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Focused on Investigating the Bacterial Spectrum, Urinary Incontinence and Immunological Biomarkers among Women with Urinary Tract Infection

Sanaa Khudhur Jameel

AL-Iraqia University\ College of Medicine, Medical Microbiology Department, Iraq.

Email: **Sanaa_Jameel@aliraqia.edu.iq**

ABSTRACT

***Background**: Urinary tract infections occur commonly in women, and 20% of them suffer from at least one urinary tract infection in their life time. The study aimed to investigate the bacterial causative agents and the prevalence of urinary incontinence, additionally quantity the serum levels of Interleukin -6 and Interleukin-17.Out of 130 women patients with suspected urinary tract infection, only 45 of them met the criteria and give bacterial growth culture. Urine samples for the isolation and identification of bacteria , confirmation of the diagnosis done by the Vitek 2 system. Blood for serum levels measurement of Interleukin -6 and Interleukin-17 compared with serum samples taken from 45 healthy women.Escherichia coli was the most common isolated bacteria among women patients represented(37.8%).From a total of 45 urinary tract infections women patients, urinary incontinence was 8 (17.77%) , and the predominant bacteria also was Escherichia coli (37.5%). Interlukin-6 and interlukin-17 showed significant differences, among the patient group than that of healthy group . In conclusion the predominant bacteria cause urinary tract infection was Escherichia coli , and urinary incontinence as a side effect of urinary tract infection . There was highly significant differences in Interleukin-6 and Interleukin-17serum level in patients compared to the control group.*

***Keywords:** Uropathogenic bacteria, Interleukin -6 , Interleukin-17, Urinary tract infection, Urinary Incontinence.*

Article Information

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INTRODUCTION

Urinary tract infections (UTI) are a common microbial illness affecting people of all ages and sexes, causing inflammation in the urinary system, and they can range from mild cystitis to severe uroseptic shock. Both males and females experience at least one UTI symptomatic infection throughout their lifetime (1). Urinary tract infections are very common; around 60% of females and 12% of males experience at least one UTI in their lifetimes. The urinary tract consists of the kidneys, ureters, bladder, and urethra organs (2).Colonization of the urinary system by uropathogenic bacteria results in a UTI. Clinically, UTIs range from simple to complex urinary tract infections somewhere in the urinary tract ,can develop when bacteria enter the urethra and travel up the urinary tract to the bladder, sometimes, the bacteria infect more distant parts of the tract, such as the kidneys (3).

The proximity of the external urethral meatus to the anus and the shorter urethral length in women compared to men are likely anatomical reasons why female sex is one of the primary risk factors for the development of UTI (4). Decreased estrogen levels, elevated postvoid residual urine, increased frequency of

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sexual intercourse, use of spermicides, family history, pregnancy, and pelvic organ prolapse are other risk factors linked to UTI in women (5). Urinary incontinence (UI) can be caused by several different factors. In some cases, UTI can worsen or manifestation of urge incontinence. Bladder spasms and increased urgency to urinate occur due to the inflammation and irritation caused by the infection. These spasms may cause involuntary urine leakage, resulting in UI episodes (6). The most predominant bacteria that caused UTI were *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella* species, and *Pseudomonas aeruginosa* (7).

Inflammatory cytokines are produced after an immunological response to the infection, which may serve as aid diagnostic indicators (8). Interleukin-6 (IL-6), IL-8 and IL-1 are usually the first cytokines released in urine after infection. They are produced in response to bacterial infections and are very necessary inflammatory mediators (1). IL-6 organizes various functions of the immune system, such as activates the synthesis of acute-phase proteins by the liver, permits the proliferation and differentiation of B- and T-cells, and activates leukocytopoiesis (9). Macrophages, fibroblasts, vascular endothelium, T-cells, and others have the ability to secrete IL-6 after stimulation by bacteria-related molecules and some other ingredients (10).

In spite of primary biological reports focused on the pro-inflammatory function of IL-17 in the comparable of autoimmune and inflammatory disorders, it has become clear that the IL-17 plays a major role far more nuancedly (11). IL-17 functions are highly organized and dependent on the tissue, and there is a clear balance between the inflammatory and safe functions of IL-17. Where IL-17 has been shown to play a role in the immune response created during fungal, bacterial, and viral infections associated with maintenance, in addition IL-17A participates in preservative and destructive mechanisms (12).

