Original Article

Estimation of TLR-4 and TLR-2 Serum Levels in UTI Patients with Some Risk Factors

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Submission: November 18, 2024 Accepted: December 1, 2024 Published: December 31, 2024

Abstract

Background: Most people of all ages are susceptible to urinary tract infections (UTIs). **Objectives:** This study investigates the correlation between urinary tract infections and serum level of TLR-2 and TLR-4 compared to healthy individuals. **Material and Methods:** The study included 90 serum specimens 45 patients suspected with UTIs and 45 individuals as control group. Samples were collected between October 2022 and March 2023 from private clinics and two hospitals in Babylon Province. Enzyme-linked immunosorbent assay (ELISA) measured serum TLR-2 and TLR-4 levels. **Results:** In this study, Among the patients, there were 28 females (62.2%) and 17 males (37.8%), indicating a higher ratio of females to males; the highest incidence of urinary tract infection occurred in the age difference (12-31 years). The analysis shows signifying a statistically significant difference in smoking behavior between the two groups as well as, indicating a significant difference in BMI distribution, suggesting that overweight status may be more prevalent among UTI patients. The immunological results revealed that the concentrations of TLR2 and TLR4 in the serum of patients were higher compared to those in healthy controls. **Conclusion:** the findings suggest that TLR2 and TLR4, along with elevated serum levels of these markers, may contribute to individual susceptibility to urinary tract infections. Additionally, BMI is identified as a contributing risk factor.

Keyword: UTIs; TLR-4; TLR-2, ELISA, innate immunity.

Introduction

Urinary tract infections (UTIs) are a major health concern that impact millions of individuals annually. The invasion of several pathogenic microorganisms into the urinary tract system is the cause of this condition. There are three types of UTIs: asymptomatic bacteriuria, acute cystitis, and acute pyelonephritis [1].

Urinary tract infections (UTIs) are among the most common bacterial infections in humans, with women being more frequently affected. This increased susceptibility in women can be largely attributed to anatomical factors, such as a shorter urethra, the lack of prostatic secretions, physiological changes related to pregnancy, and a higher risk of urinary tract contamination from fecal flora [2]. The urinary tract relies predominantly on innate immunity for its defense; cytokines and chemokines produced by epithelial and immune cells play a crucial role in recruiting neutrophils to the infection site, induction of pyrexia, and promoting the release of neutrophils [3]. Numerous resident and recruited cells that express a variety of pattern recognition receptors (PRRs) on dendritic cells, macrophages, polymorphonuclear leukocytes, and epithelial cells make up the urinary tract's innate immune system. They identify and differentiate pathogen-associated molecular patterns (PAMPs), which are linked to the bacterial cell walls' capacity to cause disease [4]. The primary distinguishing receptors in the immune system are Toll-like receptors (TLRs), which play a crucial role in the early recognition of pathogens and transmit signals that trigger a swift and powerful proinflammatory immune response. In humans, there are 11 functionally distinct TLRs [5]. When activated, TLRs initiate the expression of effector genes related to inflammatory cytokines, thereby establishing connections between innate and adaptive immunity [6].

TLRs are predominantly found on cells that are likely to encounter microbes, with each type recognizing specific pathogen-associated molecular patterns (PAMPs). TLR2 and TLR4, in particular, are closely associated with urinary tract infections (UTIs) [7]. In the bladder, high levels of TLR4 are present on the apical surface of urothelial cells and within vesicles containing intracellular bacteria. This localization enables a quick response to lipopolysaccharide detection, including the upregulation of various antimicrobial effectors [8]. The aim of this study was to investigate the correlation between urinary tract infections (UTIs) and serum levels of TLR-2 and TLR-4 in comparison to healthy subjects.

Materials and Methods

Samples Collection and Processing

A case-control study included 90 blood specimens were collected from 45 patients suspected with UTI, alongside 45 samples from a control group. Blood specimens were collected from patients admitted to private clinics and two hospitals of Babylon Province: who attended Al-Imam Al-Sadiq Hospital/ Babylon, and Marjan Teaching Hospital.

