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Investigation the correlation between pro-inflammatory cytokines (IL-1β, IL-6, and IL-8) and bacterial urinary tract infections in patients in Hilla City, Iraq

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***Abstract*— One of the most prevalent bacterial infections in the world, urinary tract infections are linked to rising antibiotic resistance. Interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8) are proinflammatory cytokines that are vital to the host immune response and may be used as biomarkers of the severity, and prognosis of infections.**
The aim of this study is to evaluate the relationship among IL-1β , IL-6 and IL-8 levels in patients with bacterial UTIs and compare their profiles with those of healthy control.
A total of 120 participants were included in this study, comprising 60 patients with urinary tract infections (UTIs) and 60 healthy controls. The age of participants ranged from 17 to 55 years. Each group included 56 females and 4 males. Samples were collected from Al-Mahaweel General Hospital and Merjan Teaching Hospital. Like Clinical evaluation urinalysis bacterial culture and identification were performed and urine cytokine concentrations were measured by enzyme-linked immunosorbent assay (ELISA). Seriously Statistical analyzes assessed differences between groups and relationships between cytokines. Urinary tract infection patients showed significant differences in urinalysis parameters, nitrite positivity was detected in 71.7% and *Escherichia coli* was identified as the dominant pathogen. IL-1β and IL-8 levels were significantly increased in patients compared to controls (P < 0.001), while IL-6 showed a non-significant trend towards higher levels. Correlation analysis showed strong positive cytokine associations in healthy individuals, but weaker or partially disrupted associations in UTI patients, suggesting altered immune regulation during infection. Increased concentrations and altered correlation patterns indicating infection-related immune dysregulation characterize the interconnected inflammatory network formed by IL-1β, IL-6 and IL-8 in bacterial UTIs.

***Keywords*— Urinary tract infection; IL-1β; IL-6; IL-8; cytokines; inflammation; biomarkers.**

I. INTRODUCTION

Urinary tract infections (UTIs) are among the most common infectious diseases worldwide and represent a significant public health concern, particularly in the context of increasing antimicrobial resistance. Understanding the underlying immunological mechanisms involved in the initiation and progression of UTIs has become essential for improving diagnostic and therapeutic strategies. Variations in host immune responses and pathogen characteristics across different age groups may influence disease severity and clinical outcomes [1].

UTIs are primarily caused by ascending bacterial infections, with Escherichia coli being the predominant etiological agent. Females are at higher risk due to anatomical factors and several additional risk factors have been identified, including urinary catheterization, sexual activity, structural abnormalities of the urinary tract, immunosuppression and chronic conditions such as diabetes mellitus [2]. These infections are particularly common in women with high rate of recurrence especially among those aged 16–35 years. Most UTIs are limited to the bladder (cystitis) and are associated with disruption of normal protective mechanisms including the vaginal microbiota dominated by lactobacilli [3].

Short-course antibiotic therapy typically lasting three days, has been shown to be effective in the management of uncomplicated UTIs. However, the selection of appropriate antimicrobial agents is increasingly influenced by local resistance patterns, particularly those of *E. coli.* Commonly used antibiotics include trimethoprim/sulfamethoxazole, cephalosporins, nitrofurantoin and fosfomycin [4].

Chemokines play a critical role in the inflammatory response by directing leukocyte migration to sites of infection. Interleukin-8 (IL-8), also known as CXCL8, is a key pro-inflammatory chemokine produced by immune and epithelial cells in response to bacterial components such as lipopolysaccharide (LPS). IL-8 is essential for the recruitment and activation of neutrophils during the early innate immune response. In UTIs, elevated urinary IL-8 levels have been strongly associated with pyuria and disease severity, highlighting its role as a marker of local inflammation [5].

Urinary IL-8 has been proposed as a non-invasive biomarker for the diagnosis and monitoring of UTIs. When used in combination with other biomarkers such as interleukin-1 beta (IL-1β), it demonstrates high sensitivity and specificity for detecting bacterial infections. Variability in patient characteristics and differences in cutoff values may limit its routine clinical application [6].

Interleukin-1 beta (IL-1β) is another key pro-inflammatory cytokine involved in the host immune response to UTIs. It is primarily produced by monocytes and

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macrophages in response to uropathogenic *E. coli* (UPEC) infection. IL-1β contributes to the recruitment of neutrophils and amplification of the inflammatory response, facilitating bacterial clearance. Excessive production may lead to tissue damage and potential renal complications [7].