The objective of the present study was to determine the bacterial causative agents of UTIs, as well as the incidence rate of UI as the side effect of UTI, additionally investigate if uropathogenic bacteria impact the serum IL- 6 and IL- 17 levels, and finally correlate IL- 6 and IL -17 concentration with patient outcome measures, to evaluate the diagnostic usage of serum levels of IL-6 and IL-17 in the assessment of UTI in females.

MATERIALS AND METHODS

Study design

This study was a case-control study carried out from November 2023 to June 2024 at Baghdad Teaching Hospital/Medical City. Included a total of 130 women patients aged more than 18 years suffering from UTI symptoms who were not receiving antibiotic treatment for a week prior to providing specimens.

Sample collection

Out of a total 130 women patients with suspected UTI , only 45 patients of them met the criteria and give positive bacterial growth culture. Two types of specimens were taken from the patients, urine samples for bacterial isolation and blood samples for serum level measurement of IL-6 and IL-17 , compared with serum level of samples taken from 45 healthy women who served as a control group of the same age and sex.

Isolation of the bacteria and Identification of the bacterial Isolates

The first step was the collection of midstream urine specimens. Bacteria were isolated by streaking of loopful of urine sample on blood agar, MacConkey and chocolate agar, the agar then incubated at 37 °C .The bacterial isolates were then identified by Gram stain

,biochemical tests, and the diagnosis was confirmed by using Vitek-2 system (Biomerieux) in accordance with the manufacturer's instructions.

Measurement of IL-6 and IL-17 serum levels in UTI patients

In order to estimate the serum levels of IL-6 and IL-17 by sandwich type of ELISA, five milliliters of venous blood were drawn from both patients and controls then the blood separate to serum . Commercial kits IL-6 and IL-17 (MYBio Source, USA) were used for the test, and instructions were followed to ensure quality control.

Statistical analysis

The statistical analysis was done using SPSS, Level of significance as not significant P>0.05, Significant P≤0.05. The following data are descriptive analyses, Minimum, maximum mean, and standard deviation (SD)for quantitative variables. The student's test was used to investigate the causes of the difference and identify any significant differences between the two categories.

RESULTS

Age distribution of the current study.

From a total of 130 female patients , only 45 females had urinary tract infection, and the current study showed that the incidence of UTI among female patients was 34.61%.

Regard the age , table 1 revealed that the age of the current study samples was normally distributed;and the prevalence of UTI incedence was elevated among the patient groups more than 61 years represented (37.9%) and elevated among the patient groups (51–60) with percentage (31.3%) ,while the female patients group at the age group 18 to 30 showed low incidence (4.4%).

Table 1: Age distribution of the current study

Age groups	No.45	
	No.	%
18-30	2	4.4
31-40	4	8.8
41-50	8	17.8
51-60	14	31.1
≥ 61	17	37.9
Total	45	100

Distribution of bacterial isolated species among patients with urinary tract infection

Regarding the urine culture assessment of the current study, Gram negative bacteria were predominant followed by Gram positive bacteria represent (88.9%, 11.1%), respectively (Table 2).

Table 2: Bacterial isolates distribution groups according to the Gram reaction.

Group	No .of bacterial isolates	%
Gram negative	40	88.9%
Gram positive	5	11.1%
Total	45	100%
P-value	--	0.0001 **
** P≤0.05		

Gram-positive bacterial isolates were *S. aureus* represented (11.1%),while Gram-negative bacteria species represented *E. coli, which* was the predominant isolated bacteria (37.8%), followed by *K.pneumoniae* , *P.Mirabilis, and Pseud.aeruginosa* (20%) ,(17.8%), (%,13.3) respectively (Table 3).

Table 3: Distribution of bacterial isolated species among patients with UTI

No.of patients with UTI=45				
No.	**Bacterial species**	**Gram reaction**	**No.**	**%**
1	*E.coli*	Gram negative	17	37.8 %
2	*K. pneumonia*	Gram negative	9	20 %
3	*P. mirabilis*	Gram negative	8	17.8 %
4	*Pseud. aeruginosa*	Gram negative	6	13.3 %
5	*S. aureus*	Gram positive	5	11.1 %
Total			**45**	**100**

E.coli :Escherichia coli / K.pneumoniae:Klebsiella pneumoniae/ P.mirabilis:Proteus mirabilis / Pseud.aeruginosa:Pseudomonas aeruginosa / S.aureus:Staphylococcus aureus

From a total of 45 UTI women patients, there was 8 (17.77%) urinary incontinence among women patients, and the most predominant bacteria was *E. coli* (37.5%), followed by *K. pneumonia*, *P.Mirabilis*(25% to each one) and *Pseud. aeruginosa* was (12.5%) as showed in table 4.

