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Research paper

Levels of IL-6 and IL-8 in complicated versus uncomplicated urinary tract infection

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Abstract

Background: Urinary tract infections (UTIs) are one of the world's most frequent infectious diseases, affecting 150 million people each year and resulting in severe morbidity and high medical expenditures. UTIs can be clinically defined as uncomplicated UTIs (uUTIs) or complicated UTIs (cUTIs) to differentiate infections of benign origin from those with a higher risk of recurrence or progression to severe pathology. The aim of this study was to evaluate interleukin-6 (IL-6) and IL-8 as potential biomarkers in differentiating complicated and uncomplicated

Methods: Ninety samples of urine were collected. There were 43 samples from healthy controls and 47 samples from UTI patients, those who have been clinically diagnosed with UTIs by the urologist. The serum IL-6 and IL-8 were measured using an enzyme-linked immunosorbent assay and commercially available kits.

Results: The levels of both IL-6 and IL-8 were significantly higher in UTI patient then in control subjects (p=0.001, p=0.001). A significant difference was observed in the mean of level IL-6 between culture positive UTI patients and culture negative UTI patients (p=0.014). In addition, the type of bacterial growth, a significant difference was observed in the mean of serum IL-6 and IL-8 level in UTI caused by gram negative bacteria in comparison with UTI caused by gram positive bacteria.

Conclusion: High levels of IL-6 are associated with the growth of gram-negative bacteria, and this indicates that gram-negative bacteria could induce stronger inflammatory responses than gram-positive bacteria. IL-8 levels are higher in UTIs caused by gram-negative bacteria. This result further supported the evidence that gram-negative bacteria are potent inducers of inflammatory responses.

Keywords: *Use about five key words or phrases in alphabetical order, Separated by Semicolon.*

1. Introduction

Urinary tract infections (UTIs) are one of the world's most frequent infectious diseases, affecting 150 million people each year and resulting in severe morbidity and high medical expenditures (McLellan & Hunstad, 2016). They are classified as pyelonephritis and a kidney infection when the infection affects the upper part of the urinary tract and manifests in severe symptoms such as abdominal pain, fever, chills, flank pain, nausea, and vomiting, which can lead to irreversible kidney damage and sepsis (González de Llano et al., 2020). When infections are confined to the lower urinary tract, urethra, and bladder, UTIs are classified as cystitis and urethritis, characterized by slight symptoms such as dysuria, suprapubic pain, and hematuria. Moreover, UTIs can be classified clinically into uncomplicated UTIs (uUTIs) and complicated UTIs (cUTIs)(Maisto et al., 2023) . Urinary tract infections (UTIs) in otherwise healthy individuals with no structural or neurological abnormalities are termed uncomplicated UTIs that are differentiated into cystitis (lower UTIs) and pyelonephritis (upper UTIs) (Jagtap et al., 2022). Complicated UTIs are associated with atypical organisms, patients considered high-risk (pregnancy, comorbidities, immunosuppression, etc.), or involve the upper urinary tract(Codelia-Anjum et al., 2023).

UTIs are caused by both Gram-negative and Gram-positive bacteria. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic Escherichia coli (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in prevalence by Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, Group B Streptococcus (GBS), Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida spp. For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is Enterococcus spp., K. pneumoniae, Candida spp., S. aureus, P. mirabilis, P. aeruginosa, and GBS (Flores-Mireles et al., 2015).

Different cells release cytokines, which are tiny, soluble proteins, in reaction to inflammation and infections. Interleukin (IL)-6 is a pro-inflammatory cytokine, the concentration of which increases in the early stage of bacterial infection. Interleukin-6 is responsible for fever and causes the growth of C-reactive protein (CRP)(Ragnarsdóttir & Svanborg, 2012). Interleukin-8 is a pro-inflammatory cytokine that rises in response to IL-1 and tumour necrosis factor α (TNF-α). Interleukin-8 is a chemokine and causes the migration of neutrophils to the site of inflammation, leading to pyuria in patients with UTI(Gokce et al., 2010)

2. Materials and methods

2.1. Subjects

This case-control study was conducted at the hospital of Imam Al-Hussein Medical City, Karbala, Iraq. Ninety samples of urine were collected. There were 43 samples from healthy controls and 47 samples from UTI patients—those who have been clinically diagnosed with UTIs by the urologist. The samples were collected between September 2022 and May 2023 from individuals of both sexes (47 girls and 43 males) who were attending the Imam Al-Hussein Medical City, Karbala, Iraq, outpatient clinic and ranged in age from 18 to 45.
Case information sheets involving age, sex, symptoms, and others were carried out for each patient.

