

Measurement of Interleukin-18 Receptor 1 and Interleukin-10 Levels as Biomarkers in Pregnant Women with Urinary Tract Infections

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ABSTRACT

This study examined pregnancy-related urinary tract infections (UTIs) and their bacteria. This study also examined how urinary tract infections affect blood and immunological indicators like IL-10 and IL-18R1. Patients and Methods: -This study involved the collection of 44 blood and urine samples from pregnant women between the ages of 18 and 41 who had urinary tract infections. The samples were obtained from a children's hospital in Al Samawa city. Additionally, 44 samples were taken from healthy nonpregnant women without urinary tract infections or chronic diseases. These samples were used as a control group, and the women were between the ages of 18 and 41. The urine specimens were analyzed and cultured and then identified by different media, biochemical tests and vitek2, along with laboratory blood tests, to identify blood variables and measure certain immune variables. Enzyme-linked immune sorbent assay (ELISA) was used to quantify the levels of IL-10 and IL-18R1. Result: -all urine samples taken from healthy nonpregnant women showed no bacterial growth. Our investigation included certain strains of bacteria, such as *Escherichia coli* (16), *Pseudomonas aeruginosa* (6), *Klebsiella pneumoniae* (2), *Staphylococcus aureus* (10), and *Enterobacter faecalis* (10). IL-10 levels were greatest in pregnant women infected with *Escherichia coli* and *Klebsiella pneumoniae*, followed by pregnant women infected with *Pseudomonas aeruginosa*, and lowest in nonpregnant women. IL-18R1 levels were greatest in pregnant women infected with *Klebsiella pneumoniae*, followed by those infected with *Staphylococcus aureus* and *Escherichia coli*, and lowest in pregnant women infected with *Enterobacter faecalis*. Conclusion: -This research revealed that pregnant women with *K. pneumoniae*, *P. aeruginosa*, and *E. faecalis* had increased IL-10 and IL-18R1 levels, while nonpregnant women had decreased IL-10 and IL-18R1 levels. These differences were statistically significant.

Keywords: Gram-Negative; Gram-Positive; IL10; IL18 Receptor 1

INTRODUCTION

Urinary tract infections (UTIs) are highly prevalent globally. Urinary tract infections (UTIs) are linked to a reduction in the general health of patients and impose a substantial impact on both medical treatment and financial resources [1]. In both symptomatic and asymptomatic bacteriuria patients, *Escherichia coli* is the major pathogen associated with urine bacteria. The presence of asymptomatic bacteriuria that is not treated has the potential to cause acute pyelonephritis in as many as 30% of mothers. This elevates the likelihood of encountering diverse complications for both the mother and the infant, such as hypertension, premature birth, restricted growth within the uterus, and low birth weight [2]. Urinary tract infections (UTIs) commonly complicate pregnancy and are present in approximately 10% of pregnancies. Asymptomatic bacteriuria (ASB) is most common. Cystitis and pyelonephritis are symptomatic infections that complicate pregnancy less frequently [3]. Urinary tract infection (UTI) is a prevalent condition caused by bacterial agents during pregnancy. If not properly diagnosed and treated, it can result in significant difficulties for newborns born to mothers with UTIs [4]. The bacteria that cause *Escherichia coli* are members of the family Enterobacteriaceae, the most harmful agent that can cause this infection during pregnancy [5]. Urinary tract infection can manifest with symptoms or without symptoms and is known as symptomatic or asymptomatic. An asymptomatic urinary tract infection refers to a patient with significant bacteria in their urine ($\geq 10^5$ colony-forming units/millilitre) and symptoms associated with a urinary tract infection. On the other hand, an asymptomatic urinary tract infection is a condition in which bacteria are present in the urine in a significant

abundance ($\geq 10^5$ colony-forming units /millilitre). Nevertheless, the patient does not exhibit any symptoms of a urinary tract infection [6]. Compared with male sex, female sex is a significant risk factor for the development of urinary tract infections (UTIs) and antibiotic-resistant bacteria (ABs), likely due to anatomical factors such as the proximity of the external urethral opening to the anus and the shorter length of the urethra. Additional risk factors linked to recurrent urinary tract infections (UTIs) in women include the use of spermicides, frequent sexual intercourse, a family history of UTIs, decreased estrogen levels, increased postvoid residual urination, urinary incontinence, and pelvic organ prolapse [7]. Three categories can be used to classify urinary tract infections (UTIs) during pregnancy. These categories are asymptomatic bacteriuria (ASB), illnesses of the lower urinary tract (cystitis), and diseases of the upper urinary tract (pyelonephritis). Lower tract bacteriuria, also known as asymptomatic bacteriuria (ASB) or cystitis, is linked to an increased risk of developing pyelonephritis from twenty to thirty percent during pregnancy. It is quite probable that this is because of the hormonal changes that occur in the urinary tract during pregnancy [8]. Numerous elements found in microorganisms function as ligands for pattern recognition receptors (PRRs). As the innate immune system's initial line of defense against invasive infections, pattern recognition receptors are a crucial component. Pathogen-associated molecular patterns (PAMPs) originating from microorganisms or host-derived DAMPs trigger signaling cascades, which in turn increase the expression of interferons, chemokines, and cytokines [9]. Each TLR targets a particular microbial substance and initiates a specific signaling

