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Positive Correlation Between Blood Serum levels of Interleukin-8 and Interleukin-1-alpha (IL-1 α) in Pregnant Women with Cystitis in Kirkuk Governorate, Iraq

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ABSTRACT

Cystitis is the most common urinary tract infections especially in pregnant women and can progress to acute nephritis in 50% of cases if not treated, increasing the risk of complications for the mother and fetus. The aim of this study is to isolate the bacterial types of cystitis in pregnant women and to assess an immunological correlation between interleukin 8 and IL-1 α .

This study included 89 samples of pregnant women with cystitis who visited private clinics and hospitals in Kirkuk Governorate for the period from February 2019 to March (2020) their ages ranged between (19-45 years old).

As a control group, 45 samples were collected from healthy pregnant women. The pregnant women were diagnosed in bed, laboratory tests were conducted. Specialized doctors identified the pathological symptoms of cystitis. Several bacterial species were isolated from pregnant women suffering from cystitis. The levels of interleukin-8 and interleukin -1 α were also measured using ELISA technique.

The results of this study showed that the highest isolation rate of 53.9% was found to represent *Escherichia coli*. While *staph sapromphiticus* was found with the lowest rate scoring 3.4%. The results showed a significant increase in interleukin-8 and interleukin -1 α level when compared to the control pregnant group as well as a positive correlation between the two interleukins.

Keywords; Interleukin 8, Interleukin 1 α , Cystitis, Pregnant women, Positive correlation

Introduction

Cystitis is most common site of urinary tract infection (1). Urinary tract infections can be symptomatic or can cause no symptoms, and the infection may cause serious complications if not treated (2). The symptoms that appear in patients with UTI are lower abdominal pain, discomfort when urinating, a feeling that the bladder is not completely empty, and urine appears opaque due to the presence of bacteria, pus and epithelial cells sloughed off from the bladder and these symptoms cause a rise in temperature (fever), shivering, chills and insomnia (3). During pregnancy, many hormonal and physiological changes occur in the reproductive system, causing disturbances in the number and type of natural bacteria to provide appropriate conditions for them and cause Diseases (4). Cystitis is more common in females than in males, accounting for 75% of cases, and it is often not severe and accompanied by some symptoms such as a low fever and burning when urinating (5). Cystitis accounts for about 1-4% of urinary tract infection (UTI) cases in pregnant women (6). Urethritis and cystitis, which are more common during pregnancy, which increases the risk of infection of other parts of the urinary tract, especially in the case of asymptomatic inflammation. This is

common in pregnant women, which progresses to cystitis in 30% of cases and acute nephritis in 50% of the cases if not treated, which increases the risk of complications for both the mother and fetus (7, 8).

Interleukin -8 (IL-8) It is a pro-inflammatory cytokine, a small essential protein belonging to the chemokine gene family. IL-8 is a permanent cytokine produced by mononuclear cells, macrophages, and endothelial cells (8). It rises early in bacterial infections and is known as interleukin-8 by the chemical attraction factor, because it primarily attracts neutrophil white blood cells to the site of infection and after these cells have arrived, it also pushes the phagocytes to the site of infection (9). There are several receptors on membrane surfaces that are able to bind to IL-8, which is an important mediator of the immune response. Important in the body's natural response and in the development of the pathological condition that leads to chronic inflammation (10,11)

Interleukin-1 alpha (IL1 α) also known as hematopoietin 1 is a cytokine of the interleukin 1 family that in humans is encoded by the IL1A gene. In general, Interleukin 1 is responsible for the production of inflammation. Some studies reported

an increase in IL-1 level In UTI patients (12, 13). In our previous serial studies, after examine the UTI induced changes in different cytokines profile, we reported different correlations between interleukins ,by applying an integrated approach to assess these different correlations in order to identify immune response profiles that were significantly different between an uncomplicated UTI and other types of UTI (14-18).

In this study the aim was to isolate the bacterial types of cystitis in pregnant women and for the first time to assess an immunological correlation between interleukin 8 and IL-1 α , in order to depict a complete picture of an interleukin response map in UTI.

