Assessment of Interleukin -6 and Interleukin -10 in Patients sera of Urinary tract Infection

Baydaa Ali Abd-Alwan Al-Tamimi*[1]; Makarim Adil Khalil[2] and Sawsan Hassan Authman[3]

[1,2] Mustansiriyah University, College of Basic Education, Department of Sciences /Iraq
[3] Mustansiriyah University, college of science, Department of Biology/Iraq
bydalybd@gmail.com
dr.sawsanH@uomustansiriyah.edu.iq

Abstract

A urinary tract infection (UTI) in humans is one of the most common ailments in developing countries. The treatment of UTI is becoming difficult because of the increasing drug resistance against the common bacteria associated with UTI. This research aimed to determine the bacteria and to detect the host cytokine response to UTI infection. A retrospective study of data (November 2020 through May 2021) from the microbiology laboratory of the General Baghdad Hospital was analyzed. Bacteria were isolated from 250 patients (43.88%) urine cultures of 139 UTI suspected urine samples. Both Gram-positive (*Staphylococcus aureus* 24.5%; S.hominis 1.6%) and Gram-negative bacteria (*Escherichia coli* 62.2%; *Klebsiella pneumonia* 6.5 %; *Pseudomonas aeruginosa* 4.9%).The level of IL_6 and IL-10 in patients sera were measured, the level of IL-6 and IL-10 increased significantly in UTI patients in comparison to healthy control. The level of IL-6 and IL-10 differ significantly in UTI patients sera according to their interval ages.,the highest level was at ≥ 30,when the mean± S.D of IL-6 and IL-10 reached to 77.75 pg/ml ± 8.62 for IL-6 and 233.66 pg/ml ±17.56 for IL-10, While the less level was at ≤ 50. The level of IL-6 and IL-10 differ significantly (p≤ 0.05) in UTI patients sera according to gram positive and negative bacteria. When it gave mean level of IL-6 ± S. E in infection with S.hominis (200 pg/ml ± 0.03) higher than other Gram positive bacteria (67.56pg/ml ± 7.82). compare with Gram negative bacteria,it gave mean level for IL-6 In serum ± S.E with K. pneumoniae was 92.90 pg/ml ± 37.52.higher than E.coli and P.aerugenosa (72.45± 12.75) and (38.18± 0.16) respectively.

Keywords: IL-6, IL-10, Urinary tract Infection

1. Introduction

Urinary tract infections (UTI) are a major clinical problem, particularly in older age groups, impacting significantly on patient well-being and global healthcare systems [1, 2].Such infections are classified as complicated or uncomplicated, depending on the presence or absence of structural or functional abnormalities of the urinary tract, but are linked to the persistent breaching of the host innate urinary tract defences by uropathogenic bacteria [3]. Continuous use of antimicrobial agents has been shown to impact UTI frequency, but at the cost of bacterial resistance, which is a major public health concern [4-6]. Age is a significant risk factor for UTI [2, 7]. However, the clinical picture is frequently confused by older. Many older patients,particularly those with cognitive impairments where history-taking is clinically challenging, are often subjected to frequent, but needless antibiotic treatment regimens that do not cure or eradicate the bacteriuria, but actually drive microbial resistance [8]. However, the clinical dilemma is considerable because leaving a suspected UTI untreated in such patients may allow the infection to progress resulting in pyelonephritis, septicaemia and in some cases death [2, 9]. Asymptomatic bacteriuria is sociated with a range of bacterial species including the Enterobacteriaceae *Escherichia coli*, *Klebsiella pneumoniae* and Gram-positive bacteria including Enterococcus sp. And Stapylococcus spp [3, 7]. Of the species isolated from urine *E. coli* is the most common identified agent with uropathogenic *E.coli* or UPEC linked to > 75% of all reported UTIs [2, 3].Protection of the lower urinary tract from microbial assault is mediated through innate defence mechanisms that include physical factors such as urine pH, ionic composition and flow. These, in conjunction with innate immune responses characterised by the constitutive or inducible synthesis of urothelial host defence molecules including antimicrobial agents, chemokines and cytokines, function to prevent infection [10, 11]. The diagnostic tools to differentiate ASB from an UTI the focus has been on urinary cytokines and chemokines, which are easily measured and presumed to reflect the immune response of the urinarytract infection. To date only IL-6, a pro-inflammatory cytokine, has been nominated as a potential biomarker of infection in older patients, but the diagnostic thresholds remain confusing [12, 13]. To address this combinations of IL-6 and, for example, leukocyte esterase have been suggested to help improve diagnosis [14]. The study was designed to detect the host cytokine response to UTI infection.

