

# Evaluation of Interleukin-6 and Interleukin-10 in Patients with Urinary Tract Infection

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## Abstract

**Background:** Urinary tract infection (UTIs) are considered to be the most common infections in humans.

**Aims:** this study aimed to evaluate the immune status of the patients with UTI by studying of (IL-6 and IL-10).

**Subjects and Methods:** A total of 49 UTI patients infected with gram –ve bacteria and 25 healthy controls were enrolled in this study. Urine samples from UTI cases was collected in AL-Imam AL-Hussain teaching hospital in Thi- Qar province, during the period from February 2014 to July 2014. Urinary isolates were identified by conventional methods.

3 ml were collected in plastic tube and then centrifuged at 3000 round per minute (rpm) for 5 min to obtain sera. And after the serum sample were collected they were divided into two apendroff tubes and stored in deep freeze at – 20° C until performing IL-6 and IL-10. (Lewis et al., 2006).

**Results:** Patients with E. coli infection accounted for 26 (53.06%); other culture results were as follows: 7 (14.29 %) patients had K. pneumonia, 9 (18.37%) patients had P. aerogenosa, 4 (8.16%) had Proteus and 3 (6.12%) patients had K.oxytoca. Measure levels of serum IL-6 and IL-10 for study group against control group, which is showed a significant increase ( $P \leq 0.01$ ) in the levels of interleukins (IL-6, IL-10) in all serum patients compared to the control group.

**Conclusion:** E- coli was most common causative agent in UTI, The high rate concentration of IL-6 and IL-10 in patients with UTI compared with the healthy control group. This refers to the injury for their role in the immune response.

**Keywords:** Urinary Tract Infection, Interleukin 6 and Interleukin 10.

## INTRODUCTION

The most typical infection in humans is thought to be urinary tract infections (UTIs) (Bien et al.,2012). According to the location of the infection, UTIs are divided into three disease categories: bacteriuria (the urine), pyelonephritis (the kidney), and cystitis (the bladder) (Foxman, 2003). Asymptomatic bacteriuria (ABU) is the medical term for urine colonization that occurs in the absence of clinical symptoms (Bien et al.,2012) Most ABU patients don't require treatment, and in many cases, the colonization of the ABU strains by other bacteria may help to prevent infection by more virulent bacteria (Trautner et al.,2003). According to Köves (2014), intestinal flora-derived Gram-negative bacteria are responsible for the majority of UTI cases. Uropathogenic E. coli strains are the primary causes in more than 80% of all UTI cases(Hooton and Stamm,1997).

Interleukins are a group of cytokines, (secreted proteins and signaling molecules) that were first seen to be expressed by white blood cells (leukocytes)( Brocker et al., 2010). The function of the immune system depends in a large part on interleukins and rare deficiencies of a number of them have been described, all featuring autoimmune diseases or immune deficiency.

Interleukine-6 (IL-6) is a pleiotropic cytokine with variable systemic functions (Barton, 1997). IL- 10 is an anti-inflammatory and immunosuppressive substance produced within the body and plays a role important in the regulation of immune responses (Moore et al.,2001).

This study aimed to evaluate the immune status of the patients with UTI by studying (IL-6 and IL-10).

## Subjects and Methods

The study population consists of 49 patients infected with Gram negative bacteria, with age ranging from (2 to 75) years and 25 individual (10 males and 15 females) healthy controls.

3 ml were collected in plastic tube and left to clot undisturbed for about 1/2 hour at room temperature, and then centrifuged at 3000 round per minute (rpm) for 5 min to obtain sera (Bishop et al.,2000). And after the serum sample were collected they were divided into two apendroff tubes and stored in deep freeze at  $-20^{\circ}\text{C}$  until performing IL-6 and IL-10. (Lewis et al., 2006).