Materials and Methods

Urinary and blood samples were collected from pregnant women suffering from cystitis who visited private clinics and private hospitals in Kirkuk Governorate. A total of 89 urine and blood samples were obtained from pregnant women, their ages ranged between 19 to 45 years old, in addition to, the collection of 45 samples saved as a control group. To diagnose bacteria and distinguish its types, a variety of laboratory supplies, tools, and equipment were used.

The method of Forbes, *etal* (19) was followed in the examination and then midstream urine samples were collected using sterile glass bottles. A total of 5 ml of diuresis was collected, and centrifuged at 3000 RPM for 5 minutes. The filtrate was disposed and a drop was taken from the precipitate and examined under a light microscope to investigate the presence of pus cells, bacterial cells, epithelial cells, and globules. Casts, red blood cells, and so on.

Different media were used to culture the samples. The incubation was carried out at 37°C for 18-24 hours. The dishes were examined to observe any bacterial growth. Then the bacterial colonies were diagnosed by studying the phenotypic and biochemical characteristics.

After observing the phenotypic characteristics of bacterial colonies developing on the culture media used, a variety of culture media were used, including `MacConkey, blood agar, nutrient agar Swabs were prepared, dyed with gram dye, and examined under a microscope for microscopic and morphological characteristics. The bacteria isolated from infected samples were also identified using: **Vitek 2 compact system** This device was used to diagnose isolates, as it is used to diagnose most types of bacteria using a device-specific diagnostic kit.

Serum Preparation

Blood samples were collected (5), and ml of venous blood was withdrawn from each sample (Subjects) by slow drawing of venous blood with a fine syringe, the blood sample was placed in a (disposable tube) and left at a temperature The room for (22) minutes for blood clotting, and then it was centrifuged for five minutes at (3000) rpm / minute, after which the blood serum was separated and it was kept until

ELISA tests were performed in order to evaluate interleukins concentrations (20).

Measurement of Interleukins concentration and ELISA technique

As for Immunological assays an enzyme-linked immunosorbent assay (ELISA) technique was used to estimate the levels of cytokines in the serum of the samples under study. Estimation of serum cytokine levels (IL-8). Diagnostic analysis kits were obtained from different origins, and the level of interleukin 1 was measured. The measurement was made according to the instructions attached to the diagnostic kit produced by Assaypro company U.S.A.

Results and Discussion

In this study, 89 blood and urine samples were collected from pregnant women with cystitis, where they were diagnosed clinically by specialized doctors. The majority of the cases had symptoms of lower back and abdominal pain with frequent urge to urinate and urinary retention, in addition to the presence of bacterial growth of more than 10⁴ cells Per 1 ml (21), primarily associated with symptoms. In addition to laboratory diagnosis, 45 samples were collected from healthy pregnant women who were not infected with cystitis. A number of bacterial species were isolated and diagnosed. The results showed different distributional rates of bacteria isolated from pregnant women with cystitis, and they were as follows and as shown in Table 1 . The proportion of *Escherichia coli* isolates reached a high of 48 bacterial isolates, accounting for 53.9% of all UTI isolates . This result agreed with many studies (22) that the emergence of these higher numbers may be due to the fact that these bacteria are present in large numbers in the stool and it is possible that they leave their home in the intestine to move to the urinary system to cause disease (23) Furthermore, these bacteria possess a number of virulence factors, the most important of which are fimbriae in uropathogenic *Escherichia coli*, which allow them to attach to specific receptor molecules in epithelial cells. (24,25).

