* . Additionally, informed consent (written or verbal) must be obtained from the patient for participation and the provision of samples, following an explanation of the nature of the research.

5. Exclusion Criteria

Structural aberrations or urinary tract obstructions (Structural/Complicated UTI): Reject patients with large kidney stones, ureteral obstruction, or congenital urinary tract anomalies, Chronic inflammatory and immune connected diseases: Reject patients with autoimmune diseases (e.g. lupus or rheumatoid arthritis) or malignancies, Renal failure and chronic kidney disease (CKD), Treatment with antibiotics or immunosuppressants, Pregnancy and breastfeeding

6. Confidentiality and Research Ethics Protocol

Sample Coding: All sample is dispensed a random numerical code directly upon receiving; patient names and personal statistics are totally separated from test tubes and laboratory archives. **Informed Consent:** Patients (or their guardians) signal a written consent procedure clearly stating that samples will be used solely for scientific research purposes, while guaranteeing the confidentiality of characters and ensuring that no personal data is revealed in academic publications. **Ethical Approval:** Obtaining approval from the applicable academic and healthcare institution's ethics committee prior to commencing sample assortment.

7. sample collection

Samples are collected from inpatients and outpatients at hospitals in Najaf Al-Ashraf Governorate (Al-Sadr Teaching Hospital and Al-Hakim General Hospital) between January 2025 and April 2025.

8. Microbiological Identification

Culture Media: Media used for bacterial isolation, such as MacConkey agar and blood agar, revealed the characteristic wave phenomenon associated with motile bacteria (swarming phenomenon). **Precise Identification:** Identification was achieved using traditional biochemical tests (oxidase, catalase, and urease positive; indole negative) and modern automated systems specifically the VITEK 2 instrument with the ID-GNB card confirming the identification of Proteus mirabilis with 100% accuracy.

9. Immunological parameters :

A. CCL2 ELISA Assay

Levels of CCL2 in participants' serum were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions. Briefly, 100 µL of standards and serum samples were added to the microplate and incubated for 2 hours at 37°C. After emptying the wells (without washing), 100 µL of detection reagent A was added, followed by incubation for 1 hour at 37°C; the wells were then washed three times with the specific wash buffer (400 µL per well).

100 µL of Reagent (B) was added, and the mixture was incubated for one hour at 37°C. The washing process was repeated five times, followed by drying; then, 90 µL of substrate solution was added, and the mixture was incubated in the dark for 10 to 20 minutes at 37°C. The reaction was stopped by adding 50 µL of stop solution, and the optical density (OD) was measured immediately at a wavelength of 450 nm.

B. Assessment of miR-370 level

a. Total RNA Extraction: Total RNA molecules containing microRNA were isolated and extracted from serum samples using the miRNeasy Mini Kit (Qiagen, Germany), which is specifically planned for microRNA applications. The manufacturer's protocol was strictly followed, and the extracted RNA was kept at -80°C until use.

b.Quantitative Reverse Transcription PCR (RT-qPCR): To measure miR-370 gene expression levels, a two-step quantitative amplification reaction was achieved using the miScript SYBR Green PCR Kit (Qiagen, Germany) with miR-370-specific primers (Forward: GCCTGCTGGGGTGGAACCTGGTAA; Reverse: GCGAGCACAGAATTAATACGAC). The U6 snRNA gene (Forward: CTCGCTTCGGCAGCACA; Reverse: AACGCTTCACGAATTTGCGT) was used as an internal reference (housekeeping) gene for data normalization.

c. Reaction Mixture Components : The total reaction mixture was ready with a final volume of 10 µL per well, according to the following constituents and proportions: 2x QuantiTect SYBR Green PCR Master Mix: 5 µL, Forward Primer: 1 µL, Reverse Primer: 1 µL, RNase-free water: 2 µL and Target cDNA (Template): 1 µL. Total reaction volume: 10 µL.

d. Thermal Cycling Conditions: The thermal amplification program was approved out using the Real-Time PCR instrument according to the following protocol: Initial Activation: At 95°C for 10 minutes (one cycle), Amplification Cycles: Contained of 40 consecutive cycles comprising the following three steps: Denaturation: At 95°C for 15 seconds., Annealing: At 55°C for 20 seconds and Extension: At 72°C for 30 seconds.

e. Statistical Analysis and Gene Expression Calculation (Data Analysis)

To analyze the amplification reaction results based on the Threshold Cycle (Ct) values, which represent the number of cycles required to reach the fluorescence signal threshold the relative change in gene expression levels (fold change) was calculated using the mathematical comparison method ($2^{-\Delta\Delta Ct}$) recognized by Livak and Schmittgen (8), in accordance with the following equations:

