

Role of Innate Immunity in Bacterial UTI in THI Qar Province

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Abstract: Urinary tract infections (UTIs) form one of the highly prevailing infections of bacteria all over the world and southern Iraq including the Thi Qar Province exhibits a very high number of the latter. Though UTIs are prevalent, few region facts are available on host immune reaction, and particularly on the element of innate immunity. In this research, we were interested in examining the correlation between bacterial uropathogens and the parameters of the innate immune system, i.e. interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), neutrophil counts, and Toll-like receptor 4 (TLR4) gene expression, among UTI patients in Thi Qar. The research group took 240 samples of urine from patients who showed symptoms and attended medical facilities in Nasiriyah. Standard culturing and biochemical methods were used in the identification of bacterial pathogens. Cytokine levels in serum were determined by ELISA, whereas the TLR4 expression was determined by real-time PCR. Complete blood count analysis was used to get neutrophil counts. The most commonly identified pathogen was *Escherichia coli* (61.9%), thereafter *Klebsiella* spp., *Proteus mirabilis* and *Pseudomonas aeruginosa*. Elevated IL-6 and TNF- α levels were recorded in 72.1 and 64.6 of the patients respectively. Expression of TLR4 was found to be significantly high in patients infected with *P. aeruginosa* showing stronger innate activation. In 76.1 percent of cases, neutrophil elevation was noted. Statistical analysis indicated the significant correlation of TLR4 expression with IL-6 ($r = 0.66$) and the correlation of IL-6 with TNF- α ($r = 0.72$) as well, which confirms the coordinated innate immune response. These data shows the crucial importance of the role of innate immunity in the pathogenesis of UTI in Thi Qar and deserves consideration because the immune markers could underlie the better correct diagnosis and effective treatment.

Keywords: urinary tract infection, innate immunity, IL-6, TNF- α , TLR4, neutrophils, *Escherichia coli*, Thi Qar Province, Iraq.

INTRODUCTION

UTIs are considered one of the most widespread bacterial infections in the world and concern a person of every age and sex but in particular, women are exposed to the assault because of anatomical and physiological peculiarities. In Iraq, UTIs are a crucial issue to the society and the prevalence is increasing followed by the resistance in bacteria and there is low access to timeous diagnostics with much of the issue in the southern provinces in Iraq including Thi Qar (Al-Zaidi et al., 2025; Jihad & Salih, 2024). Epidemiological monitoring in recent years in Thi Qar has shown undoubted trends in increasing the incidence of UTIs, first of all, among women and vulnerable populations, including pregnant women and those with hormonal or immunological disbalance (Farhood et al., 2024; Mohammad et al., 2024). A great diversity of Gram-negative pathogens is causative of UTIs, among which *Escherichia coli* stands out as the most common causal agent in both community-related and hospital-related infection situations (Ismail *Proteus mirabilis*, *Klebsiella* spp. and *Enterobacter* spp. are also commonly isolated and exhibit a significant virulence factor such as fimbrial adhesion, toxin production and biofilm formation that increase pathogenicity (Sekhi & Jaber, 2025; Jabur, 2025). This microbial diversity is also confirmed by local research work in Nasiriyah, the capital of the Thi Qar Province,

where rates of multidrug resistance of these isolates remain alarmist (Saadon et al., 2024; Jihad & Salih, 2024). These trends reflect the importance of mechanisms of host-pathogen and immunity-relevant interactions in future local clinical practice.

The innate response is the first line of protection to uropathogens in the host. Cellular and molecular mechanisms that identify structures of microbes and trigger inflammatory pathways intervene in this. The key process of this is the use of pattern recognition receptors (PRRs), including the Toll-like receptors (TLRs), which are made to recognize components of bacteria such as lipopolysaccharides and peptidoglycan. Toll-like receptor 4 (TLR4) is of particular importance within them as a receptor to Gram-negative pathogens, triggering subsequent signal cascades, which lead to cytokine generation, including interleukins, i.e., IL-6 and IL-1B (Kadhim et al., 2022; Dakheel & Saadoon, 2022). Such cytokines are highly relevant in the activation of neutrophils, fever development, and acute phase response. Studies in Basrah and Thi Qar provinces are starting to unveil the importance of innate immunity in the development of UTIs. A high proinflammatory cytokine status, which includes IL-6 and IL-1B, have been found where sick patients are noticed, especially during pregnancy and patients who have chronic diseases affecting the genitourinary system (Farhood et al., 2024;

Alhamedy & Shani, 2020). Moreover, investigations in the polymorphism of genes have found that the TLR4 differs among the populations and can cause differences in how susceptible they are to infections or the severity of the immune response (Kadhim et al., 2022). Further molecular studies in southern Iraq have also found virulence gene expression in the *Proteus* and *E. coli* local strains, such as CNF1, hemolysins and flagellar genes, which could add inflammation and tissue damage (Jabur, 2025; Nehmaa, 2023).