Moreover, *Klebsella pneumoniae* bacteria found in a total of 20 isolates, recording 23.6% of all UTI isolates. It was found that there are differences in the proportions of this bacteria in many other studies, as in the research conducted by Muften in 2000 and Julia in 2009 and other studies conducted in India (26). The proportional differences in *Klebsella pneumoniae* in UTI pregnant patients may be due to differences in isolation and purification modes or the difference in bacterial carriage by the different populations or an ethnicity effect. The reason for the presence of this bacteria may be due to its presence in the intestine, which increases its chance of transmission to the urinary system, in addition to having virulence factors, such as a capsule that protects it from unsuitable conditions, helping it in resisting unsuitable conditions, the body's immune defenses and phagocytic processes (27)

Table 1: The distribution of uropathogenic bacterial isolates in cystitis-infected pregnant women

type of bacteria	number of isolates	Bacterial rate (percentage) %
<i>Escherichia coli</i>	48	53.9%
<i>Klebseilla pnemoniae</i>	21	23.6%
<i>Pseudomonas aeruginosa</i>	12	13.5%
<i>Proteus mirabilis</i>	9	10.1%
<i>Staph. aureus</i>	7	7.9%
<i>Staph. saprophyticus</i>	3	3.4%
Pregnant women cystitis cases	89	100%

Table 2: Interleukin-8 and interleukin-1 alpha concentrations in pregnant women with cystitis

Studied group	Total number of isolates	Concentration of IL-8 (Mean±SD) pg/ml	Concentration of IL-1α (Mean±SD) pg/ml
Pregnant women cystitis cases	89	96.21 ± 31.44*	62.77± 22.49*
control	45	78.23±9.1	37.65±4.1

Table 3: Interleukin-8 and interleukin-1 alpha concentrations in pregnant women with cystitis and their relationship to bacterial species

type of bacteria	number of isolates	Concentration of IL-8 (Mean±SD) pg/ml	Concentration of IL-1α (Mean±SD) pg/ml
<i>Escherichia coli</i>	48	123.76 ± 6.4**	83.61 ± 9.3**
<i>Klebseilla pnemoniae</i>	21	117.21±4.1**	77.51±4.9**
<i>Pseudomonas aeruginosa</i>	11	101.68±3.1*	61.12±2.5*
<i>Proteus mirabilis</i>	9	73.66±2.1 ns	31.11±3.2 ns
<i>Staph. aureus</i>	7	86.91±3.3 ns	47.07±1.5 ns
<i>Staph. saprophyticus</i>	3	61.34±0.9 ns	36.19±1.1 ns
Pregnant women cystitis cases	89		
control	45	78.23±9.1	37.65±4.1

**** Highly significant $p < 0,01$**

***Significant $p < 0.05$**

ns : Non significant $p > 0.05$

Pseudomonas aeruginosa came in the third place recording 13.5% of all isolated bacteria. It is classified as an opportunistic pathogen because of its resistance to many antibiotics and antiseptics (28). Its pathogenesis is complex and multifactorial, including invasion, exotoxin A, lipase, and protease. Followed by *Proteus mirabilis*, *Staph. Aureus* and *Staph saprophyticus* recording 10.1%, 7.9% and 3.4% respectively.

Proteus mirabilis bacteria which causes urinary tract infection, causes 90% of infections (29,30) scored 10.1 % of the distributed bacterial cause of systitis in pregnant women.. This bacteria can produce the enzyme urease which hydrolyze urea to ammonia, raises the alkalinity of urine, increase the incidence of kidney stones and reduces the biological activity of antibodies (31).

Staph. sp As one of the causes of urinary tract infections, it can be attributed to a variety of factors, including the existence of an epidemiological situation as a result of the deterioration of the health situation and the difficult living and environmental conditions that society is currently experiencing,, as the lack of hygiene and the deterioration of defense mechanisms are encouraging factors for the growth of some species more than others.

Differences in bacterial isolation percentages between studies may be due to the size of the sample, the methods used in isolation and diagnosis, the nature of

the country, and the living patients and environmental conditions of the patients.

