



Purposeful scientific research is a basic pillar for the advancement of Iraq



Estimation of the concentration of some cytokines in patients with urinary tract infection

Lina Qays Yaseen Abd_Alrazak , Wa'ad Mahmood Raoof, Sabah Mohammed Salih

Biology Department, College of Science, Tikrit University, Tikrit , Iraq
drlina@tu.edu.iq

ABSTRACT

The main goal of this study is to investigate bacterial infections relationship with Interleukins 1-B, IL-6, IL-10 and IFN- γ concentrations level among persons with urinary tract infections, blood and urine samples were taken from 60 patients admitted to Azadi Teaching Hospital and Kirkuk General Hospital, from ages ranged between (15-55) years and of both gender, for the period 1st January 2015 to 1st February 2016. These were 20 positive bacterial cultures were recorded and 10 negative bacterial cultures served as control. The questionnaire list for information about the patient was recorded. Microscopic diagnosis was made and biochemical tests were conducted for the isolated samples. The Analytical Profile Index System (API) was used to diagnose the types of bacterial isolates. Five ml of venous blood samples were taken from all infected people in order to obtain serum to measure the levels of Interleukins 1-B, IL-6, IL-10 and IFN- γ concentration using an ELISA technique. The results of the current study showed IL-1 β was highly significant ($P < 0.01$) in the UTI group (2.180 ± 0.341) (1.663 ± 0.283), compared with its level in control groups and IL-10 showed a significant decline in the level of ($P < 0.05$) when compared with the Control and the results were as follows (15.0 ± 21.0) (48.2 ± 45.8) respectively. When compared to the control, IFN and IL-6 showed no significant differences. Also the current study showed the most isolated bacteria were *Escherichia coli* and *Staphylococcus Saprophyticus*.

Keywords: IL1-B, IL-6, IL-10 and IFN- γ / UTI / bacteria

Introduction

Infection of the urinary tract known as the inflammatory response of the urinary system is caused by the pathogenic bacteria present in the urinary tract inside the containers so that they appear in the urine sample taken any newly bacteremia lactation Bacteriuria and can be difficult to distinguish if they were diagnosed with polluted external or internal microbial infection [1].

Urinary Tract Infection (UTI) is a common disease that affects people of all ages, including young women, men, children, and the elderly [2]. Forty to fifty percent of adult women have a history of at least one UTI. An estimate of the UTI cases occurring globally per year is 150 million [3]. Susceptibility runs of UTI in families and UTI prone individuals experience recurrent, often clustered infection with potentially severe complications, depending on the site of infection and type of UTI [4]. UTIs which occur in men, pregnant women, and patients with immune suppression or urinary tract abnormalities, such as congenital malformations, urinary calculi, recent urologic instrumentation, indwelling catheters, neurogenic bladder, and kidney transplant and women are, more likely to be affected compared with men [5].

Infection spread is common in both men and women, but the incidence is higher in women because their physiology, the body, is regulated by many immune system factors. Pathological cases may result from abnormal immune system regulation. Both the first and second lines of defense are weakened by the immune response to infection [6].

When bacteria invade the host uroepithelial cells, inflammatory mediators such as (IL-6) and (IL-10) are produced, and the initiation of apoptotic cascades by the epithelial cells results in exfoliation of the superficial epithelial cells [7].

Material and Methods

Sample Collection

The specimens were collected from Azady Hospital, AL_Gomhore Teaching Hospital and some of external Laboratory in Kirkuk City, North of Iraq, during the period from 1th January 2015 to 1th February 2016.

During the period a total of 30 urine samples were collected from patient into a sterile container, with symptoms of urinary tract infections at a heartburn and difficulty in urine. The samples were gathering in sterile plastic bottles was a recommendation that the patient sample is gathered in the middle of urine

(Clean-Catch-Midstream Urine) Badgsl genital area by soap and water to avoid contamination of natural Ballenbat in this region [8].

Microscopic examination of Urine:

Centrifugation of urine samples was done and the swag was poured outside then the sediment applied on a clean slide, and a cover slide was applied over the urine sediment. Then slide directly under high power field [H.P.F] of light microscope to discover any epithelial cells; pus cells; bacteria cells; casts; erythrocytes and crystals [9].

Blood Sample

five ml of blood were taken from those patients used for the separation of sera involved in immunological. Were put in sterilized plane tube to clot at 37C° for (30-45) minutes, then sera were separated by centrifugation for 15 minutes at 3000 rpm. Haemolysed serum is avoided [10].

Identification of the isolated bacteria

Bacterial species were identified using standard methods such as colony morphology and gram stain (manufactured by ARCOMEX Jordan). Api 20E systems bio meriux company France) was also used as directed by the manufacturer [11].

