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ABSTRACT

Aims: In human Urinary Tract Infection (UTI) consider one of the most common bacterial infections, the purpose of this research was to assess the association between Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Tumor Necrosis Factor-alpha (TNF-α) level in *P. mirabilis* UTI cases. Methodology: 160 samples of urine and blood were gotten from persons Suffer UTI, their age reached to (10-60) years, in addition to 100 samples of urine and blood from Healthy persons as a control group. All urine samples were inoculated by sterile loop immediately on blood agar plate and MacConkey agar plate and incubated aerobically at 37°C for 24 hr. Colony Forming Units (CFUs) method was used for growing single and pure bacterial colonies for target bacteria. Enzyme-Linked Immunosorbent Assay (ELISA) kit was used for detection level of human cytokines (IL-6, IL-8 and TNF-α) in inpatient and control groups. Results: The result showed that 62.5% of samples were positive for *P. mirabilis*. The level of IL-6, IL-8 and TNF-α considerably were high inpatient which reached to (312.189±0.916, 272.417±8.699 and 93.908±0.912)respectively, compared with control group (75.416±0.721, 77.978±0.932 and 51.777±0.556) respectively. There were important connotations between age, gender and placement and *P. mirabilis* infection. Conclusion: Target cytokines piece a critical role in UTI especially in acute phase. Also, there were substantial variances in age group, gender, and residency regarding infected with *P. mirabilis* .and there are important relationships between IL-6, IL-8 and TNF-α in the patients infected with *P. mirabilis*.

Keyword: UTI, IL-6, IL-8, Chemokines, TNF-α, *Proteus mirabilis*

INTRODUCTION

Bacterial infections are a common cause of Urinary Tract Infection (UTI) in humans and happen in variable percent according to gender and age. It is responsible for 5% of all incidents of fever[(1,2)]. Infection can include parenchyma of kidney is first site was targeted by bacteria or maybe restricted alone to the inferior part of Urinary Tract (UT). Asymptomatic

Bacteriuria (ABU) is typically noticed parenthetically in high percent in population[3,4]. In ABU cases patients usually improve naturally and organize not essential treatment were eliminating the causative agents is very problematic and difficulties were frequently rare[5].

During infection and inflammation numerous cells, begin to formed cytokines that consider minor and solvable proteins. IL-6 is a pro-inflammatory cytokine, It is level rises in the initial stage of infection, it takes reformative actions which once inattentive increased the progress of the Immune system, while pro-inflammatory replies of IL-6 are facilitated by trans-signaling, it is accountable to disease that leads to development of C-Reactive Protein (CRP)[6]. IL- 8 is a pro-inflammatory cytokine which increased in reply to IL-1 and TNF-α. IL-8 is a chemokine reason to relocation of neutrophils to the apartment of irritation, causing the pyuria. in infected persons[7,8]. During advanced stages of UTI, levels of above cytokines may be rise in blood and urine[9,10,11].

Common bacteria that cause urinary tract infection are *P. mirabilis* which is gram-negative bacteria, considering the species that sweeps the agar surfaces, Overproduction and strong stampede movement are the hallmarks of this organism[12]. It is accomplished in producing symptomatic infections in the UT counting (cystitis and pyelonephritis) that is existing in suitcases of ABU, chiefly in aging and person with type 2 diabetes. Moreover, *P. mirabilis* can reason to creation of urinary stones (Urolithiasis)[13,14].

Some researchers believed the common of *P. mirabilis* which infects UT consequence for the proliferation of this microorganism from GIT while others are unpaid to person-to-person program, chiefly in healthy places[15]. This is reinforced by indication Some studies indicate that bacteria isolated from UTI are themselves isolated from stool. This microorganism also may infect other sites in a host[16]. Some research related *P. mirabilis* to rheumatoid arthritis, on the other side some researchers had not been able to prove this suggestion. Believes that induce antibodies forming by hemolysin and urease enzymes lead to Self-Antigen production that contributes to Rheumatoid Arthritis infection[17]. This research planned to assess the association between IL-6, IL-8 and TNF-α amount in *P. mirabilis* in UTI cases.