2. Material and Methods

1-Patients

Two handered and fifty two patients were enrolled and identified as having UTI with or without bacterial isolation was done between Nov. 2020 and May 2021 at the many

doi.org/10.31838/hiv22.02.35 Received: 09.01.22, Revised: 10.05.22, Accepted: 14.08.22

hospitals in Baghdad. Patients between the ages of 10 and 85 years with a clinical diagnosis of UTI were included in the study on a history of urinary tract instrumentation or acute urinary retention before onset of fever. Patients with symptoms or signs of localized infections outside the urinary tract were excluded. The diagnosis of UTI was confirmed if the patient had significant bacteriuria in freshly voided urine. Patients assigned to the group with bacteremic UTI had at least 1 positive blood culture with the same bacterial species as in the urine. The non-bacteremic UTI group had positive urine but negative blood cultures.

2- Urine cultures

Freshly voided urine samples obtained at inclusion and at each follow-up visit were cultured. Significant bacteriuria was defined as growth of a single bacterial species at a concentration of ⩾104 cfu/mL of urine [15].
Bacteria were identified by standard techniques and saved in Brain heart infusion broth with glycerol at 15 %.

3. 3-IL-6 and IL-10 analysis

Serum separated from 2 mL of venous blood was examined using ELISA. The contents of IL-6 and IL-10, were determined in strict accordance with the instructions for each ELISA kit (Shanghai Westang Biotechnology Co., Ltd., Shanghai, China). (Medgenix, Fleurus, Belgium). IL-6 concentrations of ⩾20 U/mL were considered positive [16].

Statistics

One way ANOVA test, P <.05 was considered statistically significant.

3. Results and Discussion

1-Bacterial isolation

Two handered and fifty two patients were enrolled and identified as having UTI, and 61 isolates (43.88 %) of Gram positive and Gram negatives were isolated. the diagnosis was confirmed by a positive urine culture. Bacteria were isolated from 139 (43.88%) urine cultures,out of 139 UTI-suspected urine samples. The isolated bacteria were Gram-positive (24.5%) for S. aureus and 1.6% for S.hominis and Gram-negative (62.5%) for E. coli, K. pneumoniae, and P. aeruginosa were 6.5 % and 4.9% respectively. Frequently isolated bacteria were E. coli (62.5%), followed by *Staphylococcus aureus* (24.5%). The result of bacterial isolation is presented in table -1.

Table -1: Distribution of Gram negative and gram-positive isolates in patients' urine samples with urinary tract infection

Bacterial species	Number of isolates	Percentage %
Escherichia coli	38	62.2
Klebsiella pneumoniae	4	6.5
Pseudomonas aeruginosa	3	4.9
Staphylococcus aureus	15	24.5
Staphylococcus hominis	1	1.6

Bacterial infections are a common cause of urinary disorders in humans and have become a common cause of nosocomial infections. Urinary tract infections (UTIs) accounted for 35% of hospitalized patients in Lafia, Nigeria, and 23% to 37% of patients attending general hospitals in Nepal [17]. The results coincides with Odoki et al. [18] who reported 32% positive urine samples from patients attending hospitals in Bushenie, Uganda, and Barton et al. [19] who reported 38% positive urine samples from UTI in neonates from the University Hospital of the West Indies, Jamaica [19].

2-Interleukin -6 and Interleukin -10 Levels.

The level of IL-6 and IL-10 increased significantly in UTI patients in comparison to healthy control. (Table-2). As an immune modulatory cytokine, IL-10 plays a central role in limiting the host immune response to pathogens, preventing host damage and maintaining normal tissue homeostasis [20]. In patients with UTIs, IL-10 may protect the host against exaggerated immune responses that produce inflammation and tissue damage [21].

Table -2: The Level of IL-6 and IL-10 (pg/ml) in UTI patients Sera and control group.

Level of interleukens in blood sera (pg/ml)	Patients	Healthy	P-value
IL-6			
Range	4.58- 255.4	4.80 -25.49	0.001
Mean	69.24	9.81	
Standard deviation	44.94	4.41	
Standard error	6.06	0.70	
IL-10			
Range	2.15- 536.79	2.20 -29.47	0.001
Mean	214.91	6.36	
Standard deviation	104.49	5.59	
Standard error	14.09	0.94	

3-Interleukin -6 and Interleukin -10 Levels according to Interval ages.

The level of IL-6 and IL-10 differ significantly in UTI patients sera according to their interval ages. (Table-3),the highest level was at ≥ 30,when the mean± S.D of IL-6 and IL-10 reached to 77.75 pg/ml ± 8.62 for IL-6 and 233.66 pg/ml ±17.56 for IL-10, While the less level was at ≤ 50. That was 40.83 pg/ml ± 8.23 and 209.98 pg/ml± 55.19 for IL-6 and IL-10 respectively.