Folding change $=2^{-\Delta\Delta Ct}$

$\Delta\Delta C_t = \Delta C_t$ (patients groups) – ΔC_t Control

$\Delta C_t = C_t$ (Target gene)- C_t (Reference gene\U6)

10. Statistical Analysis : Data were analyzed using SPSS (version 26.0) . Continuous variables (miR-370 and CCL2) were expressed as mean ± standard deviation and compared between groups using the independent t-test. Categorical variables were compared by the chi-square (χ^2) test. Pearson's correlation coefficient (r) was used to assess the relationship between the two markers, and receiver operating characteristic (ROC) curve analysis was achieved to assess diagnostic accuracy and calculate the area under the curve (AUC), sensitivity, and specificity. Results were considered statistically significant at $P < 0.05$.

RESULTS

Statistical analysis revealed a significant sex connected disparity in the disease frequency rate, with females 39(65%) showing a statistically significantly higher incidence rate compared to males21(35%) at $P < 0.05$. this shown in table 1.

Regarding the age distribution of the study sample , the results displayed that the 40–60 age group was the most susceptible to *P. mirabilis* induced pyelonephritis, including 25 patients (41.7%), followed by the young adult group (20–40 years) with 18 patients (30.0%), whereas the elderly group (60–80

years) ranked last with 17 patients (28.3%). This shown in table 1.

Regarding the distribution of residence amongst the study subjects (n = 60), those living in urban areas (the city) accounted for 31 patients (51.7%), associated to 29 patients (48.3%) residing in rural areas. However, this geographical difference was not statistically significant (P< 0.05), representing comparable rates of infection transmission across the two environments. this shown in table 1.

Table 1: Demographic characteristics and baseline data of the study population.

Variables	Categories	No. (%)	χ^2	p-value
Sex	Female	39(65%)	25.57	0.001**
	Male	21(35%)		
Age	20-40y.	18(30%)	51.27	0.0001**
	40-60y.	25(41.7%)		
	60-80y.	17(28.3%)		
Residence	Urban	31(51.7%)	40.00	0.001**
	Rural	29(48.3%)		

chi-square (χ^2) test, No.(number) and % (percentage).

Regarding physical examination results and clinically measured vital signs, the results displayed that costovertebral angle tendernessand suprapubic tenderness were the greatest prominent localized signs, observed in 61% (37 patients) and 63% (38 patients) and, respectively. unconsciousness in 39% (23 patients) and Dehydration was noted in 41% (25 patients), and tachycardia at 18% (11 patients), and tachypnea at 10% (6 patients). This shown in table 2.

Upon evaluating the clinical symptoms of the patients at admission (total N = 60), fever was initiate to be the record common systemic symptom, happening in 75% of cases (45 patients), followed by flank pain at 50% (30 patients), and shaking chills at 44% (26 patients). Regarding connected gastrointestinal symptoms, nausea was reported in 35% of cases (21 patients), followed by vomiting at 30% (18 patients), while diarrhea was the least frequent, occurring in 7% of cases (4 patients).Regarding localized urinary symptoms, dysuria was the maximum predominant, affecting 48% (29 patients), followed by polyuria at 20% (12 patients) and urinary urgency at 15% (9 patients); meanwhile, pollakiuria and hematuria noted the lowermost rates at 8% (5 patients each). This shown in table 2.

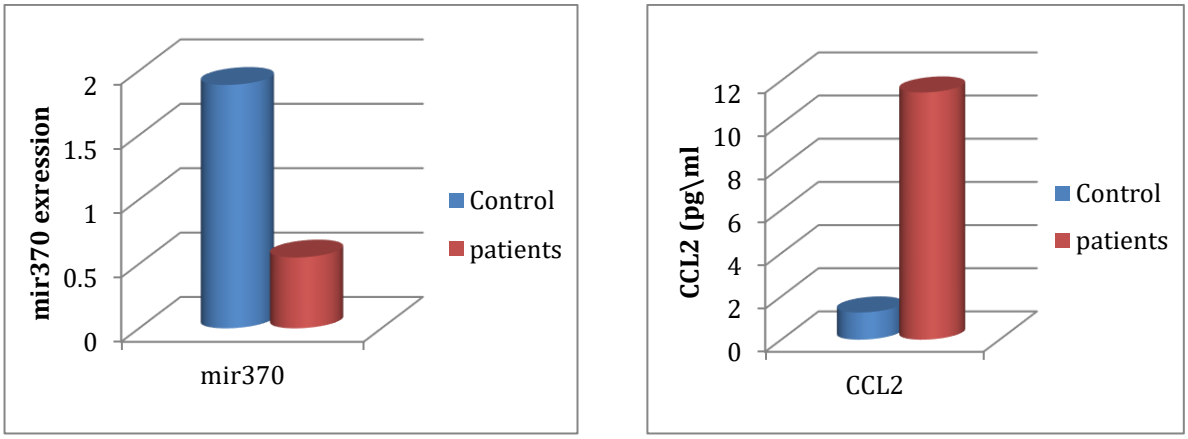
Table 2: Distribution of frequencies and percentages of clinical signs, symptoms, and severity levels among patients with acute pyelonephritis (n = 60).