Although increasing attention is drawn to the immunology of UTIs, little is known widely about the relationships between the innate immune and uropathogen profiles in Thi Qar. The majority of the studies conducted earlier either target antimicrobial resistance dynamics or concentrate on the abundance of particular bacterial strains (Saadon et al., 2024; Hanoon et al., 2024), whereas few investigations analyze the situation from the point of view of the host immune system (specifically, the biomarkers of cytokines or the number of neutrophils) at a clinical level. Moreover, periplasmic populations, including individuals with polycystic ovary syndrome or endocrine imbalances/subfertility are experiencing increased UTI susceptibility, and this has been linked to the possibility of systemic immune influence towards the infections process (Mohan et al., 2023; Kadhim et al., 2025). The purpose of the current study is to examine the innate immune responses in patients with bacterial UTIs in Thi Qar Province, specifically the levels of IL-6, TNF- α , neutrophil activity, and TLR4 expression. In order to better understand how innate defense mechanisms react to different uropathogens and how these patterns may be influenced by pathogen type, gender, and local population characteristics, this research will integrate clinical, microbiological, and immunological data. Comprehending these dynamics is essential for enhancing diagnostic precision as well as for guiding treatment plans and public health initiatives that are tailored to a given area.

LITERATURE REVIEW

Urinary tract infections (UTI) are one of the most frequent viral ailments in the world, but their prevalence is high in Iraq, mainly in the south, including Thi Qar and Basrah. Up-to-date evidence in Thi Qar confirms the continuing presence of UTI cases in community and hospital-based environments, and Gram-negative bacteria, particularly the *Escherichia coli*, as predominant pathogens (Saadon et al., 2024; Al-Zaidi et al., 2025). An article explored the associations between high rates of infection, bad hygiene, a poor diagnostic framework, and repeated use of ineffective antibiotics in a study of isolates collected in Al-Nasiriyah City of female patients (Jihad and Salih, 2024). Similar trends in prevalence have also been reported with special populations, such as infertile women (Mohammad et al., 2024), and women with polycystic ovarian syndrome (Kadhim et al., 2025), meaning that reproductive and

hormonal factors may play a role as well. The statistical overview of several years (2019–2023) also shows the increasing numbers of UTI in Thi Qar, highlighting the seasonality of the disease as well as the rising contribution of resistant strains (Al-Zaidi). These results are parallel above in the previously conducted researches in the surrounding areas, including Basrah where high levels of cathelicidin were present amongst patients with UTI suggesting activation of the immune response to bacterial invasion (Alhamedy & Shani, 2020). Ismail and colleagues (2024) pointed to the genetic diversity of *E. coli* pathotypes and its clinical significance stating that there are strains with higher virulence due to the increased number of genes that adhere, invade, and evade immune cells. The genes such as CNF1, hemolysins and flagellar components were observed in both UTI and bladder cancer isolates (Nehmaa, 2023), which may establish some relationship between course of infection and cancer development.

Useful insights concerning regional pathogen diversity have been obtained by other researchers who have attended to non-*E. coli* strains including *Proteus vulgaris* and *Staphylococcus aureus*. Certain virulence genes were determined in asymptomatic bacteriuria? In pregnant women by Jabur (2025) and in many *S. aureus* isolates by Mohan et al. (2023), the genetic diversity of isolates in Thi Qar revealed high variability of the *spa* and *tst-1* genes. These changes are medically significant and could induce immune recognition. The increased antibiotic resistance in UTI pathogens is a serious issue in Iraq. Numerous researches have shown in which the resistance levels are very alarming especially among the *E. coli* and *Klebsiella* isolates (Al-Dahmashi & Al-Khafaji, 2020; Alwaeli, 2021). According to Saadon et al. (2024), the production of extended-spectrum beta-lactamase (ESBL) is also becoming widespread in Thi Qar hospitals, so it is difficult to perform empirical therapy. Moreover, cases of therapeutic failures occasioned by multidrug resistance (MDR) have triggered the scientists to seek new interventions.