pathway that triggers a unique immune response. Several studies have shown that the administration of lipopolysaccharide (LPS), a ligand unique to TLR4, stimulates the production of prostaglandins and proinflammatory cytokines in gestational tissues, which in turn causes preterm labor [10]. LPS extraction increased the body temperature and immune parameters of rabbits, and the primary immune response and secondary immune response were associated with decreased C. reactive protein levels [11]. Pregnancy is an inherent physiological process in the female body that involves a series of immunobiological reactions. The woman's immune system is essential during pregnancy since it protects her body from dangerous bacteria and allows for the reception of genetically diverse sperm without causing an immune response. The placental tissue releases cytokines and hormones essential for regulating communication between the fetus and the mother. Recent research suggests that the production of cytokines by type 2 T helper (Th2) cells is more common during pregnancy than the production of cytokines by type 1 T helper (Th1) cells. The prevalence of Th2 cell cytokines plays a role in maintaining pregnancy. Th2 cells secrete interleukins 4, 5, 6, 10, and 13, facilitating humoral immunity [12]. Interleukin-18 (IL-18) is a cytokine that stimulates inflammation and is known for its ability to induce the production of interferon-gamma (IFN- γ).

The IL-18 receptor, referred to as IL-18R, consists of two subunits that belong to the IL-1R family. The alpha chain, also known as IL-18R α or IL18R1, has a weak attraction to IL-18. When IL-18 attaches to IL-18 R α , it causes the recruitment of the IL-18 receptor accessory protein (IL18RAP, also known as IL-18R β) to create a heterotrimeric complex. This complex has a high affinity for binding [13].

Methods and Materials

This study also examined how urinary tract infections affect blood and immunological indicators like IL-10 and IL-18R1. The samples

were obtained from a children's hospital and a specialty health clinic in Al Samawa City between September 2023 and March 2024. This study included the obtaining of 44 blood and urine samples from pregnant women aged 18 to 41 who experienced urinary tract infections across all three trimesters. In addition, a total of 44 samples were collected from nonpregnant women who were in good health and did not have urinary tract infections or chronic illnesses. The aforementioned samples were employed as a control group, consisting of females aged 18 to 41. The urine samples were examined using a dipstick and analyses under a microscope to observe sediment. In cases where the presence of pus cells indicates a bacterial infection, urine may be cultured on different media to identify the bacteria. The bacteria can then be diagnosed using the Vitek2 system. Additionally, the levels of IL10 and IL18R1 in the serum were measured using an enzyme-linked immunosorbent assay (ELISA).

Statistical Analysis

The statistical analysis of the data from the current study was performed using SPSS (Statistical Package for the Social Sciences version 26). The analyses included one-way ANOVA, least significant difference (LSD) tests, independent sample t tests, with the significance level set at $p < 0.05$.

Results

The present investigation categorized urinary tract infections in pregnant women based on the bacterial species identified in urine cultures. The study reported many types of bacteria, with *Escherichia coli* being the most prevalent species in 16 (36.3%) patients, followed by *Staphylococcus aureus* in 10 (22.8%) patients and *Enterobacter faecalis* in 10 (14.28%) patients. Six (13.6%) of these strains were *Pseudomonas aeruginosa*. The least reported species were *Klebsiella pneumoniae*, which was detected in 2 (4.5%) patients. These results are shown in Table 1.

Table 1: The Present Study Identified the Types of Bacteria Isolated from Pregnant Women Compared to those Isolated from Nonpregnant Individuals.

Isolation	NO.	Percentage
Escherichia Coli	16	36.3
Staphylococcus Aureus	10	22.8
Pseudomonas Aeruginosa	6	13.6
Klebsiella Pneumoniae	2	4.5
Enterobacter Faecalis	10	22.8
Total	44	100

The mean serum IL-10 concentration was 55.5 ± 12.9 in the pregnant with UTIs group and 16.9 ± 5.49 in the nonpregnant control group.

The average serum IL-10 level was substantially greater in the pregnant group than in the control group ($P < 0.001$), as indicated in Table 2.