Inclusion criteria

Patients with urinary tract infections (UTIs) who do not have any chronic diseases.

Exclusion criteria

1. Chronic diseases
2. Patients having antibiotics (at least 3 days).

Estimation of Toll Like receptor 2

Estimated the serum level of TLR2 and TLR4 in sera was determined, as per the manufacturing company (BT LAB, China), that uses the assay for enzyme-linked immunosorbent assay (ELISA). An equation that fits the standard curve was used to calculate the test's results.

Statistical analysis

The analysis for this study was conducted using SPSS (Statistical Package for the Social Sciences) version 23. Results are presented as mean ± SD. A paired t-test was employed for analysis, while an independent samples t-test was used to compare the systemic responses between patients and controls. ANOVA was utilized for comparisons between groups, and correlation tests were conducted to assess relationships between immunological markers. A p-value of less than 0.05 was considered statistically significant.

Ethical Approval

The College of Biotechnology at the Al-Qasim Green University ethical committee approved this study's ethical approval, obtaining verbal consent from each patient and control. A local ethics committee reviewed and approved the subject information and consent form.

Results

In the study population, the distribution of sex revealed that males constituted 37.8% of the cases, accounting for 17 out of 45 participants, while females represented 62.2%, totaling 28 out of 45 participants. Furthermore, in control group males constituted 15 (33.3%), while females represented 30 (66.7%).

Table 1: Sex distribution among UTI-infected patients.

Variable	Patients	Control
Male	17 (37.8%)	15 (33.3%)
Female	28 (62.2%)	30 (66.7%)
Total	45 (100%)	45 (100%)
P value	0.04	

However, the infection percentage increased in the age groups of (52-71) to reach 48.9% of the total number of UTI patients. While the number of cases decreased in the age groups of (12-31) years 22.2% (32-51) and years 28.9% as shown in table (2).

Table 2: Age distribution among UTI patients.

Age group	UTI Patients No (%)
12-31	10(22.2%)
32-51	13(28.9%)
52-71	22(48.9%)
X2	2.977
P-value	0.135

Figure (1) illustrates the distribution of patients and control concerning their residential addresses within Babylon province. The urban, comprises 60 (66.7%) of the total population, while the rural, and accounts for 30 (33.3%) of the total.

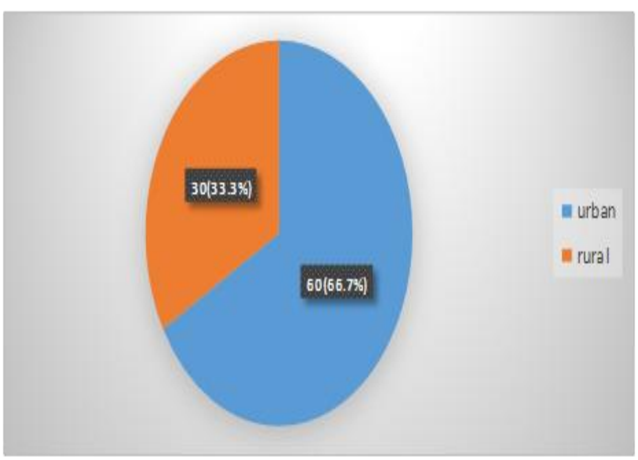

Figure 1: The distribution of study groups according to residential address

Table (4) compares the Body Mass Index (BMI) distribution between UTI patients and control subjects. Among UTI patients, 22.2% were classified as underweight (< 18.5 Kg/m²). In comparison, a significantly higher 40% of the controls fell into this category, for normal weight (18.5-24.9 Kg/m²), 26.7% of UTI patients were recorded, compared to 33.3% of controls. In the overweight category (25-29.9 Kg/m²), 51.1% of UTI patients were overweight compared to 26.7% of controls. It resulted in a p-value of 0.001, indicating a statistically significant difference in the distribution of BMI between the two groups.

