Original Article

Expression of Circulatory Interleukin-6 Concentration Associated with *Pseudomonas aeruginosa* Persistence in Recurrent Urinary Tract Infections

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Abstract

Background: The main superfamily cytokine member is interleukin-6 (IL-6). IL-6, promptly produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase responses, hematopoiesis, and immune reactions. **Objectives:** To investigate the role of IL-6 as a biomarker in recurrent urinary tract infections (RUTIs). The real-time polymerase chain reactions were performed by using specific primers with reference gene GAPDH and the target genes IL-6. **Materials and Methods:** A case–control study was done in Babylon city hospital from February 2021 to March 2022. A total of 110 patients with RUTIs with different age and sex, and healthy individuals as control group were enrolled in this study. Midstream urine was taken for culturing and identification of *Pseudomonas aeruginosa.* Blood samples were obtained from all the patients and the secreted IL-6 levels were detected by enzyme linked immunosorbent assay (ELISA). RNA was extracted for gene expression. **Results:** The expression of IL-6 was increased more than % 30-fold in UTIs compared with control group. Also, the results found that serum concentrations of IL-6 detected by ELISA assay showed significant differences at $P < 0.001$ for the patients compared with control group. **Conclusions:** Interleukin-6 gene expression shows up regulation in RUTI caused by *P. aeruginosa*.

Keywords: Gene expression IL-6, IL-6, mRNA gene, Real-time PCR, UTI

Introduction

The main superfamily cytokine member is interleukin-6 (IL-6). IL-6, promptly produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase responses, hematopoiesis, and immune reactions. IL-6 supports the innate immune response, powerfully by generating C-reactive protein (CRP), many complement system proteins, and the coagulation cascade, and also controls body thermogenesis by serving as an endogenous pyrogenes. Additionally, IL-6 encourages the development of hematopoietic precursors, encourages T and B lymphocyte differentiation and development, and regulates body thermogenesis.[1]

However, IL-6 also functions as a cytokine. It mediates anti-inflammatory and metabolic activities in skeletal muscle by increasing the expression of anti-inflammatory molecules such as IL-1ra (IL-1 receptor agonist) and IL-10 and decreasing the synthesis of pro-inflammatory molecules such as tumor necrosis factor (TNF) and IL-1ß. IL-6 initiates an anti-inflammatory reaction.[2]

In the acute phase of the immune reaction to infection, immune cells are frequently activated first and proclamation cytokines associated with inflammation, such as IL-6, TNF-α, and IL-10. Acute phase proteins are primarily stimulated by inflammation-associated cytokines, which are produced in the liver.[3,4]

Access this article online

Quick Response Code:

Website: www.medjbabylon.org

DOI: 10.4103/MJBL.MJBL_343_22

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Submission: 22-Dec-2022 **Accepted:** 11-Jan-2023
Published: 27-Apr-2023

How to cite this article: Suber BA, Alshukri MS, Judi MR. Expression of circulatory Interleukin-6 concentration associated with *Pseudomonas aeruginosa* persistence in recurrent urinary tract infections. Med J Babylon 2023;20:201-5.

The chemokine CXCL8, which is produced by *Pseudomonas aeruginosa*, plays a role in the release of acute-phase proteins, the differentiation of immune cells, as well as neutrophil migration to the site of infection.[5]

Particularly significant epithelial cells serve as a significant first route of defense and immune/inflammatory cell because they are involved in some of the initial interactions between interning pathogens like *P. aeruginosa* and the host.

Lipopolysaccharide (LPS), respectively, trigger intracellular signal pathways that produce proinflammatory cytokines.[6] In the initial stages of bacterial infection, the concentration of the pro-inflammatory cytokine IL-6 rises a cytokine that promotes inflammation and increases the comeback of TNF and IL1. In UTIs, the chemokine interleukin-8 induces neutrophil migration to the site of inflammation, resulting in pyuria.[7]

Several biomarkers encourage the identification of UTI like the levels of IL-6 and IL8 in urine and serum in children with UTI, respectively.[8] Presently, it is discovered that 35 subtypes of interleukins have a noteworthy role in the immune response and in the maturation and differentiation of B cells, the production of acute phase proteins and immunoglobulin, the stimulation of bone marrow, the expansion of meningeal cells, and the activation of macrophage monocyte phagocytic complex.[9]

Materials and Methods

Patient and clinical sample

A case–control study was done in Babylon city hospital from February 2021 to March 2022. Patients were admitted to the hospital with suspected recurrent urinary tract infections by clinical features.