Morphological Identification:

Isolated bacterial colonies were identified according to morphology, color and consistency on Nutrient and MaCconkey's agar medium and type of hemolytic on blood agar medium [12].

Microscopic examination:

Microscopic examination was used to classify bacteria to cocci, bacilli and to Gram negative or Gram positive bacteria. on the basis of their staining with gram stain, size, shape and arrangement of cells [13].

Determination of parameters

Cytokines were determinate by using its kit from komabiotech, according to the manufacturer's own technology ELISA instructions [14].

Results & Discussion

In the present study 30 urine samples (from suspected UTI) Male & female has been cultured for detecting

bacterial infection. Twenty of them, were positive and 10 were negative, (the negative group served as control group). The results showed in table (1) Forty six isolates, has been identified (gram positive and gram negative in equal number). The negative culture of Urine samples while clinical signs refers to UTI may due to present, Urethral syndrome, Ureteral obstruction, tuberculosis of the bladder or other disease must be considered. The isolated gram negative bacteria, were *Esherichia coli*, *pseudomonas* *Proteus moganii*, *proteus rettgeri*, *Klebsiella pneumoniae*. *Proteus mirabilis*, *Serratia marcescens*, *Citrobacter freundii*, *Klebsiella rhinosclomatis*, *Enterobacter liquefaciens* and *Alcaligenes faecalis*. While isolated Gram positive bacteria were, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *streptococcus pneumonia*, *streptococcus pyogenes*, *Staphylococcus simulans*, *Enterococcus faecalis* and *Micrococcus luteus*.

E. coli was isolated more than other gram negative bacteria and the percentage is low when compared with the other studies, this agreed with Hameed [15] in tikrit who found that the prevalence of *E.coli* was (20.5%).

Another study of Kadir *et al*, 2010 [16] in Kirkuk city they found that the *Escherichia coli* infection (10.8%) was the commonest among patients with urinary tract stones. A study conducted by Otajevwo & Amedu., 2015 [17] showed that the Most UTIs are caused by gram negative bacteria like *Klebsiella spp* & *E coli*. According to Vasudevn, 80-85% of UTIs are caused by *E. coli*, while 5-10% are caused by *Staphylococcus saprophyticus* [18]. 16% of the prevalence of the infection was due to *Klebsiella* species. [19].

In another study found *Staphylococcus aureus* and *Micrococcus luteus* were isolated in 4% and 5% from women with UTI, respectively. whereas *S. saprophyticus* constitutes to 5% to 10% followed by the other Gram negative rods [20].

Table 1: Distribution of bacteria isolated from UTI's according to gender

Noo	Type of bacteria	Males Isolates		Females Isolates		Total	Total
		No.	%	No.	%	No.	%
1.	<i>Staphylococcus epidermidis</i>	2	11.7 %	2	7.0%	4	8.70%
2.	<i>Staphylococcus saprophyticus</i>	3	17.6%	3	10.3%	6	13.04%
3.	<i>Staphylococcus simulans</i>	1	5.9%	1	3.4%	2	4.34%
4.	<i>Staphylococcus aureus</i>	2	11.7%	2	7.0%	4	8.70%
5.	<i>Enterococcus faecalis</i>	0	0.0%	1	3.4%	1	2.17%
6.	<i>Enterobacter liquefaciens</i>	1	5.9%	0	0.0%	1	2.17%
7.	<i>Alcaligenes faecalis</i>	1	5.9%	0	0.0%	1	2.17%
8.	<i>Micrococcus luteus</i>	1	5.9%	0	0.0%	1	2.17%
9.	<i>E. coli</i>	1	5.9%	5	17.2%	6	13.04%
10.	<i>Streptococcus pyogenes</i>	1	5.9%	1	3.4%	2	4.34%
11.	<i>Streptococcus pneumonia</i>	1	5.9%	2	7.0%	3	6.52%
12.	<i>Proteus mirabilis</i>	0	0.0%	1	3.4%	1	2.17%
13.	<i>Proteus rettgeri</i>	0	0.0%	2	7.0%	2	4.34%
14.	<i>Serratia marcescens</i>	0	0.0%	1	3.4%	1	2.17%
15.	<i>Citrobacter freundii</i>	0	0.0%	1	3.4%	1	2.17%
16.	<i>Proteus morganii</i>	0	0.0%	2	7.0%	2	4.34%
17.	<i>Pseudomonas aeruginosa</i>	2	11.7%	3	10.3%	5	10.86%
18.	<i>Klebsiella rhinoscleromatis</i>	0	0.0%	1	3.4%	1	2.17%
19.	<i>Klebsiella pneumonia</i>	1	5.9%	1	3.4%	2	4.34%
Total		17	100%	29	100%	46	100%

The current study showed a significant highly $P < 0.01$ in the level of IL-1 β concentration in UTI patients compared with its level in control groups and the results were as follows $(2.180 \pm 0.341)(1.663 \pm 0.283)$ pg/ml respectively. While IL-10 showed a significant

decline in the level of ($P < 0.05$) when compared with the Control and the results were as follows $(15.0 \pm 21.0)(48.2 \pm 45.8)$ respectively. IL-6 and IFN- γ there were no significant difference when compared with the Control group as shown in Table No. (2).