Clinical features and Findings		Frequency (n)	Percentage (%)
Physical examination	Costovertebral angle tenderness	37	61%
	Suprapubic tenderness	38	63%
	Unconsciousness	23	39%
	Dehydration	25	41%
	Tachycardia	11	18%
	Tachypnea	6	10%

Clinical features and Findings		Frequency (n)	Percentage (%)
Clinical symptoms	Fever	45	75%
	Flank pain	30	50%
	Shaking chills	26	44%
Gastrointestinal symptoms	Nausea	21	35%
	Vomiting	18	30%
	Diarrhea	4	7%
Urinary symptoms	Dysuria	29	48%
	Urinary urgency	9	15%
	Polyuria	12	20%
	Hematuria	5	8%
	Pollakiuria	5	8%

Analyses exposed a extremely statistically significant, inverse change in the expression profile of the studied biomarkers in patients with *P.mirabilis* induced pyelonephritis compared to the healthy control group. Quantitative real-time PCR (qRT-PCR) analysis demonstrated a significant decrease in the gene expression levels of miR-370; the mean relative expression was 2-ΔΔCt in the patient group (0.55 ±0.01) compared to 1.89 ± 0.02in the control group $P < 0.001$. This shown in figure 1.

Simultaneously, ELISA analysis exposed a marked and significant elevation in serum CCL2 levels in the patient group (11.45 ± 0.011pg/mL) compared to the healthy control group (1.26 ±0.017 pg/mL; $P < 0.001$). This attendant reduction in miR-370 and sharp rise in CCL2 accurately reflect the extent of the molecular and inflammatory disturbance linked with microbial pathogenesis. This shown in figure 1.



A: mir 370 expression **B: CCL2 level**

Figure 1: Statistical differences in the studied biomarkers between pyelonephritis patients and the healthy control group.

To investigate the statistical correlation between the studied biomarkers in patients with *Proteus mirabilis* induced pyelonephritis, a statistical correlation analysis was performed. The results revealed a strong, highly significant inverse (negative) correlation between the relative expression levels of miR-370 ($2^{-\Delta\Delta Ct}$) and serum CCL2 protein concentrations (pg/mL) in the patient cohort ($r = -0.645$, $P < 0.001$). This significant inverse relationship indicates that a marked decrease in miR-370 gene expression is

directly and linearly associated with a concomitant rise in blood CCL2 protein levels, suggesting the existence of an interconnected regulatory molecular mechanism between them during the active phase of infection. This shown in Table 3.

Table 3 : Statistical correlations between the studied immunological markers in the patient sample

Correlation parameters		Mir370	CCL2
mir370	r	1	-0.645**
	p	-	0.001
CCL2	r	-0.645**	1
	p	0.001	-

CCL2 protein concentrations both as individual biomarkers and combined into a dual diagnostic panel in distinguishing between patients with P.mirabilis pyelonephritis (n = 60) and a healthy control group. The results showed that the area under the curve (AUC) for the relative expression of miR-370 was 0.885 (95% CI: 0.820–0.940; P < 0.001), yielding a clinical sensitivity of 85.0% and a specificity of 82.5% at an optimal cut-off point of ≤ 0.55. Meanwhile, serum CCL2 protein levels demonstrated slightly higher diagnostic accuracy, with an AUC of approximately 0.912 (95% CI: 0.870–0.960; P < 0.001), a sensitivity of 88.5%, and a specificity of 86.0% at a cut-off point of ≥ 11.45 pg/mL. this shown in table 4.

Table 4 : Receiver Operating Characteristic (ROC) curve analysis parameters for evaluating the diagnostic performance of miR-370, CCL2, and their combination.

Parameters	AUC	CI	Specificity	Sensitivity	Cut-off	P-value
mir370	0.885	0.820-0.940	82.5%	85.0%	0.55	<0.001**
CCL2	0.912	0.870-0.960	86.0%	88.5%	11.45	<0.001**
Combined panel (mir370+CCL2)	0.954	0.910-0.990	90.5%	92.0%	-	<0.001**

ROC = Receiver Operating Characteristic curve; AUC = Area Under the Curve; CI = Confidence Interval. Combined panel likelihoods were designed by binary logistic regression . ** High significance at the 0.001 level.