A total of 110 patients with RUTIs with different age and sex, and healthy individuals as control group were enrolled in this study. A total of midstream urine was taken from patients for culturing and identification of *P. aeruginosa*, according to Forbes *et al.*[10]

Blood samples were obtained from all the patients infected with *P. aeruginosa* and put in ethylenediaminetetraacetic acid tubes. RNA was extracted for gene expression study.

Control group

2 mL of blood samples were taken from (20) healthy individuals as control group who are free from any sign and symptoms for any illness. RNA was extracted from these samples in order to be studied later.

Measuring IL-6 by ELISA

Secreted IL-6 levels were detected rendering to the kit manufacturer's guidelines (e Bioscience, USA).[11]

Ethical approval

An ethical approval was obtained by verbal consent from all patients. The committee of publication ethics at College of Medicine, University of Babylon, Iraq, approved this study under the reference number BMS/0203/016 dated on June 18, 2019.

Gene expression of IL-6: RT-PCR

After collection of blood samples from patients and healthy individuals, the total RNA was extracted according to Tri RNA Pure Kit (Geneaid Company, Korea).

The real-time qPCR reactions were performed by using specific primers with reference gene GAPDH and the target genes IL-6 with final volume mixture 20 µL. Conversion the total RNA to cDNA and amplification of DNA was done according to instructions provided by GoTaq® 1-Step RT-qPCR System (Promega, Korea). Using BRYT Green® dye, where RT-qPCR primers and conditions were summarized in Table 1. Relative expression fold was calculated ($2^{-(\Delta\Delta Ct)}$).[12]

Statistical analysis

Statistical analysis was carried out using SPSS version 23.0 (SPSS, IBM Company, Chicago, Illinois). The molecular result was exploration by using Chi-square (χ^2) test. *P* values less than (0.05) is considered. Data were expressed as mean ± SD.

Table 1: Primers sequence and condition used in qRT-PCR

Primer name	Sequence 3′-5′	Steps	Cycles	Temp./ Time
IL-6 sense	CCCCAATTTCCAATGCTCTCC	Revers transcription	1	37°C for 15 min
IL-6 anti sense	CGCACTAGGTTTGCCGAGTA	Reverse transcriptase inactivation and GOTaq DNA polymerase activation	1	95°C for 10 min
GAPDH-F	GTCTCCTCTGACTTCAACAGCG	Denaturation Annealing	40	10°C for 30 s 37°C for 30 s
GAPDH-R	ACCACCCTGTTGCTGTAGCCAA	Extension and data collection		72°C for 30 s