Table 2: The concentration of interleukins levels in patients infected with UTI

Transactions	IL-1 β (pg/ ml)	IL-6(pg/ml)	IL-10(pg/ml)	IFN- γ (pg/ml)
Patient	2.180 ± 0.341	18.5 ± 34.8	15.0 ± 21.0	31.6 ± 20.6
Control	1.663 ± 0.283	15.35 ± 5.21	48.2 ± 45.8	25.9 ± 13.3
T test	4.12**	0.40 ns	-1.96*	0.87 ns

Mean $\pm$ SE

* $P < 0.05$

** $P < 0.01$

Our results agreed with the study carried out by Földi [21] where it was found that the concentration of IL-10 levels was decreased in people with UTI compared to the control. This change marked with an elevated level of IL-10 leads to low resistance to infections. IL-6, IL-10 produced mainly by macrophages, play an important role in the initial phase of the pathophysiological processes [22]. This may be because effect of TNF α which is, effective mediator of the inflammation and incites a series of secondary cytokines (IL-6, IL-1 β) [23]. *S. aureus*-caused UTIs are typically regarded as a secondary infection to blood infections. From the bowel, the bacteria are transferred to the bladder. Following the bacterial attachment, a bio-film forms that thwarts the immune system and is the primary factor in the infection. [24]. Chemokine, IL-1 β , IL-6, were all up regulated in bladder from UPEC-infected [25]. These results agreed with patients about regulation, of IL-6 in response to UPEC [26]. The majority of cytokines and chemokines were expressed at their highest levels 24 hours after vaccination and returned to nearly baseline levels two weeks later. The type of expressed fimbriae also appears to affect the range of

chemokines secreted [22]. Polymorphonuclear cells (PMNs) are drawn in by bacterial adhesion to epithelial cells, which also causes the production of cytokines like interleukin (IL)-1 and IL-6. A variety of urinary inhibitors of bacterial adhesion are secreted, including lactoferrin, low-molecular weight oligosaccharides, serum immunoglobulin A, and bladder mucus polysaccharide. A strong innate immune response is produced when uropathogenic *E. coli* penetrates these physical barriers by attaching to the epithelium. Neutrophils are the most common early responders to infected bladders. A variety of secreted substances, including antimicrobial peptides, cytokines IL-6, TNF- α , IL-1 β , G-CSF, and IL-17, are found among the cellular infiltrates when a mammalian bladder becomes infected. Innate-like lymphocytes like T cells and Ag-presenting macrophages and dendritic cells have also been connected to the UTI host defense [27]. In another study (ÇAKIR *et al.*, 2004) in Turkey found increases the macrophage activity thereby increasing the productive capacity for the pro-inflammatory mediators [28].

Conclusion

The current study showed a significant highly ($P < 0.01$) in the level of IL-1 β concentration in UTI patients while IL-10 showed a significant decline in the level of ($P < 0.05$). IL-6 and IFN- γ there were no

significant difference when compared with the Control group. *E. coli* was isolated more than other gram negative bacteria and the percentage was highly in female.