Table (4): Distribution of patients and control group according to BMI.

Body Mass Index	UTI Patients No (%)	Controls No (%)	X2	*P*-value
Under weight < 18.5 Kg/m^2	10 (22.2%)	18(40%)	1.92	**0.001**
Normal weight 18.5-24.9 Kg/m^2	12 (26.7%)	15(33.3%)		
Over weight 25-29.9 Kg/m^2	23 (51.1%)	12(26.7%)		
X2	0.42			
***P*-value**	**0.003**			

In a recent analysis of cases, a total of 45 cases were documented, with 30 newly diagnosed cases constituting 66.67% of the total. Whereas, 15 cases, accounting for 33.33%, were relapsed cases. The findings indicate a significant proportion of newly diagnosed cases relative to relapsed ones in the overall patient population.

Table 5: Newly Diagnosed and Relapsed cases of UTI

Newly Diagnosed cases No.(%)	Relapsed cases No.(%)	Total cases No.(%)
30 (66.67%)	15(33.33%)	45(100)

Evaluation of TLR2 concentration in serum by ELISA Assay.

The results in table (6), evaluated the TLR-2 levels (in pg/ml) between case and control groups. The case group, comprising 45 participants, had a mean TLR-2 level of 7.19 ±4.88. In contrast, the control group, also with 45 participants, exhibited a significantly lower mean TLR-2 level of 2.44± 2.75. The P-value of 0.03, suggests a statistically significant difference in TLR-2 levels between the two groups. Results demonstrated that there was an increase in the serum concentrations of TLR2 in patients with UTI in comparison with the control group by using ELISA.

Table 6: Concentration of (TLR-2) pg/ml for UTI patients and controls.

	Group	No.	Mean±SD	Std. E	T-value	*P*-value
TLR-2 (pg/ml)	Case	45	7.19 ± 4.88	0.7133	0.11	0.03
	Control	45	2.44 ± 2.75	0.411		

Evaluation of TLR4 concentration in serum by ELISA Assay

The analysis of TLR-4 levels (in pg/ml) revealed significant differences between the case and control groups. The patient group, consisting of 45 participants, exhibited a mean TLR-4 level of 11.09 ±10.81. In comparison, the control group, also with 45 participants, had a mean TLR-4 level of 3.54±3.23. The calculated P-value was 0.001, indicating a statistically significant increase in TLR-4 levels in the patient group compared to the control group.

Table 7: Concentration of (TLR-4) pg/ml for UTI patients and controls.

	Group	No.	Mean±SD	Std. E	T-value	*P*-value
TLR-4 (pg/ml)	Case	45	11.09±10.81	1.65	4.218	0.001
	Control	45	3.54±3.23	0.48		

Discussion

The prevalence of UTI was higher among females than male patients. These results were in the same line with the results of the study by Ganesh, *et al*, [9], who mentioned that the rate of females with UTI was higher 55.23% in comparison with males 44.79%, also Which was in agreement with the study in Egypt that show the prevalence of UTI in female 54.8% while the prevalence of UTI in male 45.2% [10]. Females show higher percentages of infection than males, which may be attributed to the difference in their reproductive physiology, the urethra in general is shorter in women than in men, so bacteria need very little time to reach the bladder causing infection in addition to their location near the rectum [11,12].

The results presented in Table (2) indicate that the age range of patients with UTI is wide; the incidence of infection varies with age. These findings are consistent with research conducted in Ethiopia by Derese *et al.*, [13]. Also, Gachui, [14] demonstrated that male patients between the ages of 25–34 years had the highest number of UTIs. On the other hand, several studies have been found that contradict this study Gebretensaie *et al.* [15] found that the incidence of UTIs was higher among older women (over 50 years) compared to younger women. The study by Nikhila *et al.* [16] showed that around 60% of women over 50 years old had UTIs compared to about 30% of younger women.