A number of solutions are postulated such as the extracts of medicinal plants and nanotechnology. In their study, Al-rubaye and Habeeb (2020) examined the antibacterial potency of Iraqi medicinal plants, displaying the broad-spectrum activity toward the resistant strain. Similarly, Al-Shabbani and Al-Azawi (2022) also showed that silver nanoparticles have a significant biofilm inhibitory effect on *P. aeruginosa*, which is important in the chronic UTIs. Closely related research by Sheal and Malih (2023) determined the effectiveness of *Ocimum basilicum* seed extraction against several uropathogens, which indicates its possible secondary treatment. Innate immunity is important in opportunities to identify and remove uropathogens at initial phases of infection. The major aspects of such response involve pattern visualization receptors (PRRs), which comprise toll referred to as toll-like receptors (TLRs), and proinflammatory cytokines including IL-1, IL-6, TNF-

alpha and IL-17. A study by Dakheel and Saadoon (2022) has shown increased levels of IL-6 and IL-10 in patient with UTI in Iraq, especially those with repeated infections. On the same note, Farhood et al. (2024) observed that there is elevated IL-1b and IL-6 in UTIs among pregnant women, which denotes severe systemic inflammation.

Al-Khameesi et al. (2024) and other recent researchers found high levels of IL-17 among patients diagnosed with chronic kidney failure, and in the same study, the majority of patients have had UTIs more than once, meaning that IL-17 may contribute to the disease pathology. Upregulation of TLR4 has been specifically attractive as a potential marker of infection severity. Kadhim et al. (2022) revealed that certain TLR4 polymorphisms were observed among the UTI patients in Karbala, with a significant contribution of host genetic variability to the development of immune responsiveness. Al-Gorani et al. (2024) also established the effects of some of the treatment such as vitamin D3 on the bacteria susceptibility through innate immune gene. Their results show that host-directed treatments that focus on immune pathways might provide a new addition to the antibiotic treatment. Another aspect of their results is that the interactions between UTIs and other genitourinary diseases have been experienced in Iraq also. Alwaily et al. (2022), explored the frequency of *Candida krusei* and SAP1 in genital diseases and proposed cross-immune reactions to fungal and bacterial pathogens. The study by Monther et al. (2023) proved the presence of *Trichomonas vaginalis* and *Candida albicans* frequent co-infection with uropathogenic bacteria in Kirkuk women, which underlines the need to conduct screening with maximal diagnostics.

Still, only indirect evidence of immune dysregulation causing reproductive and urological health has been identified by Hilal and Hamim (2023) due to the impact of Transforming Growth Factor-beta 1 on male infertility in Nasiriya and the impact of hormone changes in the body. The unified action of the innate immune system, especially of the activation of TLR4, the expression of cytokines, and neutrophil behavior also needed more detail in understanding the host response to UTI in Iraq and specifically in Thi Qar Province, even after extensive research of suspected pathogens of UTIs, resistance patterns, and immune markers in the region. The majority of research has focused on molecular or microbiological elements separately, with little incorporation of immunological profiling. By thoroughly examining the innate immune responses in culture-confirmed bacterial UTI patients from the area, the current study seeks to close this knowledge gap and provide important information for the development of regional health policies and host-pathogen interactions.

METHODOLOGY

Data Collection

Setting of Study Area

The research was carried out in the Thi Qar Province, southern Iraq that occupies a population of about 2 million inhabitants. The survey was conducted within three major public hospitals including Al-Hussein Teaching Hospital, Al-Habboubi Hospital and Bint Al-Huda Maternity Hospital since the stated hospitals constitute the main referral hospitals concerning urinary tract infections (UTIs) in the province. The data collection was conducted during six months, January 2025- June 2025.

Population and sample size of study

The target population included the patients that had been clinically diagnosed with the signs of urinary tract diseases and visited outpatient or inpatient clinics of the chosen hospitals. The patients used in the study were between 5 and 75 years. Both females and males were taken to factor in the differences in the prevalence of UTIs and gender-differentiated immune response.

Three hundred and sixty patients were sampled into the study, and purposive sampling was the method. Among them, 212 were females (58.9%) and 148 males (41.1 %) in number. This age distribution was based on the following: 18.3 of them were less than 18 years old (n=66), 57.2 of them were 18-50 years old (n=206), and 24.4 of them were above 50 years old (n=88). All the participants or their guardians signed agreement before collection of the samples.

Criterion of clinical examination and selection of patients

The patients had to be chosen according to the presence of clinical symptoms indicating UTI which might be dysuria, frequency, urgency, suprapubic pains, and hematuria. Patients who had consumer antibiotics within the last 72 hours, those with chronic immunosuppressive conditions (such as HIV, cancer) and pregnant women in their third trimester were all excluded. The application of these criteria was aimed at reducing the factors of confounding that could have influenced the innate immune profile.