Interleukin-6 (IL-6) is multifunctional cytokine that plays a central role in immune regulation during infection and tissue injury. It is rapidly produced in response to inflammatory stimuli and contributes to host defense by promoting acute-phase protein synthesis, including C-reactive protein (CRP) as well as activation of the complement and coagulation systems. Additionally, IL-6 regulates body temperature as an endogenous pyrogen and supports the differentiation of T and B lymphocytes and hematopoietic precursors, thereby enhancing both innate and adaptive immune responses [8].

Aim: To evaluate the relationship among IL-1β , IL-6 and IL-8 levels in patients with bacterial UTIs and compare their profiles with those of healthy control.

II. Patients, Materials and Methods

A. Patients group

The patients in the current study set was made up of 60 carried out in the Al-Mahaweel General Hospital and Merjan Teaching Hospital. Clinical materials including urine was obtained from UTI patients, both male and female, ages 17 to 55, the current investigation. All patients were diagnosed by specialist doctor's urologists relying on history, clinical examinations (fever, chills, flank discomfort, Dysuria, Frequency, Urgency, Cloudy urine, Foul-smelling urine, Haematuria, Suprapubic pain, and Nocturia). Data were collected and disease-related variables were developed using a questionnaire filled out by the participants

B. Controls group

In order to balance the results of the current study, the controls group was Composed of 60 individuals from the same population who were matched for age to the patient age group (17–55 years), female and male, and who did not have UTI. It was verified that the members of this group did not have UTIs by looking at the general urine examination (GUE) and urine culture, as well as the lack of clinical symptoms of the illness.proceedings, and not as an independent document.

C. Inclusion and Exclusion Criteria

Inclusion Criteria: Individuals with clinical symptoms consistent with UTI and positive urine culture for bacterial pathogens.

Exclusion Criteria: Autoimmune disease individuals, Patients with malignancies undergoing immunosuppressive therapy,Patients with an immunodeficiency disease,Patients with asthma and other chronic infections and Patients with fungal or viral UTIs.

D. Ethical Approval

This study was approved by the Tikrit University College of Medicine Scientific Committee and conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The Babylon Health Directorate granted the required authorizations for the research at Al-Mahaweel General Hospital and Marjan Teaching Hospital. Each participant provided their informed consent before being included in the study. Data was used only for the study, and participant confidentiality and anonymity were ensured.Units

E. Materials

A variety of laboratory equipment, culture media, and immunological diagnostic kits were used in this study to look at biomarkers linked to urinary tract infections (UTIs). ELISA plate reader and washer, Vitek-2 compact identification system, autoclave, centrifuge, incubator, microscope, deep freezer, micropipettes, and urinalysis reagent strips from globally renowned manufacturers were among the standard microbiological and analytical tools in the lab.

Blood agar was used for haemolysis detection in microbiological analysis, while MacConkey agar was used for lactose fermentation and the selective isolation of Gram-negative bacteria.

A Gram-stain kit for bacterial categorization and commercially available ELISA kits for the quantitative detection of inflammatory-promoting cytokines, particularly IL-1β , IL-6 and IL-8, were used in the immunological evaluation. Coated microplates, conjugate reagents, chromogen solutions, wash buffers, and stop solution were among the standardized reagents included in each ELISA kit. These were kept at the prescribed temperature to guarantee test stability.

III. Methods

A. Urine Samples

A clean-catch midstream urine sampling method was employed for specimen collection. Prior to collection patients were instructed to cleanse external genital area and to discard the initial portion of urine to minimize contamination. Approximately 10 mL of midstream urine was collected into sterile screw-cap container.

Relevant patient information including name, age, sex, address and time of collection, was recorded at the time of sampling. All specimens were transported promptly to the hospital bacteriology laboratory and processed within one hour of collection to ensure sample integrity.

Each urine sample was aseptically divided into three aliquots: first was used for direct microscopic examination (G.U.E.), second was designated for urine culture, and third aliquot was stored at −20 °C for subsequent quantification of inflammatory cytokines (IL-1β and IL-8) using enzyme-linked immunosorbent assay (ELISA).