MATERIALS AND METHODS

One hundred and sixty urine and blood samplings were gotten from persons Suffer UTI, their age reached to (10-60) years who referred to Hospital of AL-Diwanyia During the duration from April 2016 to April 2017. Targeted patients were requested to reply ready questionnaire to make available data about age, gender and habitation. in addition to a total of 100 urine and blood specimens were obtained from a Healthy person as control group.

Urine specimens collection

Ten ml of clean and mid-stream of urine samples were collected in sterile containers (Himedia-India) from patients and control group, all containers were labeled according to

gender and age, and Habitat of each patient and healthy persons. Immediately, the urine samples were processed for bacterial cultivation and identification[18].

Isolation and identification of *Proteus mirabilis* bacteria

All urine samples were inoculated by sterile loop (Himedia-India) immediately on blood agar plate and MacConkey agar plate (Oxoid, UK). All agar plates were incubated aerobically at 37C° for 24 hr. Colony Forming Units (CFUs) method was used for growing single and pure bacterial colony; all urine samples containing less than 105 CFUs/ ml were excluded. All single and pure bacterial colonies were identified according to colony morphology, gram stain, lactose or non-lactose fermenter on MacConkey agar plate, capsule formation and according to standard biochemical tests such as motility, oxidase, catalase, Triple Sugar Iron (TSI) 21, Indole, Methyl Red, Voges-Proskauer, and Citrate (IMVIC) tests. In addition, all bacterial isolates were streaked on CHROMagar medium (Orientation, France)[19]. *P. mirabilis* was collected and all other bacterial species were neglected.

Detection of IL-6, IL-8, and TNF-α

The Serum was Separated from the blood samples and it was stored at -20C° for later use. ELISA kit was used for the detection level of human cytokines (IL-6, IL-8 and TNF-α) separated serum and done conferring to the instructions mentioned in the company brochure (diagnostic automation, inc., USA).

Statistical analysis

Chi-Square test was used to find the statistical differences between the observed frequencies and the expected frequencies of samples positive for *P. mirabilis* in relation to the age, gender and housing factor, and T-test to know the significant differences between patients group and healthy group in relation to (IL-6,IL-8 and TNF-α) at the probability level (value of $P < 0.01$). As well, Pearson correlation coefficient to see association between the studied cytokines with each other[20].

RESULTS AND DISCUSSION

From 160 Urine samplings, 100 (62.5%) were had *P. mirabilis* and 60 (37.5%) were didn't had above bacteria (Figure 1). This study disagrees with results of[21] who referred that *P. mirabilis* were isolated in (3.2%) and[22] who referred that *P. mirabilis* isolated in (21.7%), from UTI cases.

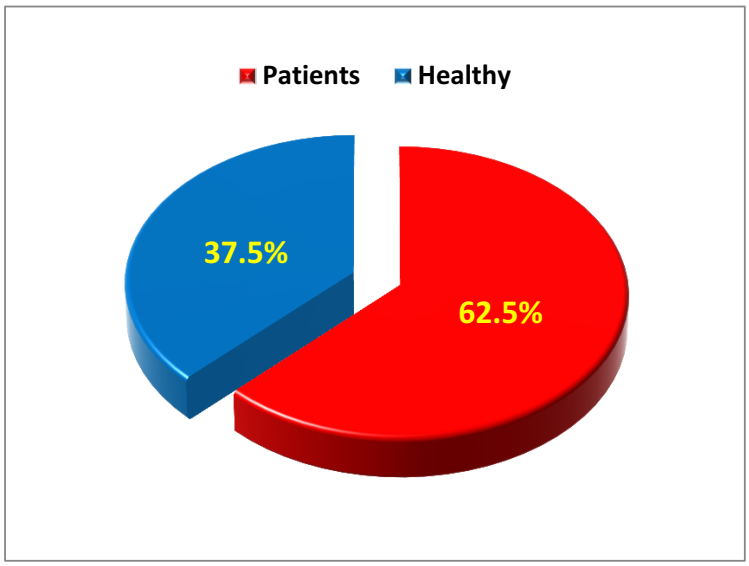

Figure 1: Distribution of UTI Patients infected and non-infected with *P. mirabilis*

These bacteria metabolically involved in urease production which act as one of the explanations, the pathogen is fruitful colonizing of urinary tract and origin infection in humans[12]. Besides that, motility of this isolate is very intricate, which is called swarming, which is a principal aspect in the accomplishment of *P. mirabilis* in causing difficult UTI and other more serious bladder and kidney infections. Generally, The urinary tract is a completely sterile place, but bacteria can come from other nearby areas of the body, perhaps cause UT disease. Pathogenic bacteria may stay inaudible in the bladder or can origin irritative symptoms like urinary urgency, 8% of women develop bacteriuria infection with symptoms[23].