In this study, both the level of interleukin-8 and interleukin-1 alpha were measured in women with cystitis. The results showed a significant difference in the level of both interleukins. The results of the present study showed that there were statistically significant increase in IL-8 and IL-1 α ($p < 0,01$) ($P < 0.05$) in the cystitis affected women when compared with control group and as shown in Table. 2. In addition, interleukin-8 and interleukin-1-alpha were measured according to the bacterial groups. The results of our study recorded that there was a significant difference that there were statistically significant differences, where the results recorded a significant increase ($P < 0.05$). (In some isolates but not others, as shown in Table N. (3). The highest level of IL-8 ($123,76 \pm 6.4^{**}$) was found in *E. coli*-induced cystitis, while the lowest level was found in *Staph. Saprophyticus* infection. Furthermore, the same bacteria showed significant increases and decreases in IL-1 α levels which was also remarkable ($83,61 \pm 9.3^{**}$ pg/ml)($36,19 \pm 1.1$ pg/ml) respectively Elevated IL-8 levels is are considered normal in infected patients because it is the cellular kinetic that initiates inflammation. The elevation of IL-8 in pregnant women with urinary tract infection may be due to the fact that IL-8 which is a chemokine , characterized by chemical attraction to the neutral

white blood cells present in the region of Inflammation represented by purulent cells, plays a key role in the final differentiation of B cells and stimulates them to produce immunoglobulin antibodies (32). Most of the *E.coli* strains present clinical symptoms, and they are represented by the serotypes H, K, and O. This bacterium's virulence factors include somatic, flagellar and envelope antigens, and they also possess two types of endotoxins in addition to (Fimbriae) which plays a role in their attachment to urinary epithelial cells, especially in *E.coli*, (24) and these cilia attach to special receptors found on epithelial cells and as a result interleukin-8 factors are released.

The elevation of IL-8 in pregnant women depends on the type of bacteria, because some bacteria that cause inflammation have virulence factors sufficient to cause infection, for example *E.coli* has many virulence factors, the most important of which is the production of hemolysin. It is a toxin that can cause holes in host cells and release iron and hemoglobin. Red blood in the microscopic examination of urine as well as other components necessary for bacterial growth. Hemolysin produced from *E.coli* degrades lymphocytes, inhibits phagocytosis and encourages chemotaxis of white blood cells, especially neutrophils (33).

As for interleukin-1, a study conducted by Ede *et al.* (2010) confirmed that interleukin-1 is one of the important mediators in immunity and in inflammatory processes by mediating inflammation and the activation of neutrophils and macrophages, as well as stimulating the growth and differentiation of T-cell, B-cell cells (34).

The study has shown a moderate positive correlation ($r: 0.6$) of IL-8 with IL-1 α (Figure 1) in different

inflammatory processes of different uncomplicated UTI. To the best of our knowledge, this is the first study using a statistically integrated approach to assess an immunological correlation between interleukin 8 and IL-1 α , in order to depict a complete picture of an interleukin response map in UTI..

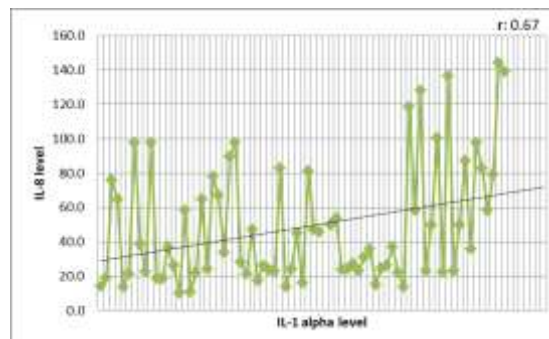


Fig. 1: Correlation between IL-8 and IL-1 α in different inflammatory processes of different uncomplicated UTI $r: 0.67$ ($0.4 < |r| < 0.7$) statistically moderate positive correlation.

Conclusions

The results showed a significant increase in the level of interleukin-8 and IL-1 α compared to the control pregnant group as well as a positive correlation between the two interleukins. Understanding the disease's physiology and having a thorough understanding of the possibilities that inflammation might produce opens up some therapeutic options. As a result, the current study stresses the significance of assessing biological indicators of inflammation in determining the degree of inflammation and determining the best treatment strategy to achieve better outcomes.