B. Urinalysis

1) Urine Physical Examination:

The attributes of urine, such as its color, smell, turbidity, and presence of foam, are evaluated, and its specific gravity is measured. The volume of urine produced over a particular period of time is also taken into consideration for functional tests.

2) Urine Chemical Examination

Urine samples were analyzed using dipstick tests. Samples were thoroughly mixed and tested at room temperature without centrifugation. Strips were briefly immersed in urine, held horizontally, and read after 30–60

seconds against the manufacturer's color chart. The main parameters assessed included leukocytes, protein, pH, glucose and nitrites, which serve as indicators of urinary tract inflammation and infection.

3) Microscopic Examination of Urine

In order to do microscopic analysis, 3–7 millilitres of urine are put into a sterile centrifuge tube and centrifuged for five minutes at 4000 rpm. A 40× objective microscope is used to examine one drop of the resultant sediment that has been put on a sanitized glass slide. White blood cells (pus cell), red blood cells, epithelial cells, crystals, bacteria, and other potential components are identified by this study.

C. Bacteriologic Examination

1) Culture Media Preparation

All culture media used in the study were prepared in accordance with the manufacturer's instructions.

Each medium was rehydrated using distilled water at the concentration specified by the manufacturer, usually 38–40 g per liter, and then heated with constant stirring to ensure complete dissolution. Following 15 minutes of autoclaving at 121°C, the media were cooled to 45–50 °C, transferred into sterile Petri dishes, and left to harden.. Blood agar was prepared by aseptically adding 5–10% blood to the cooled sterile agar base. All media were stored at 4°C to ensure optimal performance

2) Bacterial isolation

Following the genital area cleansing and centrifugation at 4000 rpm for 5 minutes, midstream urine samples significant bacteria (3-7ml) from patients with urinary tract infection were collected in sterile containers. The sediment was promptly streaked across the surfaces of blood agar, and MacConkey agar. After that, It was incubated in an aerobic environment for a 24-hour period at 37°C.

3) Bacterial Identification

a) The Gram Stain

Gram stain was used to create a smear from a young, pure culture for each bacterial isolate. Under a light microscope, Gram stain reveals the shape, size, and arrangement of bacterial cells. Gram-negative bacteria are red, and Gram-positive bacteria are blue.

Gram Stain Preparation by clean, dry glass slide was labelled and a thin smear was prepared from a young, pure bacterial colony suspended in a drop of sterile distilled water or normal saline. The slide was carefully passed through a Bunsen burner flame two or three times with the smear side up to heat-fix the smear after it had entirely dried by air.

Crystal violet stain was poured over the smear, and it was let to settle for a minute. To get rid of extra stain, distilled water was used to gently clean the slide. Gram's iodine solution was then applied to the smear for one minute in order to create a crystal violet-iodine complex. Once more, distilled water was used to gently rinse the slide.

The smear was cleaned for 10–15 seconds using 95% ethanol or acetone-alcohol. The slide was immediately washed with water to stop the decolorization process. The smear was then counterstained with safranin for 30 to 60 seconds. The slide was cleaned with water and then set upright to air dry.

b) Identifying on a Differential and Selective Medium

The *Enterobacteriaceae* family was selectively isolated using this medium. After being streaked across the MacConkey agar surface, each bacterial isolate was cultivated for a day at 37°C. Pink colonies of bacterial are seen as a positive outcome for fermenting lactose bacteria. For Gram-positive organisms, particularly *Staphylococcus species*, Mannitol Salt Agar was employed to select and differentiate mannitol fermenters. Additionally, Urea agar was used to test for urease activity, which is a distinguishing feature for some uropathogens like *Proteus spp* .

c) Identifying Bacteria with the Vitek2 Compact

Vitek2 compact was used to diagnose and identify the bacteria used in the study according to the guidelines provided by the manufacturer. The Vitec2 compact is a device for integrated microbiological identification that is accompanied with colorimetric reagent card and other hardware and software accessories. The bacterial isolates were identified using the Vitek-2 Compact's Gram-negative and Gram-positive identification tests. This is test is more precise than other biochemical identification methods. Each card is divided into 64 wells and each individual well has an exclusive substrate. These wells measure various biochemical activities such as hydrolysis, acidity, metabolic inhibitors, alkalinity and growth

D. ELISA Kits

The commercially available enzyme-linked immunosorbent assay (ELISA) kits (SunLong, China) were used for the quantitative measuring of urinary cytokines. Each of the kits was utilized accordingly using the instructions provided by the manufacturer.