5 ml of urine samples obtained from patients suspected with bacteriuria and cultured on the blood agar and MacConkey agar for diagnostic of bacteria. 1  $\mu\text{l}$  calibrated loop in vertical position in the urine, Then streak Blood agar and another loop to streak MacConkey agar plates and incubation overnight.

The culture results were interpreted as being significant and insignificant bacteriuria, according to the standard bacteriological tests. A growth of  $\geq 10^5$  colony forming units/ml was considered a significant bacteriuria (Balentine.,2012) . The pure cultures were prepared for biochemical tests to differentiate bacterial isolates which performed through

1-Colony morphology of bacterial isolates

2-Microscopic gram stain investigation

3-Biochemical tests: - Catalase test.

- Oxidase test
- API 20 test system

Statistical analysis

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2007. Numeric variables were presented as mean +SD while nominal variables were expressed as number and percentage. student test was used to compare mean difference between any two groups in case of normal distribution. Chi-square and or corrected Ch-square tests were used for the study of associations between nominal variables. Spearman Rank Correlation coefficient was used to study correlations. P-value was considered significant when it was less than or equal to 0.05.

## Results

Types of infectious micro-organism

Patients with E. coli infection accounted for 26 (53.06%); other culture results were as follows: 7 (14.29 %) patients had K. pneumonia, 9 (18.37%) patients had P. aerogenosa, 4 (8.16%) had Proteus and 3 (6.12%) patients had K.oxytoca . All control subjects were free of infection. These results are shown in table 1.

Table 1: Types of isolated bacteria in patients and control subjects enrolled in the present study

Culture	No.	%
<i>E. coli</i>	26	53.06
<i>K. pneumoniae</i>	7	14.29
<i>P. aerog</i>	9	18.37
<i>Proteus</i>	4	8.16
<i>K. oxytoca</i>	3	6.12
<b>Total</b>	49	100.00

IL6 and IL 10 levels in study groups

Mean serum IL-6 was 138.56±25.59 in patient group and 44.96±6.39 in control group. Mean serum IL-6 was significantly higher in patient group than in control group (P=0.012), as shown in table (2) and figure (1).

Mean serum IL-10 was 33.25±2.53 in patients group and 15.25±4.18 in control group. Mean serum IL-10 was significantly higher in patients group than in control group (P=0.001), as shown in table (2) and figure (2).

Table (2): IL6 and IL 10 levels in study groups enrolled in the present study

Parameter	Patients group		Control group		P-value
	Mean	SE	Mean	SE	
IL6	138.56	25.59	44.96	6.39	0.012
IL10	33.25	2.53	15.25	4.18	0.001