Table -3: The Levels of IL-6 and IL-10 (pg/ml) in UTI patients Sera and control according to their Interval ages.

Level of interleukin (pg/ml)	≥ 30 years old	30-49 years old	≤ 50 years old	P-value
IL-6				
Range	4.58— 255.42	15.32 - 106.41	12.25 - 68.63	0.004
Mean	77.75	60.13	40.83	
Standard deviation	51.02	27.71	20.16	
Standard error	8.62	7.41	8.23	
IL-10				
Range	2.15 - 536.79	48.77 - 276.99	58.06 - 397.55	0.001
Mean	233.66	170.16	209.98	
Standard deviation	103.91	83.47	135.19	
Standard error	17.56	22.31	55.19	

Total number	35	14	6	

Interleukin -6 and Interleukin -10 Levels according to Bacterial culture.

The level of IL-6 and IL-10 differ significantly in UTI patients sera according to their Bacterial culture, (Table-4).Interleukin-10 and IL-6 are parts of the immune response to urinary tract infection (UTI) due to E. coli and other bacterial species, and it is important in the early control of infection in the bladder. Defining the mechanism of engagement of the immune system by the bacteria that enables the protective IL-6 and IL-10 response is critical to exploring how we might exploit this mechanism for new infection control strategies.

Table -4: The Levels of IL-6 and IL-10 (pg/ml) in UTI patients Sera according to their bacterial culture

Level of IL_6 AND IL-10(pg/ml)	Positive culture	Negative culture	P-value
Il-6			
Range	4.58 -255.42	12.25- 101.82	0.001
Mean	75.94	57.50	
Standard deviation	52.15	25.41	
Standard error	8.81	5.68	
IL-10			
Range	74.49-536.79	2.15-291.74	0.001
Mean	251.72	150.51	
Standard deviation	96.65	86.33	
Standard error	16.34	19.30	
Total number	35	20	

In this study, the results revealed that the bacterial infection is an important reason that is sensed by and responsible for induction of IL-10 in the response to UPEC. The results show this response occurs in manner. When it gave mean level of IL-6 ± S.E in positive culture (75.94 pg/ml ± 8.81) higher than in negative culture (57.50 pg/ml ± 5.68).Similar results for IL-10 was higher in positive culture (251.72 pg/ml± 16.34) than in negative culture(150.51pg/ml± 19.30). Other studies suggest that an excess of these cytokines and other inflammatory agents may cause the body to create a so-called cytokine storm [21].

4-Interleukin -6 and Interleukin -10 Levels according to Gram positive and Gram negative Bacteria

The level of IL-6 and IL-10 differ significantly (p≤ 0.05) in UTI patients sera according to gram positive and negative bacteria. (Table-5) The results showed this response occurs in manner. When it gave mean level of IL-6 ± S.E in infection with S.hominis (200 pg/ml ± 0.03) higher than other Gram positive bacteria (67.56pg/ml ± 7.82)**.** compare with Gram negative bacteria,it gave mean level for IL-6 In serum ± S.E with K. pneumoniae was 92.90 pg/ml ± 37.52.higher than E.coli and P.aerugenosa (72.45± 12.75) and (38.18± 0.16) respectively.While results for IL-10 was higher in Gram negative bacteria than in Gram positive bacteria.

Al Rushood et al. [22] mentioned that during the acute phase, the mean urinary IL-6 level in the Cystitis group was significantly higher than that in the controls (17.8 pg/mL vs 14.8 pg/mL, P=0.03), while the serum levels were significantly higher in both the Cystitis and AP groups than in the controls (19.5 pg/mL, 19.4 pg/mL, 15 pg/mL, P=0.005 and 0.02, respectively).

Table -5: The Levels of IL-6 and IL-10 (pg/ml) in UTI patients Sera according to their bacterial species.

IL-6 and IL-10 level	S.aureus	S.hominis	E.coli	K.pneumoniae	P.aeruginosa	P-value
IL-6						
Range	16.76-106.41	200.60-200.69	4.58-255.42	33.78-199.24	37.89-38.46	
Mean	67.56	200.65	72.45	92.90	38.18	
S.D	25.92	0.05	54.09	75.05	0.29	
S.E	7.82	0.03	12.75	37.52	0.16	
IL-10						
Range	142.14-328.68	310.33-310.39	74.49-413.84	154.54-536.79	184.30-184.33	
Mean	227.55	310.36	245.80	346.99	184.31	
S.D	56.44	0.03	95.94	161.16	0.01	
S.E	17.02	0.02	22.61	80.58	0.01	
Total number	11	1	18	4	1	

Gokce et al. [23], demonstrated a significant correlation between the presence of vesicoureteric reflux and urinary IL-6 levels, which might support the suggestion that elevated IL-6 might predict acute pyelonephritis.

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