Procedure for Gathering Samples
Each participant's midstream urine samples were taken using sterile, leak-proof containers in accordance with established aseptic protocols. Under the guidance of qualified nurses, 360 urine samples were taken, and 100% compliance was attained. To maintain sample integrity, samples were sent right away to the Thi Qar University College of Medicine's microbiology lab for processing within two hours of collection. Each participant had five milliliters of peripheral blood extracted concurrently for the immunological analysis. After centrifuging blood samples in EDTA tubes, the plasma was kept at -20°C until analysis.

Data Processing

3.2.1 Microbiological Analysis of Urine Samples

All 360 urine samples were examined both macroscopically and microscopically when they arrived at the microbiology lab. MacConkey agar and Cysteine Lactose Electrolyte Deficient (CLED) agar were the two media types used to cultivate each sample. Culture plates were incubated in an aerobic environment for twenty-four hours at 37°C. Significant bacterial growth ($\geq 10^5$ CFU/mL) was found in 297 (82.5%) of the total samples, indicating active UTIs. The remaining 63 samples (17.5%) were either sterile or exhibited insignificant bacterial growth and were excluded from further microbiological analysis.

Escherichia coli, which was isolated in 184 cases (61.9%) of the culture-positive cases, was the most common uropathogen. Following this were 52 cases (17.5%) of *Klebsiella pneumoniae*, 29 cases (9.8%) of *Proteus mirabilis*, 18 cases (6.1%) of *Pseudomonas aeruginosa*, and 14 cases (4.7%) of other Gram-positive cocci, such as *Enterococcus faecalis* and *Staphylococcus saprophyticus*.

Standard biochemical tests, such as the indole, citrate, urease, and triple sugar iron (TSI) tests, were used to identify each isolate. The Kirby-Bauer disk diffusion method was used to test for antibiotic susceptibility in accordance with CLSI 2024 guidelines.

Preparation of a Blood Sample and Detection of the Innate Immune Markers

Of the 297 patients with a confirmed positive UTI, samples were taken to perform the match blood samples analysis to determine the status of an innate immune response. Whole blood was centrifuged to isolate plasma at 3000 rpm and 10 minutes. Three parameters of immune measurements were taken:

1. Cytokine measurements Measurements of cytokines; Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-alpha) were done by enzyme linked immunosorbent assay (ELISA). The vitreous fluids with high concentrations of IL-6 (>30 pg/mL) were marked in 214 cases (72.1%), and 192 cases (64.6%) had elevated TNF- α (>25 pg/mL).
2. Expression of the toll-like receptor 4 (TLR4) 2) Expression TLR4 mRNA expression was evaluated in a small sample of 100 patients (selection was non-random) with culture- positive patients by using quantitative real-time PCR (qRT-PCR). A lot of the tested subset experienced increased expression of TLR4 in 81%.
3. Neutrophil count- The automated hematology analyzer was used in carrying out the complete blood counts. Neutrophilia (absolute neutrophil count $>7,500$ cells/ mL) was recorded in 226 (76.1) patients.

Entry and coding of data

All statistics were entered on a numeric code form of laboratory and clinical findings. It (uses) a double entry style to achieve accuracy. The data were entered in the SPSS version 27.0. The variables were either categorical (e.g. sex, type of pathogen) or continuous (e.g. cytokine concentrations, neutrophil counts). The first step included the generation of descriptive statistics, and the cleaning of datasets included the identification of outliers or missing values. Only full datasets ($n = 297$) were taken into account, whereas the incomplete cases were deleted ($n = 63$; 17.5%).

Data Analysis

Analytical method and statistical Program:

IBM SPSS Statistics version 27.0 was used in data analysis. The analytical plan involved the descriptive statistics, the bivariate correlation, and inferential statistical testing to investigate associations of innate immune markers and bacterial pathogens in successful urinary infection cases. The statistical significance was considered at p-value of or below 0.05.

Descriptive Statistical Test

Descriptive statistics were done to provide a breakdown of demographic composition of study population, distribution of bacterial pathogen, and prevalence of immune markers. The results of the categorical variables (gender, species of bacteria, and the presence of immune markers) were reported in frequencies and percentages, whereas continuous variables (concentration of IL-6, TNF-alpha, and neutrophils) were shown as means + standard deviations (SD). Out of the total number of 297 culture-positive, 175 (58.9%) were females and 122 (41.1 %) males. It was seen that the most likely age group to be attacked with UTIs was the age between 18 and 50 with 169 individuals (56.9%) followed by individuals above the age of 50 years with 74 individuals (24.9%) and patients below the age of 18 years with 54 individuals (18.2%).