1) Human IL-1β Test

Human IL-1β ELISA kit (SunLong, China; Cat. No: SL0984Hu; sensitivity: 0.5 pg/mL).

2) Human IL-6 Test

Human IL-8 ELISA kit (SunLong, China; Cat. No: SL1001Hu; sensitivity: 0.5 ng/mL).

3) Human IL-8 Test

Human IL-8 ELISA kit (SunLong, China; Cat. No: SL1004Hu; sensitivity: 1.5 pg/mL).

IV. RESULTS

A. Baseline Characteristics of the Study Population

Out of the 120 participants included in the study, 60 were patients with culture-confirmed bacterial urinary tract infections (UTIs) and the other 60 were healthy controls. No significant differences were observed between the groups

with respect to age and sex distribution ($p > 0.05$). 93.3% of participants in both groups were female.

Compared to the control group, patients with urinary tract infections had statistically and clinically higher rates of hypertension and diabetes ($p = 0.013$ for both). Urinalysis outcomes were significantly different between the groups, with nitrite positive in 71.7% of UTI patients and nitrite negative in all control group participants ($p < 0.001$). None of the control samples had bacterial proliferation, while Escherichia coli was the most common uropathogen and was found in 53.3% of UTI patients, followed by *Staphylococcus saprophyticus* and *Enterococcus faecalis* ($p < 0.001$). This is summarized in Table1.

TABLE1. COMPARISONS OF CHARACTERISTICS, CYTOKINES AMONG STUDY GROUP

Characteristic	Healthy Control N = 60[1]	UTI Patients N = 60[1]	p-value[2]
Age (years)			0.551
Mean ± SD	32.0 ± 10.25	30.9 ± 10.82	
Median (Min, Max)	30.0 (15.00, 54.0)	27.0 (17.00, 55.0)	
Sex			>0.999
Female	56.0 (93.3%)	56.0 (93.3%)	
Male	4.0 (6.7%)	4.0 (6.7%)	
BP			0.013
No	60.0 (100.0%)	53.0 (88.3%)	
Yes	0.0 (0.0%)	7.0 (11.7%)	
DM			0.013
No	60.0 (100.0%)	53.0 (88.3%)	
Yes	0.0 (0.0%)	7.0 (11.7%)	
Pregnant Status			0.324
No	44.0 (78.6%)	48.0 (85.7%)	
Yes	12.0 (21.4%)	8.0 (14.3%)	
N/A	4	4	
Marital Status			0.034
Not Married	26.0 (43.3%)	15.0 (25.0%)	
Married	34.0 (56.7%)	45.0 (75.0%)	
Nitrite			*<0.001*
Negative	0.0 (0.0%)	17.0 (28.3%)	
No	60.0 (100.0%)	0.0 (0.0%)	
Positive	0.0 (0.0%)	43.0 (71.7%)	
Gram Stain			*<0.001*
Control	60.0 (100.0%)	0.0 (0.0%)	
Negative	0.0 (0.0%)	47.0 (78.3%)	
Positive	0.0 (0.0%)	13.0 (21.7%)	
Bacteria Type			*<0.001*
Healthy Control (no growth)	60.0 (100.0%)	0.0 (0.0%)	
E. coli	0.0 (0.0%)	32.0 (53.3%)	
E. coli & klebsiella pneumoniae	0.0 (0.0%)	1.0 (1.7%)	
E.faecium	0.0 (0.0%)	1.0 (1.7%)	
E.fecalis	0.0 (0.0%)	5.0 (8.3%)	
Enterobacter cloacae	0.0 (0.0%)	2.0 (3.3%)	
Klebsiella pneumonia	0.0 (0.0%)	3.0 (5.0%)	
Morganella morganii	0.0 (0.0%)	2.0 (3.3%)	
Proteus mirabilis	0.0 (0.0%)	3.0 (5.0%)	
Pseudomonas aeruginosa	0.0 (0.0%)	4.0 (6.7%)	
S.saprophyticus	0.0 (0.0%)	7.0 (11.7%)	
Pus Cell (H.P.F)			*<0.001*
Mean ± SD	2.4 ± 0.96	18.2 ± 9.04	
Median (Min, Max)	2.0 (1.00, 5.0)	15.5 (7.00, 50.0)	
IL-1B (pg/ml)			*<0.001*
Mean ± SD	25.9 ± 6.79	35.7 ± 10.78	
Median (Min, Max)	24.4 (16.25, 44.8)	32.6 (19.85, 62.4)	
IL-6 (ng/L)			0.001
Mean ± SD	21.8 ± 4.65	25.0 ± 6.00	
Median (Min, Max)	20.7 (12.75, 31.4)	23.7 (16.13, 41.9)	
IL-8 (pg/ml)			*<0.001*
Mean ± SD	58.1 ± 15.33	86.2 ± 27.38	
Median (Min, Max)	53.2 (36.22, 96.5)	85.4 (43.58, 155.2)	