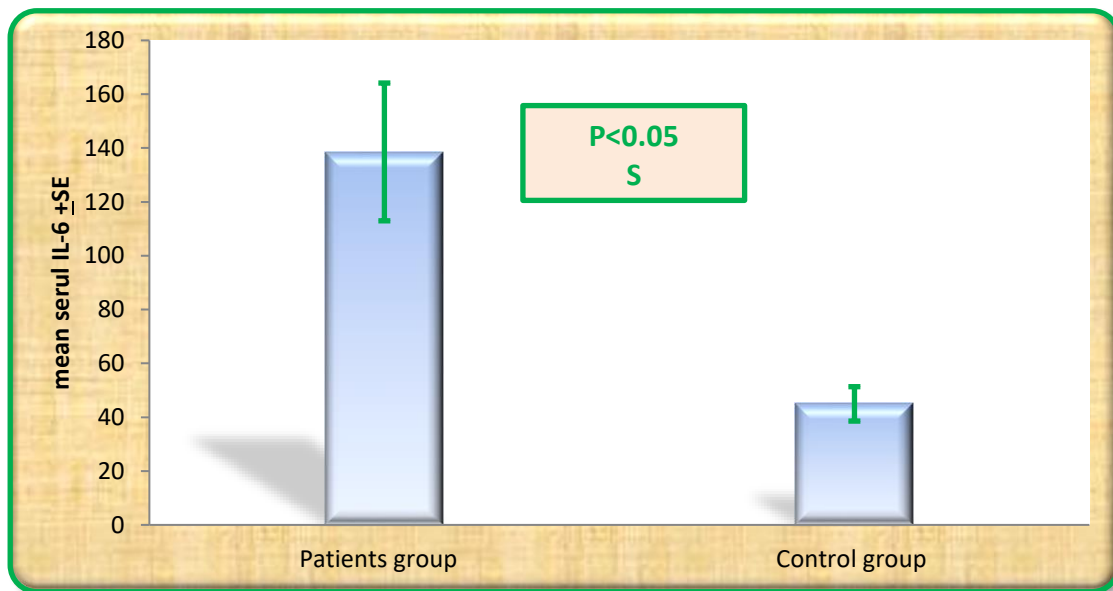


Figure (1): Mean serum IL-6 in study groups

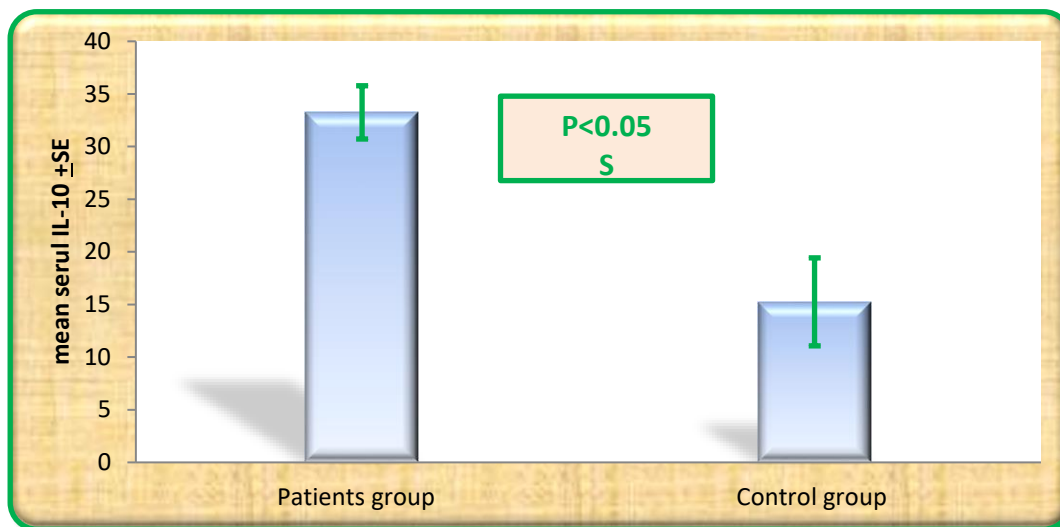


Figure (2): Mean serum IL-10 in study groups

## Discussion and conclusion

### Bacterial isolates

Microorganisms isolated from (49) Gram -ve culture-proven bacteriurics are shown in table (3-4).

*E. coli* form the majority of strains isolates 53.06% while *pseudomonas aerogenosa*; *Klebsiella pneumonia*; *protus sp.*; Represents 18.37% , 14.29%, 8.16% respectively .these finding are different from that of other works (AL- Dujaily et al.,2003)

*E. coli* is an important pathogen in urinary tract, particularly uropathogenic strains through possessing adhesion pili and other adheissins that predispose bacterial binding to the urothelium ( Jasmina et al. 2001). In addition to that *E. coli* possess many other tools make it potent pathogen to urinary tract and other sites of the body (Brooks et al. 2007). So for the above mentioned criteria *E. coli* took the first rank of isolation from urinary tract infection in this study.

### High level of IL6 and IL 10

The results of the current study show that the mean serum IL-6 was significantly higher in patient group (138.56+25.59) than in control group (44.96+6.39 (P=0.012), as shown in table (3-7) and figure (3-3).