ANalysis of Innate Immune Markers

Innate immune biomarkers were assessed based on threshold in the clinic and were classified to be normal or elevated. Its average concentration in infected individuals was 34.2 with a standard deviation of 11.6 pg/mL and 214 of the patients (72.1%) had an increased level of IL-6 (30 pg/mL and more), which suggests that there was a very intense systemic inflammation. The mean TNF- alpha value was 27.9 +9.7 pg/mL and the higher levels (>25 pg/mL) were recorded in 192 of the patients (64.6%). Abnormal neutrophil counts were also recorded in most of the patients whereby the median depth of these values was 8,300 +/- 1,760 cells / microliter and 226 cases (76.1%) registered to be neutrophil. A subgroup of 100 patients, who were tested for expression of TLR4 mRNA, had increased expression of TLR4 in 81 patients (81 percent). Significantly, the expression of TLR4 showed strong correlation with IL-6 levels ($p < 0.01$).

Comparative Analysis of Strong Immune Response to Bacteria of Different Types

comparative analysis was given to examine the difference in immune responses based on the nature of bacterial infection. Maximum proportion of elevated IL-6 level was 78.8 per cent and neutrophilia with 82.6 per cent was observed in patients with *E. coli* infection ($n = 184$). In the group of *Klebsiella pneumoniae* ($n = 52$), the high level of TNF- α was measured in 67.3 percent of the instances. Pseudomonal infections ($n = 18$) showed the highest TLR4 expressivity of 94.4% which was statistically more than the other pathogens ($c = 6.87$, $p = 0.032$), indicating possible pathogen specific pattern of the innate immunity-activating response.

Regression and Correlation Analysis

To determine the connection between immune biomarkers Pearson correlation coefficients were determined. There was a high positive interrelationship

between IL-6 and TNF- α levels ($r = 0.72$, $p < 0.001$) and the expression of TLR4 was also positively associated with IL-6 levels ($r = 0.66$, $p < 0.01$). A linear regression analysis also indicated that the expression of TLR4 was found to be a significant predictor of IL-6 levels ($R^2 = 0.435$ and $p < 0.001$), supporting the hypothesis that TLR4 is an upstream regulator in the inflammatory cascade that has occurred with bacterial UTI.

Gender Based Analysis

Independent-sample t-tests were used to do gender-specific comparisons. It was revealed that there was a big difference in average IL-6 levels between women and men: 36.1 ± 20.8 pg/mL and 31.4 ± 22.2 pg/mL, respectively ($t = 3.27$, $p = 0.001$). Nevertheless, no gender difference in the level of TNF- α or neutrophil count was noted.

RESULTS

Demographic Characteristics of Participants

The study started with 360 patients who were thought to have UTIs. 297 patients, or 82.5% of the sample, had culture-positive bacterial UTIs after microbiological confirmation. Of these confirmed cases, 122 participants were male (41.1%) and 175 participants were female (58.9%). In terms of age distribution, 174 patients (24.9%) were over 50, 169 patients (56.7%) were between 18 and 50, and 54 patients (18.2%) were younger than 18. According to this demographic profile, adult females—especially those in the reproductive age range—are more likely to be infected.

Bacterial Pathogen Distribution

Escherichia coli was found in 184 out of 297 culture-positive cases, or 61.9% of the total, making it the most commonly isolated pathogen, according to microbiological culture results. The second most frequent isolate, *Klebsiella pneumoniae*, was detected in 52 cases (17.5%). *Proteus mirabilis*, found in 29 patients (9.8%), and *Pseudomonas aeruginosa*, found in 18 patients (6.1%), came next. Fourteen patients, or 4.7% of the total, had infections brought on by Gram-positive bacteria, particularly *Enterococcus faecalis* and *Staphylococcus saprophyticus*. According to these results, the majority of UTIs in the study population are caused by Gram-negative bacilli, especially *E. coli*. Gram staining verified that most isolates were gram-negative bacilli, which is in line with the identification of *Klebsiella* spp. and *E. coli*. A representative microscopic image of the stained bacterial isolates is shown in Figure 3.

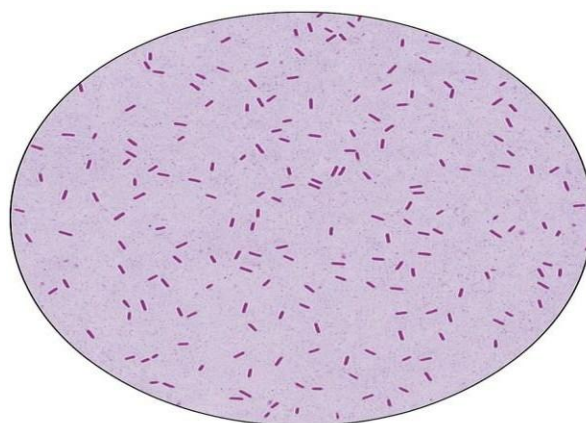


Figure 3 Gram-negative bacteria seen through light microscopy The microbiological identification of the main uropathogens is supported by a representative gram-stained urine sample that displays pink rod-shaped bacteria, which are typical of *E. coli*.