Combining together biomarkers into a single predictive model (using binary logistic regression) resulted in high diagnostic accuracy, with AUC reaching 0.954 (95% CI: 0.910–0.990; P < 0.001). At this optimal threshold, sensitivity rose to 92.0% and specificity to 90.5%, demonstrating the clear superiority of the joint biomarkers over individual biomarkers in diagnosing *P. mirabilis* kidney infections.

DISCUSSION

The higher incidence of pyelonephritis among females observed in this study is attributed to anatomical and physiological factors greatest notably the shortness of the female urethra and its proximity to the perianal region as these factors facilitate the retrograde migration of microorganisms into the urinary tract (1, 9). the unique swarming motility of P. mirabilis enhances its ability to breach these

short anatomical barriers in females and reach the renal tissue (10).

Demographic indicators in the current study reveal that individuals aged 40 to 60 constitute the demographic group most affected by the disease. This prevalence among middle-aged and older adults aligns with existing literature documenting an increase in structural and functional urinary tract disorders during these stages of life. Within this cohort, factors such as renal stone formation (specifically struvite calculi), undiagnosed anatomical abnormalities, and a decline in local mucosal immunity facilitate bacterial colonization and ascending migration (11,12). Furthermore, the relatively high prevalence observed in the older age group (60–80 years) is closely linked to the phenomenon of immunosenescence and the increased need for clinical medical interventions such as the insertion of urinary catheters which create an ideal environment for the formation of *P. mirabilis* biofilms (13).

The results of the current study showed no statistically significant difference ($P<0.05$) in the incidence rates of *P.mirabilis* pyelonephritis between urban and rural patients. This similar distribution indicates that exposure to the pathogen is not directly influenced by urban lifestyles or specific rural environmental factors. The results confirm that the disease is associated with physiological predispositions and prior common medical procedures such as urinary catheterization or antibiotic use rather than geographical location (14). The slight increase observed among urban residents (51.7%) may be attributed to higher population density and the easy access that city-center residents have to teaching hospitals and medical centers (15, 16).

Clinical manifestations reveal a predominance of systemic symptoms—such as fever (75%) and chills (44%) induced by Gram-negative bacterial endotoxins (LPS), which trigger an immune response by activating immune cells. This systemic inflammatory process coincides with a sharp rise in serum CCL2 levels; this chemokine acts as an immunological bridge, recruiting macrophages and monocytes that secrete pyrogenic cytokines, and also induces nausea and vomiting through direct effects on the central nervous system (17).

Localized symptoms such as flank pain, costovertebral angle tenderness, and suprapubic tenderness are directly linked to the ability of *P. mirabilis* to produce the enzyme urease. This enzyme causes urine alkalinization and the precipitation of phosphate, magnesium, and ammonium crystals, leading to the formation of struvite stones; these stones contribute to acute mechanical obstruction and distension of the renal capsule and pelvis, resulting in severe pain and tenderness (18). Tissue destruction and mechanical obstruction are associated with reduced expression of miR-370; its loss deprives renal cells of their natural ability to suppress inflammatory and cellular oxidative stress pathways, thereby exacerbating interstitial tissue damage (19).

Dehydration, loss of consciousness, and hypotension indicate the progression of the disease from localized pyelonephritis to severe urosepsis and septic shock. This progression involves the release of microbes and their enzymatic products into the bloodstream, leading to acute systemic vasodilation and impaired perfusion of the brain and vital organs. These events coincide with elevated serum levels of the CCL2 protein and the breakdown of the miR-370-mediated local immunosuppressive mechanism, ultimately resulting in an uncontrolled, life-threatening systemic inflammatory response (20).

A strong, statistically significant inverse correlation ($r = -0.645$, $P < 0.001$) exists between reduced miR-370 expression and elevated serum CCL2 levels, indicating a disruption in the molecular regulatory axis

linking microRNAs to immune response mediator proteins during ascending infection. Mic370 is classified as a renoprotective miRNA that acts as an endogenous inhibitor, preventing the overactivation of inflammatory signaling cascades. Studies have demonstrated that miR-370 directly targets genes within inflammatory signaling pathway specifically the NF-κB pathway, which is the master regulator responsible for transcribing genes that encode cytokines and chemokines (19).