2.2. Methods

Ninety individuals had venipunctures to obtain blood samples. Using a disposable syringe and sterilization procedures, five millimeters of venous blood were extracted, moved to a gel tube, and allowed to clot. Centrifugation was then used to separate the serum for five minutes at 1500 rpm. The serum has been collected in Eppendorf tubes and then stored at -20 °C. The serum IL-6 and IL-8 were measured using an enzyme-linked immunosorbent assay and commercially available kits. (Cat. No E0090Hu, Cat. No E0089Hu, BT LAB, China).

3. Results

This study had 90 cases, of whom 47 were UTI patients and 43 were controls. Table (1) presents the individuals' clinical features along with their demographic information.
In terms of age, there were 18 to 45-year-old patients in this study, with a mean±SD value of 32.47±7.83, and 18 to 42-year-old controls, with a mean±SD value of 31.33±7.87.
In the experimental group, there were 22 male patients (46.8%) and 25 female patients (53.2%). In contrast, the control group had 21 male patients (48.8%) and 22 female patients (51.2%). Based on their correlation with structural abnormalities of the urinary tract and/or accompanying comorbidities (such as diabetes and kidney stones), urinary tract infections have been divided into two categories: complicated UTIs and uncomplicated UTIs. Thirteen patients (29.8%) had complicated UTIs, compared to thirty-three patients (70.2%) with uncomplicated UTIs.
In relation to the results of the urine culture, 36 patients (76.6%) had positive results, while 11 patients (23.4%) had negative results.
Based on the type of organism that was isolated, 22 patients (61.1%) had growing gram-negative bacteria, whereas 14 patients (38.9%) had growing gram-positive bacteria.

Table 1: Demographic and Some Clinical Characteristics of the Studied Subjects

Age [mean(SD)]	Case		32.47(7.83)
	Control		31.33(7.87)
Sex [No(%)]	Case	male	22 (46.8%)
		female	25 (53.2%)
	Control	male	21 (48.8%)
		female	22 (51.2%)
Classification of UTI [No(%)]		Complicated UTI	14 (29.8%)
		Uncomplicated UTI	33 (70.2%)
Result of Urine Culture [No(%)]		Culture Positive	36 (76.6%)
		Culture Negative	11 (23.4%)
Type of Organism [No(%)]		Gram Negative	22 (61.1%)
		Gram Positive	14 (38.9%)

Table (2) shows a comparison between the patients and controls in respect to serum levels of IL-6 and IL-8.
The levels of both IL-6 and IL-8 were significantly higher in UTI patient then in control subjects (p=0.001, p=0.001).

Table 2: Comparison Between the Patients and Controls

Parameters	Case (UTI Patients) Mean ± SE	Healthy Control Mean ± SE	P. value
IL-6 (ng/l)	166.96 ± 8.18*	37.82 ± 4.72	0.001 **
IL-8 (ng/l)	454.14 ± 23.91*	99.81 ± 26.79	0.001 **

- Statistical Test: student T Test.
- NS: Non significant P. value
- *: Significant P. value
- **: Highly Significant P. value

Table (3) shows distribution of IL-6 levels according to classification of UTI, results of urine culture and type of bacterial growth.
According to classification of UTI, no significant difference was observed in the mean of level IL-6 between complicated UTI and uncomplicated UTI (p=0.354).
Regarding the result of urine culture, a significant difference was observed in the mean of level IL-6 between culture positive UTI patients and culture negative UTI patients (p=0.014).
In addition, the type of bacterial growth, a significant difference was observed in the mean of serum IL-6 level in UTI caused by gram negative bacteria in comparison with UTI caused by gram positive bacteria (p=0.001).