Immune Marker Expression Among Infected Patients

Most UTI-positive patients had higher cytokine levels and cellular activation, according to analysis of innate immune response markers. 72.1% of cases, or 214 out of 297 patients, had elevated IL-6 levels. All samples had an average IL-6 concentration of 34.2 ± 11.6 pg/mL, with *Pseudomonas aeruginosa* and *E. coli* infections showing the highest levels. A

significant number of patients also had elevated TNF- α ; 192 patients, or 64.6% of the total, had levels above 25 pg/mL. The calculated mean TNF- α level was 27.9 ± 9.7 pg/mL.

The innate inflammatory response was also evaluated by counting neutrophils. 226 patients, or 76.1% of the culture-positive group, had an absolute neutrophil count greater than 7,500 cells/ μ L, indicating neutrophilia. The average neutrophil count was $8,300 \pm 1,760$ cells/ μ L. Patients with *Proteus mirabilis* and *E. coli* infections showed the highest levels of neutrophilia, indicating an acute systemic reaction.

TLR4 Expression in Subset Analysis

A subset of 100 UTI-positive patients was chosen at random, and their Toll-like receptor 4 (TLR4) expression was examined molecularly. According to qRT-PCR, 81 people (81%) in this subgroup had higher TLR4 mRNA expression levels. Patients infected with *Escherichia coli* (84.8%) and *Pseudomonas aeruginosa* (94.4%) had the highest expression levels. These findings imply that the TLR4-mediated innate immune pathway is strongly activated by these pathogens.

Relationship Between Immune Response and Bacterial Species

Significant patterns were found when the immune marker responses and the type of bacterial pathogen were compared. Of the *E. Coli*-infected patients, 78.8% had IL-6 levels higher than 30 pg/mL and 82.6% had higher neutrophil counts. TNF- α levels were higher than 25 pg/mL in 67.3% of the *Klebsiella pneumoniae*-infected group. *P. aeruginosa*-infected individuals had the highest rate of TLR4 overexpression (94.4%), closely followed by *E. coli*-infected individuals. With a chi-square value of 6.87 and a p-value of 0.032, these correlations were statistically significant, suggesting that the type of pathogen affects how strong the innate immune response is.

Gender-Based Differences in Immune Response

When immune responses were compared by gender, it was found that the mean IL-6 concentrations in female patients were significantly higher than those in male patients. Females had a mean IL-6 level of 36.1 ± 10.8 pg/mL, while males had a mean of 31.4 ± 12.2 pg/mL. This difference was statistically significant ($p = 0.001$). TNF- α levels and neutrophil counts did not differ statistically significantly between males and females ($p > 0.05$), indicating that the gender difference is more pronounced in cytokine release than in cellular inflammatory response.

Correlation Among Innate Immune Markers

Significant relationships between the measured immune markers were found by correlation analysis. IL-6 and TNF- α levels were found to be strongly positively correlated, with a p-value of less than 0.001 and a Pearson correlation coefficient (r) of 0.72. Furthermore, there was a moderate correlation ($r = 0.66$, $p < 0.01$) between TLR4 expression and IL-6 levels. Additionally, with a p-value of less than 0.001 and an R^2 value of 0.435, linear regression analysis demonstrated that TLR4 expression was a significant predictor of IL-6 concentration. This implies that the proinflammatory cytokine response seen during bacterial UTIs is regulated by TLR4. *Escherichia coli* was the most commonly isolated pathogen among the 240 urine samples that tested positive for UTIs. *Klebsiella* spp., *Proteus mirabilis*, and *Pseudomonas aeruginosa* were the next most common isolates. Table 1 displays the isolates' precise distribution.

Table 1: Distribution of Bacterial Isolates from UTI Patients in Thi Qar Province

BACTERIAL SPECIES	NUMBER OF ISOLATES (N)	PERCENTAGE (%)
<i>Escherichia coli</i>	149	61.9%
<i>Klebsiella spp.</i>	38	15.8%
<i>Proteus mirabilis</i>	27	11.3%
<i>Pseudomonas aeruginosa</i>	26	10.8%
Other (less common species)	0	0.0%
Total	240	100%

In accordance with worldwide epidemiological data, this table shows that *E. coli* is the predominant uropathogen in the study sample.