[1]n (%)

[2]Welch Two Sample t-test; Fisher's exact test; Pearson's Chi-squared test

B. *Association of IL-1β, IL-6 and IL-8 with Bacterial Characteristics*

Table 2 illustrates the distribution of urine pro-inflammatory cytokines (IL-1β, IL-6, and IL-8) according to bacterial type. Cytokine concentrations are expressed as mean ± standard deviation (SD) and median (minimum–maximum). Differences between groups were assessed using the Kruskal– Wallis rank sum test.

1) IL-1β Levels According to Bacterial Type

The mean urine IL-1β level in the healthy control group (no bacterial growth) was 25.9 ± 6.79 pg/ml, representing the lowest value among all groups. In comparison, patients with bacterial infections demonstrated higher IL-1β concentrations, with a statistically significant difference observed across bacterial types ($p < 0.001$). Among Gram-negative isolates, *Klebsiella pneumoniae* showed markedly elevated IL-1β levels (55.2 ± 12.46 pg/ml), followed by *Morganella morganii* (56.2 ± 0.00 pg/ml) and *Escherichia coli* (34.6 ± 7.98 pg/ml). Similarly, infections caused by *Pseudomonas aeruginosa* exhibited increased IL-1β levels (34.3 ± 6.00 pg/ml).

In contrast, relatively lower IL-1β levels were observed in infections caused by *Staphylococcus saprophyticus* (31.6 ± 7.29 pg/ml) and *Enterococcus faecium* (31.1 ± NA pg/ml).

2) IL-6 Levels According to Bacterial Type

The mean IL-6 level in the healthy control group was 21.8 ± 4.65 ng/L. Although increased IL-6 levels were observed in several bacterial groups, the overall difference among bacterial types did not reach statistical significance ($p = 0.059$). infections caused by *Morganella morganii* showed the highest mean IL-6 concentration (41.9 ± 0.00 ng/L) followed by *Klebsiella pneumoniae* (28.9 ± 5.94 ng/L) and *Enterobacter cloacae* (24.1 ± 5.66 ng/L). Moderate IL-6 elevations were also observed in *Escherichia coli* infections (25.2 ± 6.00 ng/L) compared to controls.

3) IL-8 Levels According to Bacterial Type

The mean IL-8 level in the healthy control group was 58.1 ± 15.33 pg/ml while significantly higher IL-8

concentrations were observed in patients with bacterial infections. A statistically significant association was found between bacterial type and IL-8 levels ($p < 0.001$).

The highest mean IL-8 levels were detected in infections caused by *Klebsiella pneumoniae* (105.0 ± 8.67 pg/ml) and *Enterobacter cloacae* (97.3 ± 55.18 pg/ml) followed by *Escherichia coli* (90.2 ± 29.58 pg/ml) and *Pseudomonas aeruginosa* (81.6 ± 17.17 pg/ml). These elevated levels indicate enhanced neutrophil recruitment and activation, reflecting a robust chemokine- mediated inflammatory response. Lower IL-8 concentrations were observed in infections caused by *Staphylococcus saprophyticus* (69.7 ± 20.60 pg/ml) and *Morganella morganii* (69.4 ± 0.00 pg/ml), although these values remained higher than those of the control group.