Table 2: Evaluation of the Immune Parameter Interleukin-10 in Pregnant Women with Utis and Nonpregnant Women

Immune Parameter	Pregnant with UTI No. 44	Nonpregnant No. 44	P. Value
	Mean $\pm$ S. D		
IL10	55.5 ± 12.9	16.9 ± 5.49	$< 0.001^{**}$

The mean serum IL-18R1 level was 24.5 ± 7.82 in pregnant women with urinary tract infections (UTIs), while it was 10.5 ± 3.64 in the control group of nonpregnant women.

The average serum IL-18R1 level was considerably greater in the pregnant group than in the control group ($P < 0.001$), as indicated in Table 3.

Table 3: Evaluation of the Immune Parameter Interleukin-18R1 in Pregnant Women with Utis and Nonpregnant Women

Immune Parameter	Pregnant with UTI No. 44	Nonpregnant No. 44	P. Value
	Mean $\pm$ S. D		
IL18R1	24.5 ± 7.82	10.5 ± 3.64	$< 0.001^{**}$

The present study revealed that pregnant women infected with *E. coli* and *K. pneumoniae* had the highest level of IL-10, followed by pregnant women infected with *P. aeruginosa*, while the lowest level of

IL-10 was detected in nonpregnant women, with a p value < 0.05 , as shown in figure (1).

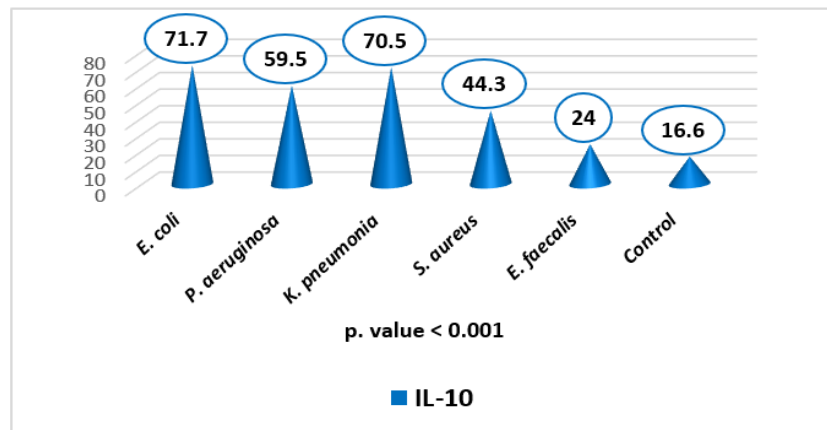


Figure 1: IL-10 Concentrations in Nonpregnant Women and Pregnant Women with Utis Caused by Different Bacteria.

The present study revealed that pregnant women infected with *K. pneumoniae* had the highest level of IL-18R1, followed by pregnant women infected with *S. aureus* and *E. coli*, while pregnant women

infected with *E. faecalis* had the lowest level of IL-18R1, with a p value < 0.05, as shown in figure (2).

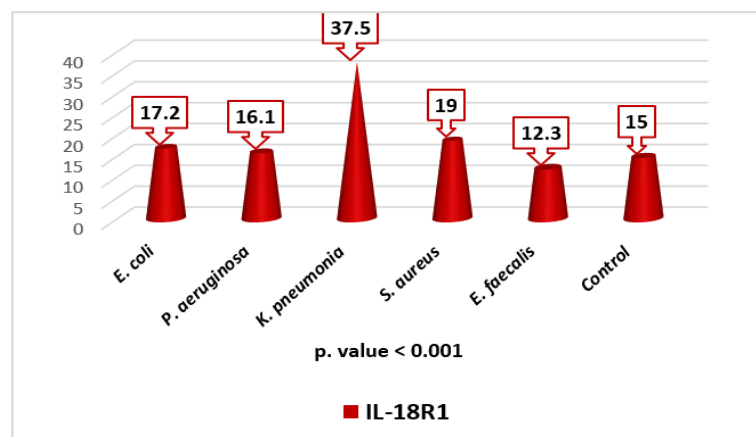


Figure 2: IL-18R1 Concentrations in Nonpregnant Women and Pregnant Women with Utis Attributable to Various Bacterial Species