References

- 1-Vandepitte J. ;Verhaegen, J. ; Engbaek, K. ; Rohner, P. ; Piot, P. and Heuck, C. C. (2003). Basic laboratory Procedures in Clinical bacteriology. 2nd ed. WHO. Geneva
- 2-Mukherjee M. Koley S. Mukherjee S. Basu S. Ghosh B. Chakraborty S. (2015). Phylogenetic background of *E. coli* isolated from asymptomatic pregnant women from Kolkata, India. *J Infect Dev Ctries*; 9(7):720-724.
- 3-Chamberlain, R. N. (2009). The big picture Medical Microbiology. The McGraw-Hill Companies.
- 4- Richard, M.H. (1995) "Microbiology & Immunology". 4th ed. Heidelberg press, New Pharm. Res., 4(7), 2361.
- 5-Winn, W.C. et al. (2006). Koneman's color atlas and textbook of diagnostic Microbiology. 6th ed. Wolters Kluwer/Lippincott. Williams and Wilkins. Philadelphia, USA. PP: 82-87
- 6-Vazquez JC, Villar J. (2003) Treatments for symptomatic urinary tract infections during pregnancy. *Cochrane Database Syst Rev* 4:CD002256. p. 1-55

- 7- Haider G, Zehra N, Munir AA, Haider A. Risk factors of urinary tract infection in pregnancy. *J Pak Med Assoc.* 2010 Mar;60(3):213-6. PMID: 20225781.
- 8-El-Kashif M. M. L. Urinary Tract Infection among Pregnant Women and its Associated Risk Factors: A Cross-Sectional Study. *Biomed Pharmacol J* 2019;12(4).
- 9-Modi W, Dean M, Seuanez H, Mukaida N, Matsushima K, O'Brien S (1990). "Monocyte-derived neutrophil chemotactic factor (MDNCF/IL-8) resides in a gene cluster along with several other members of the platelet factor 4 gene superfamily". *Hum. Genet.* 84 (2): 185-7
- 10-Brown A, Hooton J, Schaefer C, Zhang H, Petkova E, Babulas V et al. (2004). "Elevated maternal interleukin-8 levels and risk of schizophrenia in adult offspring". *Am J Psychiatry* 161 (5): 889-95.
- 11-Frasca L, Nasso M, and Spenseri F (2008) :INF- γ arms human dendritic cells to perform multiple