Our result agreed with result of Benson et al. (1994) and Jacobson et al. (1994) whom found that the serum IL-6 concentrations are elevated mainly in patients with UTI.

Also agreed with result of (Abdulmohymen et al. 2010) whom Bacterial infected patients with and without bladder cancer revealed a significant increase in the mean serum IL-6 levels by ELISA.

IL-6 is secreted by a number of different cell types, and IL-6 blood levels are elevated in numerous infectious, inflammatory, and autoimmune diseases and in cancer in association with increased synthesis of other cytokines stimulated by infection, trauma, and immunological challenge. (Jones et al., 2001).

Elevated plasma and urine levels of IL-6 have been demonstrated in cancer and inflammatory diseases of the urinary tract (Andrews et al., 2002).

So that (Otto et al., 1999) suggest that the IL-6 was produced at the site of infection in the urinary tract can reach the bloodstream and contribute to the systemic disease in patients with nonbacteremic febrile UTI.

Urinary tract cytokine responses are initiated when bacteria reach the mucosal surface (de Man et al.,1989). Attachment to epithelial cells activates a first cytokine cascade that includes IL-6, IL-1, IL-8, and other chemokines (Hedlund et al.,1996).

study believe that invasion of lipid A component of endotoxin and P fimbriae present in *Escherichia coli* and other gram negative bacteria induce inflammation and release of IL-1 $\beta$ , IL-6, and IL-8 cytokines in the invaded area and local inflammatory responses cause cytokines to spill into general circulation, resulting in detectable levels in biological fluids, such as serum and plasma (Mohkam et al., 2008).

In the early stage of acute pyelonephritis, when bacteria attach to the kidney epithelial cells, alpha hemolysin induces calcium oscillations intracellularly which activates the proinflammatory cytokines IL-6 and IL-8 (Uhlen and Laestadius et al., 2000) and in the bladder, sloughing of the uroepithelium and bladder haemorrhage is initiated (Smith and Rasmussen et al., 2008).

The results of the current study show that the mean serum IL-10 was significantly higher in patient group (33.25 $\pm$ 2.53) than in control group (15.25 $\pm$ 4.18) (P=0.001), as shown in table (3-7) and figure (3-4).

The results of this study were agreement with Duell et al. (2012) Found IL-10 were higher in patients with UTI than in control. The regulatory cytokine, Interleukin-10 (IL-10), which is produced during UPEC infection in murine models of UTI and in patients with UPEC cystitis and pyelonephritis, has been a focus of several recent pathogenesis studies (Duell et al.,2012).

IL-10 regulates immune responses during many infections, predominantly by shifting immune responses towards a Th2-centric adaptive immune outcome that may benefit the host, and sometimes the pathogen (Couper et al., 2008). IL-10 is produced by a wide variety of leukocytes (Duell et al.,2012) and can be secreted by multiple intracellular trafficking pathways under different conditions (Stanley et al.,2012)

The presence of neither type 1 fimbriae nor bacterial attachment is required for IL-10 production in the mixed cell coculture model of human bladder also other components of bacteria that may trigger IL-10 include LPS (Chanteux et al.,2007).

IL-10 have revealed protective roles in UPEC infection UPEC induces IL-10 in the bladder during acute UTI, and this has been proposed to down-regulate inflammatory responses shortly after infection via monocytes/macrophages (Duell et al.,2012). patients with UPEC cystitis secreted significant urinary IL-10 and produced high levels of IL-10 systemically during UPEC urosepsis IL-10.

In conclusion, we measured concentrations of proinflammatory cytokine (IL-6) and anti-inflammatory cytokine (IL-10) in the sera of patients with gram-negative urinary tract infections. Our results demonstrate that there was positive correlation between them, and that due to the production of proinflammatory cytokines can be inhibited by so-called antiinflammatory cytokines, of which IL-10 is the most potent (Boyle, 2005).

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