References

- [1] **Barini-García, M.** and Whitmore, K.(2008). Urinary Tract Infection (Frequently Asked Questions), Department of Health and Human Services, Office on Women's Health. U.S.A.
- [2] **Soto M S.** Importance of Biofilms in Urinary Tract Infections: New Therapeutic Approaches . Hindawi Publishing Corporation Advances in Biology. 2014., Article ID 543974, 13 pages .
- [3] **Yu y,**Patricia S,Cynthia B,Nicolas C,Karen E and Rembert P.Dignosing inflammation and infection in the urinary system via proteomics. Journal of transduction medicine .2015.13:111.
- [4] **Godaly G,**Ines A and Catharina S.Innate immunity and genetic determinants of urinary tract infection susceptibility .wolters Kluwer health.2015;vol 28 (1).
- [5] **Kim G & Joseph T.** Urinary Tract Infection Update. American Journal of Clinical Medicine. 2012 ; Vol 9 (2).
- [6] **Shimeld, A.L.** (1999). General methods for Identification of bacteria . 1st ed. Delmar publisher, New York. U.S.A.
- [7] **Reygaert WC.** Innate Immune Response to Urinary Tract Infections Involving *Escherichia coli*. J Clin Cell Immunol.2014. 5: 280.
- [8] **Vandepitte, J, Verhaegen, J, Engbaek, K.; Rohner, P.; Piot, P. and Heuck, C.** (2003). Basic laboratory procedures in clinical bacteriology. Second edition, World Health Organization, Geneva, Switzerland pp.30-32.
- [9] **Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Piot P, Heuck CC.** Basic laboratory procedures in clinical bacteriology. 2nd ed. World Health Organization. Geneva.2003..p. 14-24,71.
- [10] **Lewis, S.M.; Bain, B.J., and Bates, I.** (2001). Practical Haematology. 9th ed. Churchill Living stone, London.
- [11] **Reynolds, J.** (2004). API20E, Richaland College.University of Hawaii.
- [12] **Otajevwo, F. D & Amedu , S. S** Community Acquired Urinary Tract Infection Prevalence in a Tertiary Institution Based in Evbuobanosa, Edo State, Nigeria. Global Journals Inc. (USA). 2015; Volume 15 Issue 3.
- [13] **Alfred, E. B.** (2005). Bensons microbiological applications in laboratory manual in general microbiology. 9th ed. McGraw – Hill. Companies. U.S.A.
- [14] **Tietz,** Reference Information for the Clinical Laboratory. In Textbook of Clinical Chemistry, 3rd ed Burtis, C.A., Ashwood, R.A. W.B. Saunders: Philadelphia, 1999; 1831.
- [15] **Hameed S H.** The relationship between Urinary Tract Infection and sex hormones in women. M.s.c.thesis.. College of science , University of tikriti.2007.
- [16] **Kadir M A, Ibrahim M N and Salih N M.** prevalence of urinary tract infections in patients with renal stones.2010.
- [17] **Otajevwo, F. D & Amedu , S. S** Community Acquired Urinary Tract Infection Prevalence in a Tertiary Institution Based in Evbuobanosa, Edo State, Nigeria. Global Journals Inc. (USA) . 2015; Volume 15 Issue 3.
- [18] **Vasudevan R .** Urinary Tract Infection: An Overview of the Infection and the Associated Risk Factors. J Microbiol Exp .2014; 1(2).
- [19] **Taneja N, Chatterjee SS, Singh M, Singh S, Sharma M .**Urinary tract infection in a tertiary, care centre from north India. Indian J Med Res.2010., 131: 101-105.
- [20]**Zalmanovici T A, Green H, Paul M, Yaphe J, Leibovici L** (2010) Antimicrobial agents for treating uncomplicated urinary tract infection in women. Cochrane Database Syst Rev (10): CD007182.
- [21] **Földi Viktor.** The effect of fluid resuscitation and antioxidant treatment, on the burn trauma, induced inflammation and oxidative stress 2011. Ph.D. thesis. University of Pécs, Faculty of Medicine.
- [22] **Balog A, Gál J, Gyulai Z, Zsilák S, Mándi Y.** Tumour necrosis factor-alpha and heat-shock protein 70-2 gene polymorphisms in a family with rheumatoid arthritis. Acta Microbiol Immunol Hung 2004; 51: 263-9.
- [23] **Ingersoll M, Kline K, Nielsen H , Hultgren S.** G-CSF induction early in uropathogenic *E coli* infection of the urinary tract modulates host immunity. Cell microbial 2008.,10(12):2568-2578.
- [24] **SEDARAT Z & Taylor –Robinson A.** Biofilm Formation by Pathogenic Bacteria: Applying a *Staphylococcus aureus* Model to Appraise Potential Targets for Therapeutic.BMC J. 2022 .11(4): 388.
- [25] **ÇAKIR B & YEGEN B.** Systemic Responses to Burn Injury Turk J Med Sci 34 (2004) 215-226 TÜB -TAK.
- [26] **Sundvall et al.** interleukin- 6 concentration in the urine and dipstick analyses were related to bacteriuria but not symptoms in the elderly : across sectional study of 421 nursing home residents . BMC Geriatrics 2014, 14:88.
- [27] **Sivick Kelsey E., Matthew A. Schaller, Sara N. Smith and Harry L. T. Mobley .**The Innate Immune Response to Uropathogenic *Escherichia coli* Involves IL-17A in a Murine Model of Urinary Tract Infection. J Immunol.2015. 184:2065-2075.
- [28] **çakir b & yegen B .** Systemic Responses in Injury Turk J Med Sci 34 2004. 215-226 TÜB -TAK .

44