Table 3: Distribution of IL-6 Levels According to Classification of UTI, Results of Urine Culture and Type of Bacterial Growth

Variables		IL-6 (ng/l) Mean (SE)	P. Value
Classification of UTI	Complicated	180.99 (19.53)	0.354 NS
	Uncomplicated	161.01 (8.22)	
Urine Culturing	Culture Positive	179.53 (8.10) *	0.014 *
	Culture Negative	125.85 (18.53)	
Type of Growth	Gram Negative	207.80 (8.06) *	0.001*
	Gram positive	135.10 (6.39)	

- NS: Non significant P. value
- *: Significant P. value
- **: Highly Significant P. value

Table (4) shows distribution of IL-8 levels according to classification of UTI, results of urine culture and type of bacterial growth. According to classification of UTI, no significant difference was observed in the mean between complicated UTI and uncomplicated UTI (P=0.305).

Regarding, the result of urine culture, no significant difference was observed in the mean between culture positive and culture negative(P=0.191).

However, regarding, type of bacterial growth, a significant difference was observed in the mean of level IL-8 in patients infected with gram negative bacteria then patients infected by gram positive bacteria (p=0.025).

Table 4: Distribution of IL-8 Levels According to Classification of UTI, Results of Urine Culture and Type of Bacterial Growth

Variables		IL-8 (ng/l) Mean (SE)	P. Value
Classification of UTI	Complicated	334.76 (55.64)	0.305 NS
	Uncomplicated	270.58 (29.18)	
Urine Culturing	Culture Positive	437.34 (29.58)	0.091 NS
	Culture Negative	509.11 (28.65)	
Type of Growth	Gram Negative	489.44 (31.68) *	0.025 *
	Gram positive	355.48 (51.66)	

- NS: Non significant P. value
- *: Significant P. value
- **: Highly Significant P. value

4. Discussion

Interleukin-6 (IL-6) is a compound characterized by omnidirectional interactions in the processes occurring in the human body. It is considered a cytokine that strongly activates the immune system and enhances inflammatory response, although considering some of its effects, it may be classified as anti-inflammatory interaction(Wojdasiewicz et al., 2014).

These results of serum levels of IL-6 were consistent with Pirdel, who found a significant difference between the patient and the control (p = 0.008).(Pirdel & Pirdel, 2021).

In addition, a study conducted by Al-Kaabi found that both serum levels of IL-6 and IL-8 had a significant difference (p = 0.000), which is in agreement with our study (Al-Kaabi & Al-Khalidi, 2020).

In this study, IL-6 showed no significant difference between a complicated UTI and an uncomplicated UTI (P = 0.354). findings could suggest that higher IL-6 serum levels are independent of the type of UTI.

However, in urine culture of UTI a significant difference between culture positive UTI and culture negative UTI p=0.014. these result were consistent with Al Rushood et al, that found a significant difference between culture positive UTI and culture negative UTI p=0.005 (Al Rushood et al., 2020).

In addition, regarding type of bacterial growth, a significant difference was observed in the mean of serum IL-6 level in UTI caused by gram negative in comparison with UTI caused by gram positive (p=0.001). These findings were in line with Darogha et al, that found a significant difference in UTI caused by gram negative in comparison with UTI caused by gram positive p=0.000(Darogha et al., 2021).

Interleukin-8 is a chemokine and causes migration of neutrophils to the place of inflammation, leading to pyuria in patients with UTI (Krzemień et al., 2016). In the current study, no significant differences could be observed in the levels of IL-8 between the deferent groups of patients (complicated versus uncomplicated UTI; culture positive versus culture negative UTI). The appearance of interleukin-8 (IL-8) in blood serum usually indicates an inflammatory response in the body. While according to the type of organism, a significant difference could be observed between gram negative caused by UTI and gram positive UTI p=0. 025.these results indicate that Gram-bacteria can induce higher levels of IL-8 secretions.

Importantly, as shown in table 9 there was a significant positive correlation between IL-8 serum levels and number of pus cells in urine (r=0.579, p=0.000).

Many studies have reported elevated levels of IL-8 in the serum of patients with urinary tract infection, including study by Al Rushood et al, who reported that the levels of IL-8 elevated in UTI patient (Al Rushood et al., 2020). These results were also similar to a study by Abbas et al.who found that levels of IL-8 in serum were elevated in patients with UTI (Abbas et al., 2022). However, these study disagree with the study of Mahyar et al.who found that serum IL-8 is not a reliable marker for prediction of vesicoureteral reflux (VUR) in children with first febrile urinary tract infection (UTI) (Mahyar et al., 2015).

The potential causes of the differences between this study and study of Mahyar et al. may be attributed to type of disease and age group.

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