In figure (1) the distribution suggests that there may be differences in the prevalence or detection of the condition being studied between urban and rural areas. Laxminarayan *et al*,. [17] confirmed that the prevalence of infectious diseases such as urinary tract infections is higher in urban areas due to high population density and easier transmission of infections among residents. Understanding the variation between these studies requires considering multiple factors such as healthcare availability, living conditions, and lifestyle [18].

Increased weight can lead to physiological changes in the body, such as higher blood sugar levels, which can increase the risk of bacterial infections in the urinary tract. Obesity can affect the immune system and make it less effective at fighting infections, which can increase the likelihood of UTIs [19]. Excess weight can put more pressure on the bladder and urinary system, which can cause issues with completely emptying the bladder and increase the risk of infection. Anglim *et al*,. [20] found that women with higher BMI had a significantly higher risk of recurrent UTIs.

In the present study in Table (5), shows the expression of TLR2 correlated with activation by the TLR2 ligand peptidoglycan, which aligns with the findings of Zhu *et al*. [21], who reported that TLR2 receptor concentrations were higher in patients with urinary tract infections (UTIs) compared to controls. This suggests that, regardless of whether TLR2 is released in small quantities during bacterial infections, the cytokine production cascade that occurs during such infections differs from the classical pro-inflammatory cytokine cascade typically observed during acute inflammation. Abdalhussin, *et al*. [22] demonstrated that the TLR2 level elevated in the urine of patients in comparison to the control with a high statistical significance.

Elevated levels of Toll-Like Receptor 4 (TLR4) in the urinary tract are indicative of an active immune response to a bacterial infection, particularly a urinary tract infection (UTI) [23]. According to Aksu, *et al.*, [24], urine TLR4 may be used as a useful biomarker in predicting UTI and subsequent pyelonephritis in children with UTI; and may be predicted using 1.28 ng/mL as the cut-off for urine TLR4 with 68% sensitivity and 65% specificity. In the UTI group, urine TLR4 levels were significantly higher in pyelonephritis than in cystitis ($p < 0.0001$). In contrasting studies, some studies have reported lower or inconsistent levels of TLR-4 in various infections, suggesting that TLR-4 responses can vary based on the type of pathogen and the individual's immune system [25]. This activation leads to the production of cytokines and other inflammatory mediators, which help in controlling the infection but can also contribute to the symptoms of inflammation and tissue damage seen in UTIs [26].

Conclusion

The study highlights several significant findings related to urinary tract infections (UTIs), revealing important demographic and biological trends, BMI is identified as a contributing risk factor. Additionally, the elevated levels of TLR-2 and TLR-4 in individuals with UTIs compared to healthy controls point to an enhanced immune response in these patients, underlining the immune system's role in UTI pathology.

Acknowledgments

The authors would like to express their gratitude to Al-Qasim Green University and the College of Biotechnology for their assistance and for providing the necessary facilities for this work.

Conflict of interests

There are non-conflicts of interest.

References

[1] Tullus K, Shaikh N. Urinary tract infections in children. The Lancet, 2020; 395(10237): 1659-1668.

[2] Al-Shahrani FT, Belali MO. Factors influencing the higher incidence of urinary

tract infections in women: A comprehensive review. Urology Annals, 2024, 16(1), 5-12.

[3] Al-Saadi MA, Sulaiman MA, Rashid KA, Abdulrahman NK, Abba AA. The role of innate immunity in urinary tract infections: A review. Journal of Infection and Public Health, 2020, 13(8), 1218-1225.

[4] Hug F, Hermann M, Boehm A. Innate immunity and urinary tract infections: Role of pattern recognition receptors. Nature Reviews Urology, 2018, 15(8), 485-497.

[5] Mukherjee S, Mehta SK, Sahu, RK. The role of TLRs in host defense and inflammation in urinary tract infections. Nephrology Dialysis Transplantation, 2019, 34(9), 1573-1580.