Table 4: Distribution of bacterial isolates in Urinary incontinence patients.

No.	Bacterial species	Urinary Incontinence N(%)	
1	***E. coli***	3	37.5
2	***K. pneumoniae***	2	25
3	***P.mirabilis***	2	25
4	***Pseud. aeruginosa***	1	12.5
5	***S. aureus***	0	0
Total		8	100

Immunological parameters IL -6 and IL- 17 among patients with UTI compare with control group.

There was no Pearson Correlation between IL-6 and IL-17 in women patients compared to the control group (Table 5).

Table 5: Pearson correlation between the age, IL-6 and IL-17

Correlations		Age	IL-6	IL-17
Age	Pearson Correlation	1	.010	-.058
	Sig. (2-tailed)		.946	.689
	No.	45	45	45
IL-6	Pearson Correlation	.010	1	-.091
	Sig. (2-tailed)	.946		.529
	No.	45	45	45
IL-17	Pearson Correlation	-.058	-.091	1
	Sig. (2-tailed)	.689	.529	
	No.	45	45	45

Regarding IL-6, significant differences were identified, as the mean value was found to be significantly higher among the patients women group than that of healthy women (212.57 vs. 15.8), respectively, with significant differences of 0.0001. Also, interlukin-17 was significantly higher among the patient women group than that of healthy women (725.74 vs. 117.7), respectively, with significant differences *in P* values of 0.0001 .There were no significant differences of age in correlation to the IL-6 and IL-17 in women patients compared to the control group; the mean value was (39.18 vs. 386.78), with significant differences *P* values of 0.16 (Table 6).

Table 6: Correlation between the age, IL-6, and IL-17, comparison with control group

Statistics	Age control	Age patients	IL-6 patients	IL-6 control	IL-17 Patients	IL-17 control
Number	45	45	45	45	45	45
Mean	386.78	39.18	212.57	15.8	725.74	117.7
Std. Error of Mean	2.48	2.67	7.51	0.73	20.97	28.07
Median	30.9	31.5	239.26	16.27	749.5	65.68
Std. Deviation	17.56	18.9	53.11	5.16	148.35	198.49
Minimum	14	13	23.5	4.58	51.32	22.2
Maximum	85	85	273.8	25.65	951.1	883.74
P value	0.16 Non-sig		**0.0001**		**0.0001**	

Correlation between IL-6 , IL-17 and bacterial species

Table 7 showed that there was no correlation between the species of bacteria and IL-6 serum levels,and no significant differences in the IL-6 production regarding bacterial species that *P* value 0.08 . Interleukin-17 showed no significant differences in serum level and bacterial species , also there was no correlation between bacterial species and IL-17 secretion that *P value* was 0.071.

Table 7:Correlation between IL-6 , IL-17 and bacterial species

Bacterial species	IL-6 Mean±SD	IL-17 Mean±SD
E. coli	690.11±114.2	226.32±44.9
K.pneumonia	760.9±155.2	195.6±33.2
Pseud. aeruginosa	730.12±149.7	185.24±61.2
P. mirabilis	747.59±181.2	221.68±41.2
S. aureus	746.84±171.3	226.38±57.2
***P* value**	0.08	0.071

DISCUSSION

From a total of 130 female patients, 45 had UTI, and the current study showed that the incidence of UTI was 34.61% and had a high prevalence among aged women because the immune system became weak among elderly women. Also, hormonal disturbances occur in women during menopause , and the bladder function decreases in older women. This result agrees with Vazquez-Montes *et al.* (13) and Harrington and Hoot (14).

This study showed that the Gram-negative bacteria were the predominant (88.9%), while the Gram-positive bacteria were (11.1%). These results are compatible with the results of the study conducted by Flores-Mireles *et al.* (15),and the most common bacterial species was *E. coli* (37.2%),these were agree with Govindarajan and Kandaswamy (17).The study conducted by Asmat*et al. (16); they* recorded that there was a high rate of bacterial isolation of *E. coli.* The important virulence factors produced by *E. coli*, like toxins, capsule polysaccharides, flagellum, biofilm, fimbriae, adhesions, hemolysins, and siderophore, play a major role for evasion from host defenses, and adhere of uropathogenic *E. coli*(UPEC) to host epithelial cells; this process remains the most important determinant of pathogenicity (18, 19, 20).