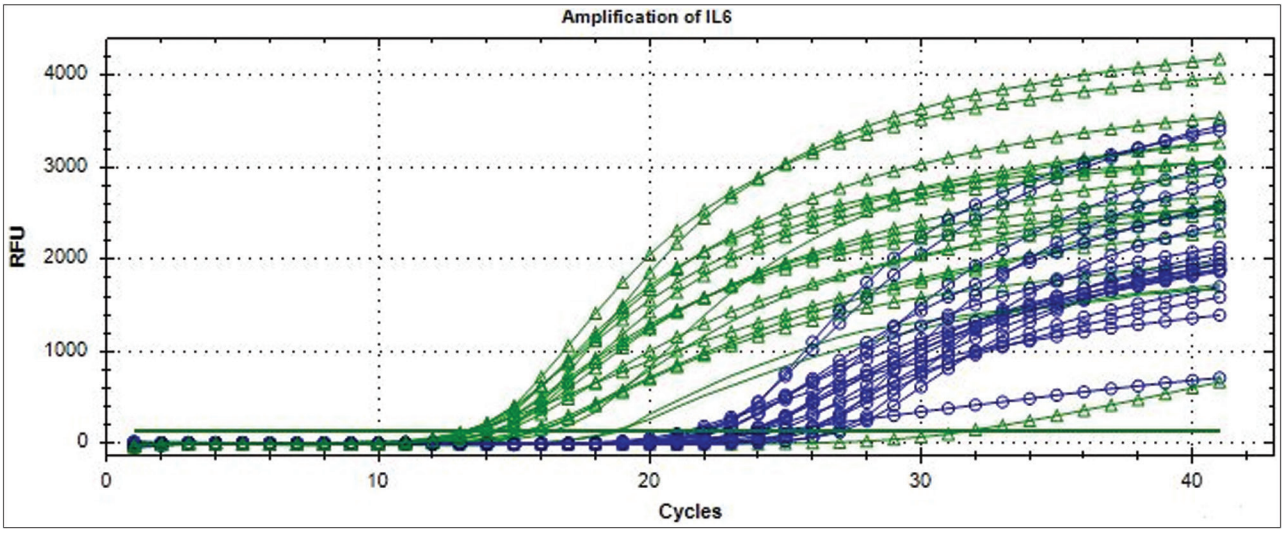

Figure 1: The level IL-6 expression. This is the first run for 15 samples, represents amplification of Reference gene (GAPDH), represents amplification of samples RUTI Patients, represents amplification of control samples

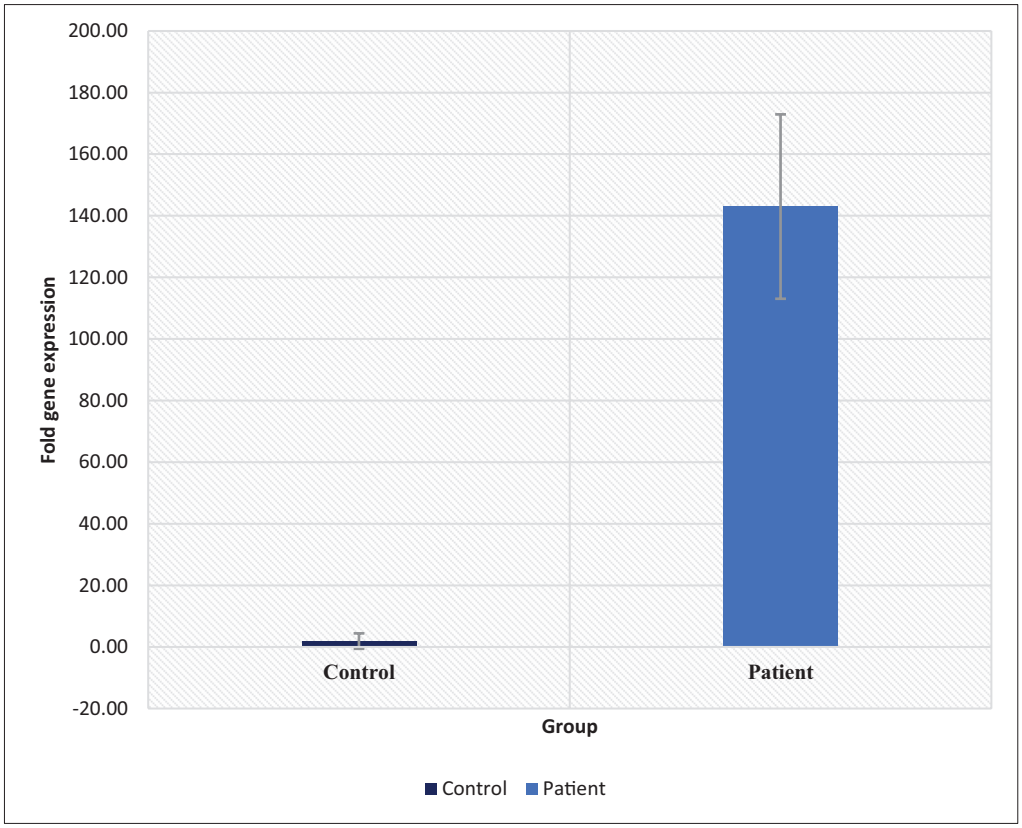

Figure 2: IL 6-fold gene expression between control and RUTI patients versus the reference gene (GAPDH)

Results

Gene expression IL-6 level using real time PCR

A total of 30 blood samples were collected. RNA was extracted to revision the gene expressing of IL-6 by means of RT-PCR (ΔΔCt). The results found that the level of expression to IL-6 gene in the patients' samples as well as in control samples, normalized by means of house-keeping gene GAPDH, represents a significant difference at $P \leq 0.05$, as shown in Figures 1 and 2, Tables 2 and 3.