[6] Qiu Y, Yang Z, He R. Signal transduction pathways activated by TLRs in the urinary tract: A delicate balance between pro- and anti-inflammatory responses. Frontiers in Immunology, 2019, 10, 1101.

[7] Morris JC, Schaefer R, Meisner T. Toll-like receptor interactions with bacterial components: Insights into host-pathogen interplay. Infection and Immunity, 2019, 87(5), e00808-18.

[8] Wu R, Zhang H, Li K. The role of TLR4 in urinary tract infections: A potential therapeutic target. Current Urology Reports, 2017, 18(2), 21.

[9] Ganesh S, Tiwari R, Dhanwar A. A hospital-based study to assess the prevalence of urinary tract infections among females and males. Journal of Clinical and Diagnostic Research, 2019, 13(5), DC01-DC04.

[10] Amin A, Zaki ME, Elashry A. Prevalence and antibiotic susceptibility of urinary tract infections in females and males in Egypt. *Egyptian Journal of Medical,* 2020.

[11] Tan CW, Chlebicki MP. Urinary tract infections in adults. *Singapore medical journal*, 2016, 57(9), 485.

[12] Kenny TS, Liu F, Arias A. Female anatomy and urinary tract infections: A comparison with male patients. Journal of Urology, 2017, 198(6), 1392-1398.

[13] Derese A, Abate A, Yirga B. Prevalence and antimicrobial susceptibility of uropathogens among women with urinary tract infection in Ethiopia. BMC Infectious Diseases, 2016, 16(1), 1-7.

[14] Gachui J. Age-related prevalence of urinary tract infections in males: A retrospective study. African Health Sciences, 2017, 17(3), 750-756.

[15] Gebretensaie AG, Meret A, Mengistu S. Urinary tract infections among older women: A comprehensive overview. Ethiopian Journal of Health Sciences, 2022, 32(6), 1137-1145.

[16] Nikhila K, Kumar A, Reddy KC. Prevalence of urinary tract infections in postmenopausal women: A clinical study. Journal of Menopausal Medicine, 2023, 29(2), 123-129.

[17] Laxminarayan R, Duse A, Wattal C. Antibiotic resistance: A global threat. The Lancet Infectious Diseases, 2020, 20(3), e62-e64.

[18] Chudasama YJ, Khunti K, Dhalwani NN. Urban–rural differences in the prevalence of urinary tract infection: Cross-sectional data from the Health Survey for England. BMC Urology, 2020, 20(1), 1-7.

[19] Wang X, Wu X, Zhang S. Obesity and its impact on the risk of urinary tract infections in adults: A meta-analysis. Obesity Reviews, 2020, 21(6), e13045.

[20] Anglim J, McCarthy M, McCarthy J. The relationship between body mass index and recurrent urinary tract infections in women. International Journal of Urology, 2022, 29(1), 35-40.

[21] Zhu Z, et al. Single nucleotide polymorphisms in TLR signaling pathways and cancer risk: A meta-analysis. Cancer Epidemiology, Biomarkers and Prevention, 2013, 22(4), 657 -670.

[22] Abdalhussin H, Jafar N, Alrifai S. Assessment of TLR2 and TLR4 in urine of bacteria isolated from urinary tract infection of Baghdad hospitals. HIV Nursing. 2022, 22(2):3444-3448.

[23] Mertowski S, et al. The role of TLR4 in urinary tract infections: A systematic review. Infectious Diseases in Clinical Practice, 2020, 28(6), e423-e428.

[24] Aksu A, Yılmaz E, Fidan A. Urine TLR4 levels as a biomarker for predicting urinary tract infections in children. Pediatric Nephrology, 2023, 38(2), 345-351.

[25] Jaeger T, et al. Variability in TLR4 expression during bacterial infections: Implications for patient outcome. Clinical Microbiology and Infection, 2015, 21(8), 762-769.

[26] Ambite JL, et al. Cytokine production and tissue damage associated with TLR-4 activation in urinary tract infections. Infection and Immunity, 2021, 89(9), e00027 -21.