TABLE 2. Association Between Bacterial Type and Cytokines

Bacterial Type	**IL-1β (pg/ml)**			**p-value***
		Mean ± SD	**Median (Min, Max)**	
Healthy Control (no growth)	**N = 60**	2 5.9 ± 6.79	24.4 (16.25, 44.8)	**<0.001**
E. coli	**N = 32**	3 4.6 ± 7.98	32.6 (19.85, 53.7)	
E. coli & klebsiella pneumoniae	**N = 1**	3 6.0 ± NA	36.0 (35.98, 36.0)	
E.faecium	**N = 1**	3 1.1 ± NA	31.1 (31.13, 31.1)	
E.fecalis	**N = 5**	3 6.4 ± 13.87	30.2 (28.59, 61.1)	
Enterobacter cloacae	**N = 2**	2 5.4 ± 0.35	25.4 (25.11, 25.6)	
Klebsiella pneumoniae	**N = 3**	5 5.2 ± 12.46	62.4 (40.78, 62.4)	
Morganella morganii	**N = 2**	5 6.2 ± 0.00	56.2 (56.15, 56.2)	
Proteus mirabilis	**N = 3**	3 3.0 ± 21.99	20.4 (20.35, 58.4)	
Pseudomonas aeruginosa	**N = 4**	3 4.3 ± 6.00	36.2 (25.61, 39.4)	
S.saprophyticus	**N = 7**	3 1.6 ± 7.29	30.5 (22.16, 44.2)	
Bacterial Type	IL-6 (ng/L)			
		Mean ± SD	Median (Min, Max)	**p-value**
Healthy Control (no growth)	N = 60	2 1.8 ± 4.65	20.7 (12.75, 31.4)	**0.059**
E. coli	N = 32	2 5.2 ± 6.00	24.1 (16.13, 37.9)	
E. coli & klebsiella pneumoniae	N = 1	2 0.7 ± NA	20.7 (20.71, 20.7)	
E.faecium	N = 1	2 2.5 ± NA	22.5 (22.54, 22.5)	
E.fecalis	N = 5	2 2.1 ± 3.27	23.4 (16.45, 24.5)	
Enterobacter cloacae	N = 2	2 4.1 ± 5.66	24.1 (20.09, 28.1)	
Klebsiella pneumoniae	N = 3	2 8.9 ± 5.94	25.4 (25.42, 35.7)	
Morganella morganii	N = 2	4 1.9 ± 0.00	41.9 (41.91, 41.9)	
Proteus mirabilis	N = 3	2 3.0 ± 4.08	20.6 (20.60, 27.7)	
Pseudomonas aeruginosa	N = 4	2 0.9 ± 2.26	21.5 (17.91, 22.7)	
S.saprophyticus	N = 7	2 3.7 ± 2.55	23.7 (20.81, 28.5)	
Bacterial Type	**IL-8 (pg/ml)**			**p-value**
		Mean ± SD	**Median (Min, Max)**	
Healthy Control (no growth)	**N = 60**	5 8.1 ± 15.33	53.2 (36.22, 96.5)	**<0.001**
E. coli	**N = 32**	9 0.2 ± 29.58	88.9 (43.58, 155.2)	
E. coli & klebsiella pneumoniae	**N = 1**	1 04.6 ± NA	104.6 (104.55, 104.6)	
E.faecium	**N = 1**	6 4.4 ± NA	64.4 (64.37, 64.4)	
E.fecalis	**N = 5**	7 6.4 ± 28.44	81.0 (44.24, 116.1)	
Enterobacter cloacae	**N = 2**	9 7.3 ± 55.18	97.3 (58.24, 136.3)	
Klebsiella pneumoniae	**N = 3**	1 05.0 ± 8.67	100.0 (99.95, 115.0)	
Morganella morganii	**N = 2**	6 9.4 ± 0.00	69.4 (69.38, 69.4)	
Proteus mirabilis	**N = 3**	9 0.3 ± 27.52	74.4 (74.41, 122.1)	
Pseudomonas aeruginosa	**N = 4**	8 1.6 ± 17.17	83.1 (59.12, 101.0)	
S.saprophyticus	**N = 7**	6 9.7 ± 20.60	58.0 (49.38, 99.5)	

C. Correlation analysis between pro-inflammatory cytokines in healthy controls and UTI patients

Figure 1 illustrates the correlations between selected pro-inflammatory cytokines in healthy controls (red) and UTI

patients (blue). Pearson's correlation coefficient (r) was used to evaluate the strength and direction of the associations while p-values indicate statistical significance. Linear regression lines are shown for each group.