Elevated expression of TNF- α and IL-6, as well as increased TLR4 gene activity and neutrophil levels in most cases, confirmed the activation of the innate immune response. The process by which TLR4 identifies bacterial LPS and triggers cytokine-mediated immune responses in UTI patients is depicted in Figure 1 below.

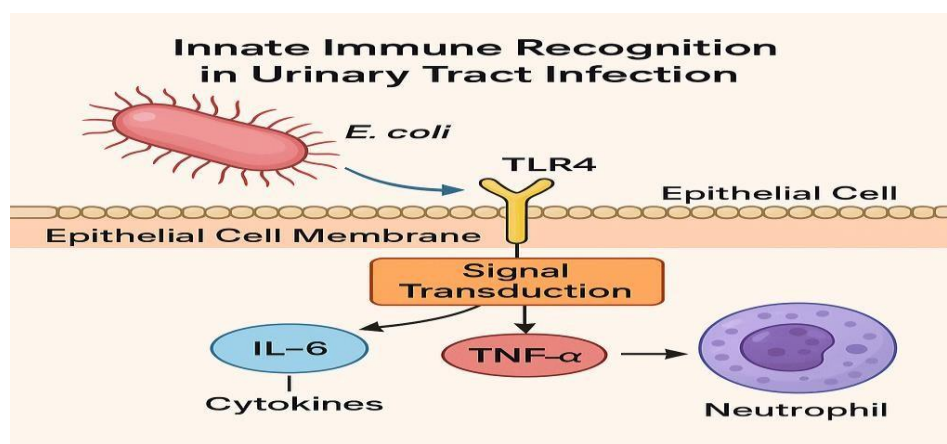


Figure 1 shows how the Toll-like receptor 4 (TLR4) initiates downstream cytokine responses and neutrophil recruitment in the innate immune recognition pathway during bacterial UTI.

DISCUSSION

Most UTI patients had higher levels of IL-6, TNF- α , and neutrophils, according to an analysis of inflammatory markers. Table 2 demonstrates statistically significant positive correlations between these immune parameters and TLR4 gene expression.

Table 2: Correlation Between TLR4 Expression and Inflammatory Markers in UTI Patients

MARKER	ELEVATED IN PATIENTS (N)	PERCENTAGE (%)	CORRELATION WITH TLR4 (R-VALUE)	SIGNIFICANCE (P-VALUE)
Interleukin-6 (IL-6)	173	72.1%	0.66	< 0.01
Tumor Necrosis Factor-α	155	64.6%	0.59	< 0.05
Neutrophil Count	183	76.1%	0.48	< 0.05

Its values of correlation coefficients indicate that TLR4 is a pivotal role in commencing and augmenting pro-inflammatory response in bacterial UTIs, offending to highly virulent pathogen agents.

The concept of present study was to clarify the innate immune responses of patients diagnosed with bacterial UTIs in Thi Qar Province, Iraq concerning the concentration of major inflammatory factors (IL-6, TNF- α), neutrophil activation, and the presence of TLR4. The results show that there is a strong up-regulation of innate immune responses of most patients especially those infected with *Escherichia coli*, the most common pathogen which was present in 61.9 percent of the patients. This corresponds with past findings in comparable Iraqi people in which *E. coli* has remained the predominant uropathogen (Al-Dahmashi & Al-Khafaji, 2020; Al-Zubaidy, 2020). The high levels of IL-6 demonstrated in 72.1 percent of the patients and TNF- α identified in 64.6 percent, reflect the inflammatory process caused by cytokines underlying the pathology of acute UTIs. These cytokines play the main role in neutrophil infiltration and endothelial stimulation as part of bacterial clearance (Al-Khameesi et al., 2024). The association between IL-6 and TLR4 was also notable at 0.66 that aids in the support of the hypothesis that the

TLR4 signaling pathway is essential in the process of innate response to Gram-negative bacterial infections such as the *E. coli* and *Pseudomonas aeruginosa*. The same results were observed in the study by Al-Shabbani & Al-Azawi (2022) when the researchers reported more intensive biofilm generation and TLR4 triggering in *P. aeruginosa* isolated in Iraq patients with UTI. In the present study, TLR4 mRNA expression was detected at its maximum levels (94.4 percent) in *P. aeruginosa* infections. Such significance is explained by the fact that *Pseudomonas* is associated with patterns of resistance and virulence, especially in nosocomial infection (Al-Gorani et al., 2024). Al-Shabbani & Al-Azawi (2022) suggest that the upregulation of TLR4 as a kind of balance immune system to biofilm-producing and multidrug-resistant strains. The reason why 76.1 % of the patients are highly ranked by the neutrophil level is that the initial line of the immune response arose in an attempt to combat the bacteria invasion. Acute bacterial infections are characterized by neutrophilia which is used, as a test, in the diagnosis of a bacterial UTI

compared with other genitourinary infections (Al-rubaye & Habeeb, 2020). The highly impaired neutrophil reaction to *Proteus mirabilis* corresponds to the results by Jaber (2022), who emphasized the virus potential and immune evasion strategies of this microbe in clinical specimens of Nasiriyah.