The outcomes of this research appear there are a major connection between age and *P. mirabilis* incidence, whereas the occurrence percent was high (80%) in the age group (51-60) year (Table 1). UTI is the greatest communal reason for bacteremia in older adults. Many factors underwrite to improved infection and impermanence in the elderly, counting comorbid conditions, an increase in the number of aggressive events achieved and reduced physiologic investments[24].

Table 1: Age, Gender and Residence distribution in patients infected with *P. mirabilis*

Parameters		Total	*P. mirabilis* positive	Percentage	X^2	P-value
Age	10-20	11	3	27.27%	48.300[a]	0.000
	21-30	10	6	60%		
	31-40	36	20	55.56%		
	41-50	58	35	60.34%		
	51-60	45	36	80%		
Total		160	100	62.5%		
Gender	**Male**	64	39	60.93%	4.840[b]	0.028
	Female	96	61	63.54%		
Total		160	100	62.5%		
Residence	**Rural**	115	79	68.7%	33.640[c]	0.000
	Urban	45	21	46.67%		
Total		160	100	62.5%		

[a]. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 20.0.
[b,c]. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 50.0.

So there is substantial alteration between males and females concerning the occurrence of infection with *P. mirabilis* when record-high percent (63.54%) in female while (60.93%) in males (Table 1). The incidence of urinary tract infection increases with increased sexual activity and age[(25)]. In women, UTI was increased after menopausal due to pelvic prolapse, reduced estrogen, Also, the lack of *Lactobacillus* sp. in the vagina leads to colonization of pathogenic bacteria, as well as some chronic diseases such Diabetes Mellitus (DM)[(26)].

(Table 1) showed that rural residency was extraordinarily infected with *P. mirabilis* (68.69%) associated with urban placement (46.66%) this maybe belong to the aspects which affluence achievement of infection such as insufficient existing resources and unfortunate cleanliness.

About the chemokines serum TNF-α concentration in patients infected with *P. mirabilis* was expressively higher, in contrast, to control group (93.908±0.912, 51.777±0.556) correspondingly, (Table 2). This outcome may be accredited to the tough immune comeback to *P. mirabilis* improved the immune system which was reproduced in an improved blood concentration of TNF-α that had an imperative role in host resistance to *P. mirabilis* infection, but a great level of this cytokine may be reason to pathogenesis of bacteria. TNF-α play important role in host defense against diseases produce by the virulence factors in pathogenic bacteria such as lipopolysaccharide (LPS) and Bacterial Super Antigens (BSA)[(7,27)]. Production of TNF-α is molecularly regulated and several studies have shown that an increased concentration can be a cause of a person's susceptibility to disease[(28)].

Table 2: Distribution of (IL-6, IL-8 and TNF-α) concentration of in infected group and healthy group.

Variables	Groups		t-value	Sig. (2-tailed)
	Patients (n=100) Mean ± S.D.	Healthy (n=100) Mean ± S.D.		
IL-6 pg/ml	312.189±0.916	75.416±0.721	2030.642	0.000**
IL-8 pg/ml	272.417±8.699	77.978±0.932	222.240	0.000**
TNF-α pg/ml	93.908±0.912	51.777±0.556	393.672	0.000**

S.D. = Standard deviation
**A statistical difference is significant at the 0.01 level (2-tailed)

The severity of the diseases is strongly related to the virulence factors in the bacteria, but these However, these elements are individual were not enough to illustrate the various diseases resulting from infection with *P. mirabilis*[12]. The immune status of host play important role in development of illness when *P. mirabilis* contact with host cells lead to stimulus release of such as TNF-α[7]. Also, the amount of IL-6 and IL-8 were expressively high in patients that reach to (312.189±0.916, 272.417±8.699) respectively, associated with uninfected group (75.416±0.721, 77.978±0.932) respectively (Table 2, Figure 2).