- effectors function J of Immunology,180(3) :1471-1481.
- 12- Glen C. Ulett, Richard I. Webb, Kimberly B. *et al*, Group B *Streptococcus* (GBS) Urinary Tract Infection Involves Binding of GBS to Bladder Uroepithelium and Potent but GBS-Specific Induction of Interleukin 1 α , *The Journal of Infectious Diseases*, Volume 201, Issue 6, 15 March 2010, Pages 866–870, <https://doi.org/10.1086/650696>
- 13-Reyes, L., Reinhard, M. & Brown, M.B. Different inflammatory responses are associated with *Ureaplasma parvum*-induced UTI and urolith formation. *BMC Infect Dis* 9, 9 (2009). <https://doi.org/10.1186/1471-2334-9-9>
- 14-Chateen I Ali, Sheelan A Anwar, Sinan B Issa. Study of UTI among pregnant women in Kirkuk province . *Tikrit Medical Journal*.2007;13(2):39-43.
- 15-Nidaa W, Chateen I. Ali, Sabah M S. Isolation and Identification of bacteria causing urinary tract infections in children in Kirkuk city. *Tikrit Journal of Pure Sceince*.2017; 22(2): 35-44 (Feb. 2017)
- 16-Chateen I. Ali Pambuk, Sabah M. Salihand Fatma Mustafa Mohammed, Emergence of Metallo- b-Lactamase producing Isolates of *Pseudomonas aeruogenosa* Urinary Tract Infection in Children, *J Pure Appl Microbiol.*, 2019; 13(4):2309-2316. <https://doi.org/10.22207/JPAM.13.4.46>
- 17- Hameed, N. W. S., Salih, S. M. & Pambuk, C. I. A. (2022). Evaluation of interleukin-6 level in the sera of children with UTI in Kirkuk city, Iraq. *Journal of Current Researches on Health Sector*, 12 (1), 63-74.
- 18- Baneen Ali Asker, Chatin Iz-Alden Ali. Correlation between IL-1 β , IL-18 and some hematological variable in uropathogenic *E. coli* infected UTI patients. *Teikyo medical Journal* (2022) ; 45 (01), 4695-
- 19 -Forbes, B.A.; Sahm, D.F. and Weissfeld, A.S. (2007). *Baily and Scott's Diagnostic Microbiology*. 12th ed. Mosby (Elsevier). USA, P: 171-178.
- 20- Lewis, S.M. and Bain, B.J. (2001). *Dacie and Lewis, Practical Haematology*. 9th ed. Churchill Livigstone., pp. 447-477
- 21-Vazquez JC, Villar J.(2003) Treatments for symptomatic urinary tract infections during pregnancy.*Cochrane Database Syst Rev* ;4:CD002256. p. 1–55
- 22-Schaeffer, A. J.; Foxman, B. and Tracy, R.N.(2007). What I need to know about Urinary Tract Infections, National Institutes of Health and National Kidney and Urologic Diseases Information Clearinghouse Health and Human Services. U.S.A.,:07–4807.
- 23- Foster, R.T.Sr. (2008). Uncomplicated urinary tract infections in women. *Obstet. Gynecol. Clin. North Am.*, 35(2): 235-248
- 24-Braunwald, F.; Fauci, A.; Kasper, E.; Hauser, C.; Longo, W. and Jameson, B. (2001). *Harrison's Principles of Internal Medicine*, 15th edition , London., pp: 1620-1626.
- 25-Janda, J.M. Abbott, S.L. (2006). *The enterobacteria*. 2nd ed. ASM Press, USA, P:181-188.
- 26- Brooks, G.F.;Carroll, K.C.; Butel, J.S.; Morse, S.A. (2007). *Jawetz, Melnick ,and Adelbergs. Medical microbiology*.24thed. McGraw-Hill. USA. PP:88-94.
- 27-Heghe, M.; Ethel, S. and Bhat, G.K. (2006). Bacterial Adherence and Hummoral response in women with symptomatic urinary tract infection. *Indian J. Microbiol.*, 24(1): 30-33.
- 28-Pceditor, C. (2008). *Pseudomonas: Genomics and molecular biology*. 1st ed. Caister Academic Press.
- 29-Aronoff, S.C.; Quinn, J. and Stern, C. (1997). Longitudinal serum IgG response to *Pseudomonas cepacia* surface antigens in cystic fibrosis. *Pediatric . Pulmonal.*, 11: 269-293.
- 30-Swiezko, A.S.; Kirikae, T. and Kinke, E. (2000). Biological activities of Lipopolysaccharide of proteus and their interaction with polyxyin B and an 18-kda Cationic Antimicrobial Protein (CAP 18) Derived peptide. *J. Med. Microbiol.*, 49: 127-138.
- 31-Lawrenson, R.A. and Logie, J.W. (2001). Antibiotic failure in the treatment of urinary tract infections in young women. *J. Antimicrob. Chemother.*, 48(6): 895-901.
- 32-Scarborough DE ,(1990). Cytokine modulation of pituitary hormones secretion, *Ann NY Acad sci* 594:169-18
- 33- Raksha, H. ; Srinivasa, H. and Macaden, R.S. (2003). Occurrence & characterization ofuropathogenic *E.Coli* in UTI, *Indian J. Med. Microbiol.*, 21(2):102-107
- 34-Ede H, Burnette B, Shimada H, Hope R, and Monahan J (2010): Interleukin-1 induced interleukine-6 production in A549 cells is mediated by both phosphatidylinositol 3- kinase and IL-1r associated kinase -4 . *Cell biology Int. Immed. Publi.* ;CB120100247.