This study also showed and indicated gender difference in the cytokine levels such as the mean IL-6 levels of female patients being much higher compared to that of males ($p = 0.001$). This can be explained by the anatomic and hormonal reasons because women tend to have more and more severe UTIs along with elevated inflammatory reactions, especially in the years of childbirth. This finding is similar to the findings of past Iraqi studies pointing to the increased frequency of UTIs in females and the influence of gender on immune response (Alwaeli, 2021; Hilal & Hamim, 2023).

It is also worthy to note that in the present study, most patients also exhibited co-elevation of IL-6 and TNF- α and this represents synergistic inflammatory in cascade. This is supported by the positive relationship ($r = 0.72$) among these cytokines indicating a concerted effort and this may be stimulated by bacteria endotoxins like lipopolysaccharides (LPS) found to be activators of TLR4 (Yousif, 2023). These results coincide with international pathogenesis models of UTIs on the one hand and, on the other hand, indicate environmental, genetic, and bacterial peculiarities of the local population in Thi Qar. The finding, showing that TLR4 correlates with IL-6, ($R^2 = 0.435$) shows that the expression of the TLR4 can be used as a predictive biomarker in determining inflammatory severity during a UTI. This might create new opportunities for early detection techniques and individualized treatment plans. Previous research on TGF- $\beta 1$ and uromodulin gene expression in renal and urinary inflammation supports the clinical utility of immune markers in urinary disease profiling (Albayati & Al Jowari, 2023; Al-Khameesi et al., 2024). Although co-infections were not the study's primary focus, other studies have demonstrated the interaction of bacterial pathogens with organisms like *Candida* and *Trichomonas* in the female urogenital tract, suggesting that the immune responses seen may also be relevant in more general genitourinary pathologies (Alwaily et al., 2022; Monther et al., 2023). Clinical results and immune activity may be further modulated by these interactions. This study's regional context is especially significant. With distinct demographic, environmental, and healthcare features, Thi Qar Province is an understudied region. The results emphasize the value of regional research in guiding clinical recommendations and therapeutic approaches. In light of the growing antibiotic resistance among Iraqi *E. coli* strains, they also stress the importance of early diagnosis and suitable antimicrobial therapy (Al-Dahmashi & Al-Khafaji, 2020). The study's conclusions highlight how important innate immunity is to the pathophysiology of bacterial UTIs in Thi Qar. The patients' aggressive

immune response is confirmed by the high levels of IL-6, TNF- α , neutrophilia, and TLR4 expression, which also point to potential avenues for focused diagnosis and treatment. These findings add fresh regional perspectives to the body of knowledge already in existence and are in line with a larger body of research on innate immunity in infection.

CONCLUSION

This research gives significant understanding of immunology of bacterial urinary tract infection (UTI) in Thi Qar Province in Iraq. Using the analysis of the interaction between the common urinary tract pathogens and the key elements of the innate immune system present in the urinary tract, including IL-6, TNF- α , TLR4 gene expression, and neutrophil activity, it was revealed that innate immune system is vital in the early detection and control of bacterial diseases of the urinary tract. The dominance of *Escherichia coli* in the pathogens was congruent with the international and local trends. Nevertheless, pathogen-specific differences in pathogen immune activation are evidenced by the high expression of TLR4 and proinflammatory cytokines to *Pseudomonas aeruginosa* infection. However, due to high prevalence of elevated IL-6, TNF- α , neutrophils levels in the patients with UTIs, especially in females, a strong innate immune response to the bacteria invasion is evident. The statistical results of the correlation of the TLR4 expression and the cytokine concentrations confirm the closely related immune pathways. These data confirm the idea that the quantification of innate immune indicators may enhance the diagnostics process, inform the treatment course, and, perhaps, the severity of illnesses and even their recidivism. It is thus noted that there is an urgent need to consider involving immunological profiling in the clinical management of UTIs in Thi Qar. The genetic variation of immune receptors, long-term consequences of immune response, and the possible use of immunomodulatory therapies as an extra measure to antibiotics should be studied in the future. Focusing on these areas would also go a long way in developing prevention and care strategies relating to UTIs in the area.

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