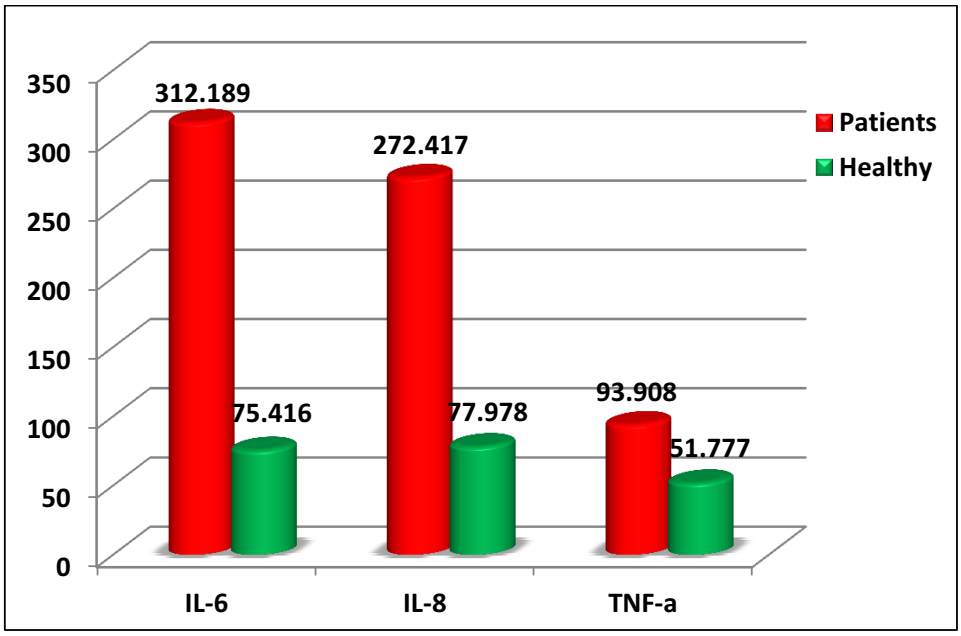

Figure 2: Level of (IL-6, IL-8 and TNF-α) in infected group and healthy group.

Above chemokines are important apparatuses of the inflammatory immune retort to pathogenic bacteria, and moreover, research data offer understanding into the method by

which tissue is damaged by bacteria and these data additional provision evidence that inflammation theaters a important part in *P. mirabilis* pathogenesis[28].

In (Table 3) we shoe there were significant expulsion correlation between IL-6, IL-8 and TNF-α were correlation factor were ranged between 0.998-1.

Table 3: Correlation between (IL-6, IL-8 and TNF-α) in patients.

Parameters	IL-6	IL-8	TNF-α
IL-6	1	0.998**	0.999**
IL-8	0.998**	1	0.998**
TNF-α	0.999**	0.998.**	1

**. Correlation is significant at the 0.01 level (2-tailed).

Both IL-6 and IL-8 are chief tools for starting and intensifying the inflammatory events, some researcher has revealed that IL-1β-511 polymorphisms are related to improved IL-1β making, then connected to expansion of disease and is up controlled the attendance of bacteria and it theaters an significant part in the beginning and magnification of inflammatory retort to infection[29].

When UTI occurs, specific White Blood Cells (WBCs) yield cytokines which chemotactneutrophilis to the damage site and control on host defense against pathogen these cytokines include IL-8, Monocyte Chemoattractant Protein-1 (MCP-1), C-C Motif Chemokine Ligand 5 (CCL5), TNF-α, Gamma interferon (IFN-γ), IFN-β, IL-1β, IL-6, IL-10, and IL-17[10,30]. Nevertheless, these emplacements become lessened with age progresses. alteration in stage leads to alterations in the innate immune response to infection and therefore reason to alterations inability of pathogen to establishment in a specific site in the host[31,32].

CONCLUSION

Our study exposed that target cytokines piece a critical role in UTI, especially in acute phase. Also, there were substantial variances in age group, gender, and residency regarding infected with *P. mirabilis*. and there are important relationships between IL-6, IL-8 and TNF-α in the patients infected with *P. mirabilis*.

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