ELISA for measuring IL-6

The result showed increasing secreted IL-6 levels produced according to that have been indicated highly significant at P value = 0.001 when compared with control and gender association as shown in Table 4 and Figure 3.

Table 2: Expression of IL 6-fold gene between control and RUTI patients versus the reference gene (GAPDH)

Groups	No.	Expression levels (2^−(ΔΔCt))		
		Mean	SD	SE
Controls	20	1.91	2.51	0.56
Patients	30	142.99	29.93	5.47
P value		<0.0001*		

* Represent a significant difference at $P \leq 0.05$

Table 3: Fold gene expression (Ct) for IL-6 between control and RUTI patients versus the reference gene (GAPDH)

Groups	No.	Ct		
		Mean	SD	SE
Controls	20	24.6480	1.13072	.25284
Patients	30	17.5207	1.13191	.20666
P value		<0.0001*		

* Represent a significant difference at $P \leq 0.05$

Table 4: Secreted IL-6 ELISA for measuring levels produced according to patient and control

	Case	No.	Mean	Std. deviation	Std. error mean	*P* value
IL-6	Control	20	186.01455	14.450275	3.231180	0.001
	Patient	30	286.65140	81.470098	14.874337	

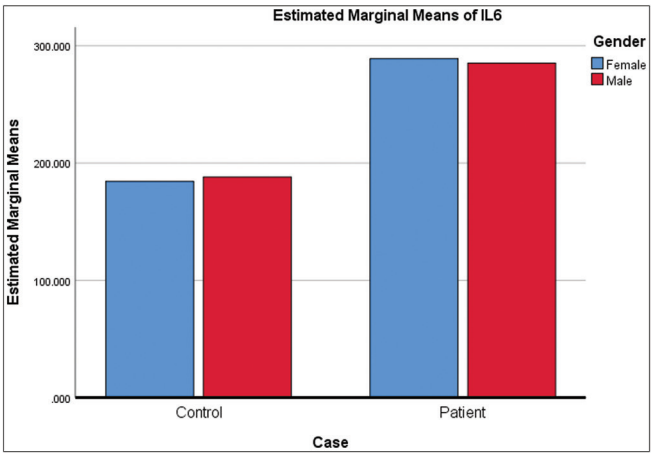

Figure 3: Secreted IL-6 ELISA for measuring levels produced according to patient and control and gender association

Discussion

A wide range of stimuli (bacterial infections, inflammation, tissue injury response, and other cytokines and growth factors), IL-6 gene expression are easily elevated in cells. The promoter IL-6 is turned on at the molecular level via a number of signaling mechanisms.[13] The study was conducted (IL-6) as an indication of bacterial urinary tract contagion and agreed with other study that suggested (IL-6) as a marker of bacterial urinary tract infection.[14]

In the course of acute inflammatory reactions in urinary tract infections, cellular and molecular processes and interactions successfully reduce the risk of approaching harm or infection brought on by microorganisms invading.[15]

Gene expressing of (IL-6) can be second hand as a biomarker for initial UTI detection because it is unique of the greatest significant components of the inflammatory reaction and released by urothelial cells in comeback to exposure to UTI-causing substances.[16]

The detection of cytokines in the serum and gene expressing have been employed in this work to aid in the diagnosis and follow-up of numerous urological disorders. However, when compared to the controls group in the current investigation, the IL-6 mean levels were substantial ($P = 0.001$). This result agreed with other clinical and test findings, were performed by the measurement of IL-6 in the blood and was found in studies to be useful in separating upper from lower involvement and aiding in the diagnosis of UTIs.[17]

Olsyzna *et al.*[18] study on UTI patients showed that urinary IL-6 was increased in the UTI group.