Panel A shows the correlation between IL-1β and IL-6 levels. In healthy controls, a strong positive correlation was observed ($r = 0.64$, $p = 4.7 \times 10^{-8}$), indicating a robust physiological association between these cytokines under normal immune conditions. In contrast, UTI patients exhibited a moderate positive correlation ($r = 0.41$, $p = 0.0012$).

Panel B presents the relationship between IL-8 and IL-6. Healthy controls demonstrated a weak-to-moderate but statistically significant positive correlation ($r = 0.33$, $p = 0.0097$), whereas the correlation in UTI patients was weak and not statistically significant ($r = 0.23$, $p = 0.079$).

Panel C depicts the correlation between IL-8 and IL-1β. A moderate positive correlation was observed in healthy controls ($r = 0.43$, $p = 0.00055$), reflecting coordinated baseline cytokine signaling. Similarly, UTI patients showed a moderate and statistically significant correlation ($r = 0.38$, $p = 0.0028$).

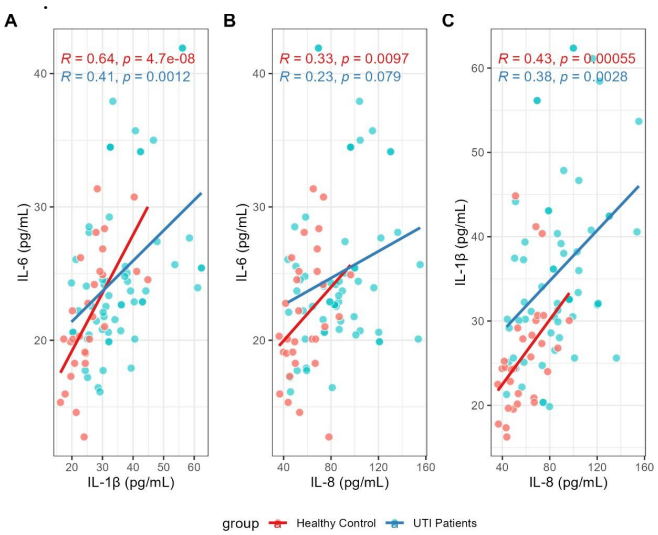

Fig.1: Correlation analysis between pro-inflammatory cytokines in healthy controls and UTI patients[This figure illustrates the correlations between selected pro-inflammatory cytokines in healthy controls (red) and UTI patients (blue). Pearson's correlation coefficient (r) was used to evaluate the strength and direction of the associations, while p-values indicate statistical significance. Linear regression lines are shown for each group]

V. Discussion

The present study investigated the demographic, clinical, microbiological and immunological characteristics of patients with urinary tract infection (UTI) in comparison with healthy controls. The mean age and gender distribution were comparable between the two groups, with more than 90% of participants being female, reflecting the well-established higher prevalence of UTIs among women [9]. Comorbid conditions, particularly hypertension and diabetes mellitus, were significantly more frequent among UTI patients ($p = 0.013$ for both), suggesting that these factors may increase susceptibility to infection or contribute to disease severity [10]. Pregnancy and marital status differed significantly across certain subgroups, supporting previous evidence that physiological and social determinants may influence UTI risk [11-12].

Urinalysis and microbiological findings demonstrated marked differences between patients and controls. Nitrite positivity was detected in 71.7% of UTI cases but was absent in controls ($p < 0.001$), confirming the presence of bacterial infection. Microbiological analysis revealed a predominance of Gram-negative organisms, with Escherichia coli accounting for 53.3% of isolates, followed by Klebsiella pneumoniae and Pseudomonas aeruginosa. Gram-positive pathogens, including Staphylococcus saprophyticus and Enterococcus faecalis, were identified less frequently. These findings are consistent with previous epidemiological studies identifying E. coli as the most common uropathogen worldwide [13-14].

Markers of inflammation were significantly elevated in UTI patients. Pus cell counts, indicative of neutrophilic activity, were substantially higher in patients (18.2 ± 9.04 per high-power field) compared to controls (2.4 ± 0.96, $p < 0.001$). These baseline findings provided a foundation for further evaluation of immunological biomarkers associated with infection [15].