Upon infection with *P. mirabilis*, the bacteria secrete potent virulence factors such as endotoxins (LPS) and the enzyme urease leading to severe local alkalinization and the destruction of renal interstitial and epithelial cells. This intense cellular stress suppresses and down-regulates the expression of miR-370. Consequently, the cells lose a natural inhibitor, thereby enabling the unrestricted activation of the NF-κB pathway within the nucleus; this triggers a massive induction of CCL2 transcription and its subsequent release into the bloodstream (18, 21).

Chemokines CCL2 acts as a potent chemoattractant, recruiting monocytes and macrophages from the bloodstream into injured kidney tissue to elicit an immune response. In the absence of miR-370-mediated immune regulation, this recruitment process intensifies, leading to excessive cellular accumulation and the release of massive amounts of inflammatory chemokines—a phenomenon known as a "chemokine storm"—which drives the progression of the condition toward sepsis (22).

The deterioration of the patient's condition and the worsening of renal tissue damage in cases of pyelonephritis do not stem from direct bacterial virulence; rather, the negative correlation ($r = -0.645$) between the two immunological markers protective miR-370 (↓) and CCL2 secretion (↑) establishes the miR-370/CCL2 axis as a diagnostic target for predicting the extent of renal tissue damage and preventing septic shock.

The diagnostic performance of the two immunological markers was evaluated using ROC analysis a statistically significant procedure to determine the accuracy of these biomarkers in the current study. The results demonstrate excellent to exceptional discriminative performance for both miR-370 and CCL2 when analyzed individually; however, combining them into a unified predictive panel resulted in a marked improvement in diagnostic accuracy, achieving exceptional performance.

The high discriminatory capacity of miR-370 is attributed to its sensitivity to early cellular changes associated with renal pathology. Under normal physiological conditions, this miRNA acts as an immune guardian protecting renal tissues; however, when these tissues are attacked by *P. mirabilis*, miR-370 expression levels decrease. This alteration serves as a sensitive immunological indicator reflecting the extent of localized renal tissue damage prior to disease progression and the onset of significant structural complications (23, 24).

The high diagnostic value of serum CCL2 reflects its role as a pivotal immunological marker in the body's immune response; damaged renal cells secrete high levels of this chemokine to recruit macrophages a process triggered by specific immune signals and elevated concentrations serve as a strong indicator of acute, active inflammation (22, 25).

The statistical results regarding the combination of immunological markers demonstrated a successful and superior Area Under the Curve (AUC) profile, validating the principle of diagnostic integration. Relying on a single marker might overlook complex aspects of the disease; conversely, combining the immunological marker miR-370 whose down-regulation reflects intracellular

dysfunction with CCL2 whose up-regulation reflects the immune and inflammatory response provides the physician with a comprehensive perspective. This offers a complete picture of the disease's progression, spanning from its immunological origins to its clinical manifestations.

This academic approach aligns with modern medical recommendations confirming that integrating biomarkers (epigenetic/miRNA biomarkers) with traditional protein markers (protein/cytokine markers) significantly decreases the likelihood of diagnostic error and enhances clinical sensitivity and specificity(26,27,28). Consequently, the proposed dual panel (mir-370 + CCL2) serves as a greatly accurate, non-invasive tool for identifying acute pyelonephritis and triaging dangerous cases, thereby obviating the need for composite diagnostic procedures.

CONCLUSION

The present study demonstrates that acute pyelonephritis caused by *P.mirabilis* is related with a reciprocal molecular and immunological inequity: specifically, a down-regulation of the reno-protective miR-370 and a marked raise in serum levels of the inflammatory CCL2, which are strongly inversely connected (r = -0.645). merging these 2 biomarkers into a unified diagnostic panel produces exceptional diagnostic performance surpassing that of individual markers with a discriminative accuracy (AUC) of 0.954 and clinical sensitivity and specificity exceeding 90%, making this a promising, non-invasive tool for the early and accurate diagnosis of the illness.

RECOMMENDATION

Recommend adopting the joint dual-marker panel (miR-370 + CCL2) as a high precision, non-invasive showing tool in medical research laboratory and hospitals for the early diagnosis and fast triage of patients with acute kidney inflammation; this too opens new molecular avenues for discovering the potential use of miRNA mimics as a likely therapeutic strategy to overwhelm inflammatory cascades and stop renal damage and septic shock.

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Author Contribution :Layla S. Abdul-Hassan was responsible for methodology, study design, statistical analysis, understanding of results, and recruiting the manuscript.

Ethics Approval and Consent to Participate : The manuscript was agreed by the Ethics Committee of the Najaf Health Directorate. Written learned consent was got from each participant previous to the study. All measures were lead in accordance with the guidelines of the local study committee and the Declaration of Helsinki (1964) and its following amendments.

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