The results were compatible with the study of Rama *et al.*,[19] they showed that IL-6 was not a good biomarker for urinary tract infection and differentiation in the upper tract involvement from the lower tract.

In urothelial cells, IL-6 induced Stat3 phosphorylation, and universal IL-6 injection increased urothelial Stat3 phosphorylation and antimicrobial peptide production.[20]

Chronic IL-6 reduction also resulted in severe pyelonephritis and an increase in the number of bacteria in the kidneys. Thus, infected urothelium expresses antimicrobial genes through a transcriptional pathway driven by IL-6/Stat3 signaling, which is crucial for preventing epithelial invasion and spreading infection. Thus, it has significant roles in regulating epithelial attack and ascending infection.[21]

The onset of chronic cystitis is accompanied by biomarkers of local and systemic acute inflammation 24 h after infection, including severe pyuria, bladder inflammation with mucosal injury, and a distinctive serum cytokine signature consisting of elevated IL-5, IL-6, G-CSF, and IL-8 in those with a history of chronic cystitis. Chronic cystitis incidence varies according to host strain and infectious dosage.[22]

Both Wawrysiuk *et al.*[23] and Sundvall *et al.*[24] demonstrated that cytokines are essential elements of the indigenous host-cell comeback and that they are talented biomarkers for distinguishing upper from lower UTIs. Additionally, they may be helpful in assessing the degree of local immune reaction during simple lower UTIs, which might aid in choosing a non-antibiotic course of treatment.

Rego *et al.*,[25] showed that LPS-stimulated bone marrow-derived T cells, IL-6 production is positively regulated

by two different CCAAT/enhancer-binding protein and NF-B pathways and an SHP-1-independent NF-B pathway as *P. aeruginosa*.[26] Several factors have a role in the pathogenesis such as genetics (family history), diet, and environmental factors.[27,28]