Cytokine analysis demonstrated significantly increased levels of IL-1β and IL-8 in UTI patients relative to healthy controls. IL-8 concentrations increased from 58.1 ± 15.33 pg/mL in controls to 86.2 ± 27.38 pg/mL in patients ($p < 0.001$), while IL-1β levels rose from 25.9 ± 6.79 pg/mL to 35.7 ± 10.78 pg/mL ($p < 0.001$). These findings support the role of these cytokines as key mediators of the inflammatory response to urinary pathogens [16].

Correlation analysis revealed distinct patterns between groups. A strong positive correlation between IL-1β and IL-6 was observed in healthy controls ($r = 0.64$, $p = 4.7 \times 10^{-8}$), whereas a moderate but significant correlation was found in UTI patients ($r = 0.41$, $p = 0.0012$), as illustrated in Figure 1A. This suggests coordinated cytokine regulation under physiological conditions, with partial disruption during infection.

For IL-8 and IL-6 (Figure 1B), healthy controls exhibited a weak-to-moderate positive correlation ($r = 0.33$, $p = 0.0097$). In contrast, UTI patients showed a weaker, non-significant correlation ($r = 0.23$, $p = 0.079$), indicating a disturbance in cytokine interaction during active infection.

In the case of IL-8 and IL-1β (Figure 1C), both groups demonstrated moderate positive correlations, although the association was stronger in controls ($r = 0.43$, $p = 0.00055$) than in UTI patients ($r = 0.38$, $p = 0.0028$). This pattern suggests that while cytokine co-regulation is preserved in disease, it is somewhat attenuated.

Cytokines exhibited consistent positive correlations, supporting the concept of a coordinated inflammatory signaling network. However, reduced correlation strength and loss of statistical significance in certain cytokine pairs among UTI patients indicate that acute infection may disrupt immune regulatory balance. This disruption likely reflects heterogeneity in immune responses driven by factors such as bacterial load, pathogen type and host-specific immune variability.

These findings are biologically plausible, as infection-induced immune activation often involves differential pathway engagement and variable mediator production, which may weaken linear relationships between cytokines despite their overall elevation. Supporting this interpretation, previous studies have reported significant increases in IL-6 and IL-8 levels in UTI patients, confirming their central role in disease pathogenesis [17].

Infection-related biomarkers such as IL-1β and IL-8 have demonstrated strong diagnostic performance in distinguishing symptomatic from asymptomatic UTIs particularly when used in combination [18]. Positive correlations between IL-6, IL-8 and systemic inflammatory markers such as C-reactive protein have also been documented, reinforcing the interconnected nature of the cytokine network [19].

Evidence from pediatric UTI studies similarly shows elevated IL-6 and IL-8 levels associated with increased inflammatory activity and correlations with classical markers such as erythrocyte sedimentation rate (ESR), white blood cell count and CRP (17). These findings further validate the biological relevance of the correlations observed in the present study.

The observed reduction in correlation strength among certain cytokine pairs in UTI patients may reflect immune response heterogeneity during infection. Gram-negative uropathogens are known to induce stronger IL-6 and IL-8 responses compared to Gram-positive organisms, highlighting pathogen-dependent variability in cytokine signalling [20].

Collectively these results support the concept that cytokine inter-correlation is a fundamental feature of inflammatory processes, while variations in correlation strength may differentiate between health and disease states or reflect differences in inflammation severity [21]. The current findings align with growing evidence that IL-1β, IL-6 and IL-8 function as an integrated inflammatory network in UTIs, with preserved but partially dysregulated coordination during infection. This underscores the potential utility of multi-cytokine profiling not only for diagnostic purposes but also for understanding immune dynamics and assessing disease severity [22].

VI. Conclusion

Pro-inflammatory cytokines IL-1β IL-6, and IL-8 show positive associations, suggesting, a coordinated inflammatory response during immune activation. Cytokine correlations were stronger and more consistent in healthy individuals, but they were reduced or partially disrupted in patients with urinary tract infections, showing variability in immune regulation during active infection. More extensive and long-term study is required to better understand the temporal dynamics, pathogen-specific effects, and prognostic usefulness of cytokine networks in urinary tract infections.

Conflict of interest

Authors declare that they have no conflict of interest.

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