Klebsiella pneumoniae, the second uropathogenic bacteria in the current study, which was isolated from 9 (20%) women from a total of 45, has two types of pilli types used by bacteria for biofilm formation and bladder colonization (17). *Proteus Mirabilis isolates* were found in 8 of the total 45 with a percentage(17.8%), *P. mirabilis have* fimbriae (PMFs), which aids the bacteria for colonization in the bladder and kidney (21). Non-Agglutinating fimbriae (NAFs), which aid bacteria in adhering to uroepithelial cells (22). *Pseudomonas aeruginosa* isolated from 6(13.3%) women patients the major virulence factor for these bacteria were biofilms, extracellular polysaccharides, elastase, exoenzyme S, and hemolytic phospholipase C and also produces the elastane enzyme's protease activity, which causes tissue destruction by releasing nutrients essential for bacterial growth (23).

The results of the present study revealed that *S. aureus* were 11.1%; *S. aureus* is a rare cause of UTIs. In spite of being rare, it is considered very dangerous because it leads to life-threatening septicemia (24). Additionally, the coagulase enzyme has the ability to convert soluble fibrinogen into insoluble fibrin, and this enzyme is specific for *S. aureus* used to differentiate *S. aureus* from other *Staphylococci* spp. (25).

The present study showed that UI among 45 women patients with UTI was 8 (17.77%) and the most predominant bacteria was Gram-negative, represented by *E. coli* (37.5%). Followed by *K. pneumonia and P. Mirabilis accounted (*25%) while *Pseud. aeruginosa represented* (12.5%)(Table 4*)*. UTI was one of the important predisposing factors causing UI; if the infection included the bladder and if was untreated, it could lead to UI.

A possible side effect of UTI is UI, UTI is a bacterial infection of the urinary tract that may involve the ureters, bladder, urethra, or kidneys (26). When infection reaches the bladder, irritation or swelling of the bladder may happen, leading to an incidence of UI. Predominant, people who have UTI suffer from an increase in multiples of feeling the need to urinate, which can lead to an involuntary loss of urine. Another explanation was that UTI can cause a variety of symptoms, including transient urinary incontinence. When the bacteria get into the urethral opening and reach the bladder, it causes the bladder to become swollen and irritated, and urine can leak out (27).

There were no significant differences in age in correlation to the IL-6 and IL-17 in women patients compared to the control group (Table5 and Table 6). That the immune response to uropathenic bacteria is equal in all ages, or may be the bacterial pathogens have a specific strategy for escape from the immune response and cause UTI.

Table 6 showed a significantly increased serum level of IL-6 in patients women than that of the control group.

Table 7 showed that there was no correlation between the species of bacteria and IL-6 serum levels,and no significant differences in the IL-6 production regarding bacterial species, the results of present study agree with Ching *et al.* (28). The urothelium produces interleukins as a response to the UTI (29) and interleukin-6 is fast-produced specifically as a response to UTI by urothelial cells . Lipopolysaccharide (LPS) and P-fimbriae are antigenic structures eliciting urothelial IL-6 production (30). In bladder urothelium, LPS links the Toll-like receptor to stimulate an intracellular signaling cascade and IL-6 secretion. IL-6 secretion may aid in amplifying the immune host response to UP bacteria by stimulating antimicrobial defense action in a rounded urothelial cell (31).

Table 6 showed elevated interlukin-17 serum levels in patients than that of the control group. These results are similar to the study done by Ahmadikia *et al.* (32) .The current study showed there was no correlation between IL-17 secretion and bacterial species (Table 7).The function of IL-17 was trigger antimicrobial peptide and chemokine expression, attracts and stimulates neutrophils and monocytes, and destroys the infected cells (33).

CONCLUSION

The current study showed that the most common bacteria cause UTI was *E. coli*, and the incidence of UI as a side effect of UTI. Additionally, uropathogenic bacteria did not impact serum IL-6 and IL-17 levels. Finally , there were highly significant differences in IL-6 and IL-17 serum levels concentrations between female patients compared to healthy women. These results are important to assess the diagnostic utility of serum levels of IL-6 and IL-17 as potential biomarkers in the diagnosis of UTI in females.

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