Conclusions

The role of IL-6 in intermediating pro-inflammatory according to gene expression and phenotypes in recurrent urinary tract infection. These findings provide preclinical justification for clinical investigations of IL-6 in RUTI.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol 2018;9:754.
2. Eckardt K, Görgens SW, Raschke S, Eckel J. Myokines in insulin resistance and type 2 diabetes. Diabetologia 2014;57:1087-99.
3. Al-Eqaby JHH, Al-Charrakh AH, Mustafa AAM. Resistance to third-generation cephalosporins among hospitalized patients with monomicrobial enterobacteriaceae bacteremia (mEB) in Baghdad, Iraq. J Pharm Negat Results 2022;13:2309-17.
4. El Haddad H, Chaftari AM, Hachem R, Chaftari P, Raad II. Biomarkers of sepsis and bloodstream infections: The role of procalcitonin and proadrenomedullin with emphasis in patients with cancer. Clin Infect Dis 2018;67; 971-7.
5. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol 2014;6:a016295.
6. Pomerantz JL, Baltimore D. Two pathways to NF-kappaB. Mol Cell 2002;10:693-5.
7. Gokce I, Alpay H, Biyikli N, Unluguzel G, Dede F, Topuzoglu A. Urinary levels of interleukin-6 and interleukin-8 in patients with vesicoureteral reflux and renal parenchymal scar. Pediatr Nephrol 2010;25:905-12.
8. Mahyar A, Ayazi P, Maleki MR, Daneshi-Kohan MM, Sarokhani HR, Hashemi HJ, *et al*. Serum levels of interleukin-6 and interleukin-8 as diagnostic markers of acute pyelonephritis in children. Korean J Pediatr 2013;56:218-23.
9. Godaly G, Otto G, Burdick MD, Strieter RM, Svanborg C. Fimbrial lectins influence the chemokine repertoire in the urinary tract mucosa. Kidney Int 2007;71:778-86.
10. Al Charrakh AH, Al Awadi SJ, Mohammed AS. Detection of metallo-β-lactamase producing *Pseudomonas aeruginosa* isolated from public and private hospitals in Baghdad, Iraq. Acta Med Iran 2016;54:107-13.
11. Forbes BA, Daniel FS, Alice SW. Bailey and Scott's Diagnostic Microbiology. 12th ed. Maryland Heights: Mosby Elsevier Company;2007.
12. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402-8.
13. Darogha SN, Azeez SH, Abdullah ZG. Evaluation of procalcitonin and interleukin-6 as a marker of bacterial urinary tract infection. Cell Mol Biol 2021;67:203-13.
14. Aziziaram Z, Bilal I, Zhong Y, Mahmod A, Roshandel M. Protective effects of curcumin against naproxen-induced mitochondrial dysfunction in rat kidney tissue. Cell Mol Biomed Rep 2021;1:23-32.
15. Masajtis-Zagajewska A, Nowicki M. New markers of urinary tract infection. Clin Chim Acta 2017;471:286-91.
16. Brauner A, Söderhäll M, Jacobson SH, Lundahl J, Andersson U, Andersson J. *Escherichia coli* induced expression of IL-1α, IL-1β, IL-6 and IL-8 in normal human renal tubular epithelial cells. Clin Exp Immunol 2001;124:423-8.
17. Otukesh H, Javadi Larijani F, Fereshtehnejad SM, Hosseini RO, Salman Yazdi R. Evaluation of urinary and serum concentration of interleukin-6 (IL-6) in children with urinary tract infection: Differentiation between pyelonephritis and acute cystitis. Razi J Med Sci 2010;17:7-14.
18. Olszyna DP, Vermeulen H, Baan AH, Speelman P, van Deventer SJ, Gouma DJ, *et al*. Urine interleukin-8 is a marker for urinary tract infection in postoperative patients. Infection 2001;29:274-7.
19. Rama N, Rasoul NK, Alireza E. Comparing serum levels of Interleukin-6 (IL-6) in acute pyelonephritis versus acute in 6 months to 12 years old children. Int J Child Adolesc 2017;3:348-54.
20. Ching CB, Gupta S, Li B, Cortado H, Mayne N, Jackson AR, *et al*. Interleukin-6/Stat3 signaling has an essential role in the host antimicrobial response to urinary tract infection. Kidney Int 2018;93:1320-9.
21. Schilling JD, Mulvey MA, Vincent CD, Lorenz RG, Hultgren SJ. Bacterial invasion augments epithelial cytokine responses to *Escherichia coli* through a lipopolysaccharide-dependent mechanism. J Immunol 2001;166:1148-55.
22. Chen SL, Hung CS, Pinkner JS, Walker JN, Cusumano CK, Li Z, *et al*. Positive selection identifies an in vivo role for FimH during urinary tract infection in addition to mannose binding. Proc Natl Acad Sci USA 2009;106:22439-44.
23. Wawrysiuk S, Naber K, Rechberger T, Miotla P. Prevention and treatment of uncomplicated lower urinary tract infections in the era of increasing antimicrobial resistance-non-antibiotic approaches: A systemic review. Arch Gynecol Obstet 2019;300:821-8.
24. Sundvall PD, Elm M, Ulleryd P, Mölstad S, Rodhe N, Jonsson L, *et al*. Interleukin-6 concentrations in the urine and dipstick analyses were related to bacteriuria but not symptoms in the elderly: a cross sectional study of 421 nursing home residents. BMC Geriatr 2014;14:88.
25. Rego D, Kumar A, Nilchi L, Wright K, Huang S, Kozlowski M. IL-6 production is positively regulated by two distinct Src homology domain 2-containing tyrosine phosphatase-1 (SHP-1)–dependent CCAAT/enhancer-binding protein β and NF-κB pathways and an SHP-1–independent NF-κB pathway in lipopolysaccharide-stimulated bone marrow-derived macrophages. J Immunol 2011;186:5443-56.
26. Alubaidi GT, Hasan SM. Stem cells: Biology, types, polarity, and asymmetric cell division: A review. Med J Babylon 2022;19:318-23.
27. Al-Kaif LA, Al-Saadi MA, Al-Charrakh AH. Effect of SARS-CoV-2 infection on HBV-infected patients: Reactivation. Med J Babylon 2022;19:736-46.
28. Behayaa HR, Juda TM, Mohammed SB. The effect of androgen hormones in acne pathogenesis: A review. Med J Babylon 2022;19:345-9.