Discussion

A similar conclusion was reached in a prior study carried out in Samarra city in 2020; the most often detected bacterial species in urine samples from pregnant women was *E. coli* 38 (54.28%). differing counts of bacteria and species among the reported samples, with Klebsiella spp. accounting for 16 (22.85%) patients and Pseudomonas spp. accounting for 10 (14.28%) patients. Proteus spp. were identified in 6 (8.57%) patients, which is the lowest reported species [14]. The results of previous studies agreed with our results for isolated bacteria. The variations in these ratios are caused by the quantity of samples collected throughout the study period of certain bacterial species. These differences may also be caused by variations in the studied geographic area. It is essential to investigate the potential role of IL-10 in pregnant women, given that immune responses may influence the prevalence and pathology of UTIs. Therefore, comparable but larger single-sex patient studies are required to investigate whether sex influences the cytokine/ASB levels of IL-1 β , IL-5, IL-8, and IL-10 [15]. Unlike the study conducted in Diyala City 2021, which collected samples from 45 pregnant women who appeared healthy, this study collected samples from 15 pregnant women who were infected at each of the three phases of pregnancy. Significantly lower levels of IL-10 (50.1296 ± 4.8345 pg/ml) were detected in pregnant women with UTIs ($p = 0.05$) than in the control group of healthy pregnant

women (55.6142 ± 12.7402 pg/ml) [16]. This study's specimen population comprised both pregnant women with UTIs and nonpregnant women, while the study's Diyala city population group included both types of pregnant women. Pregnancy induces an increase in the levels of anti-inflammatory cytokines, including IL-4 and IL-10 [17]. The innate immune response and adaptive immunological response are both stimulated by interleukin-18. IL-12 plays a crucial role in facilitating the production of interferon-gamma (IFN- γ) by many cells, including T helper 1 (Th1) cells, macrophages, natural killer (NK) cells, natural killer T (NKT) cells, B cells, dendritic cells (DCs), and nonpolarized T cells from the immune system. In the absence of IL-12, the presence of IL-18 and IL-2 increases the production of type 2 T helper (Th2) cytokines by natural killer (NK) cells, nonkiller T (NKT) cells with a CD4+ phenotype, and even Th1 cells. In addition, the combination of IL-18 and IL-3 increases the production of IL-4 and IL-13 by mast cells and basophils [18]. The protein IL-1 receptor-related protein, often referred to as IL-18R α , is a molecule that specifically attaches to IL-18 and is located on the cell's external surface. However, the degree of connection is superficial. The recruitment of IL-18R β , also called accessory protein-like [AcPL], is necessary for activating the intracellular signaling pathway and forming a high-affinity binding site [19]. An identical conclusion was drawn in an earlier study conducted in 2012. Increased IL-10 production appears to indicate that it is involved in the body's natural defense mechanism against UTIs [20]. To facilitate contact with

leukocytes, gram-negative bacteria attach themselves to endothelial cells that express Toll-like receptor 4 (TLR-4) [21]. On the other hand, gram-positive (G+) bacteria are host cells that express Toll-like receptor 2 (TLR-2) via lipoteichoic acid. These cells then produce pneumolysin, which causes significant endothelium damage [22]. Thus, these phasic Th1/Th2-driven phases of inflammation and tissue regeneration may substantially contribute to the chronicity of infection. However, the antagonistic functions of Th1/Th2 are well defined in rodents, whereas their effects on humans are less noticeable. Additionally, IL-10 is produced by a diverse array of immunological and nonimmune cells. The present study revealed that pregnant women infected with *E. coli* and *K. pneumoniae* had higher levels of IL-10 than pregnant women infected with *P. aeruginosa*. These findings are consistent with those of a prior study that examined the levels of the anti-inflammatory cytokine IL-10 in the blood samples of individuals with gram-negative urinary tract infections [23]. Because of its strong anti-inflammatory properties, IL-10 is thought to play a crucial role in maintaining pregnancy by inhibiting activated monocytes from producing proinflammatory cytokines [24]. When monocytes and macrophages were first found to release IFN γ -inducing factors, IL-18 was initially thought to be mainly produced by these and other myeloid cells [25]. On the other hand, in recent times, it has been demonstrated that the epithelial lining of various organs, such as the lungs, colon, and oral cavity, generates a considerable quantity of IL-18 in response to infection and injury [26]. The tubular epithelium in the kidney is an important area for the synthesis of IL-18 in individuals who have chronic kidney illness or who have undergone renal transplantation. This is also the case in models of bacterial lipopolysaccharide challenge, obstructive kidney injury, and lupus nephritis [27]. IL-18 attaches to IL-18R α , a protein that was initially identified as related to IL-1R due to its resemblance to the IL-1/Toll receptor family. Signal transduction mediated by the IL-18R

complex is carried out by the IL-18R β subunit, another member of the IL-1R family [28]. Interleukins play important roles in other diseases in addition to microbes [28, 29], especially in the kidney [30]. Different changes occur during pregnancy, which may also play a role in the results [31]. In addition, IL18 plays an important role in lung disease. In cancer, the regulation of interleukins is also very important [32].

Conclusion

IL-10 and IL-18R1 were highly expressed in pregnant women who contracted UTIs compared with nonpregnant controls, especially in those infected with *K. pneumoniae*. We express our most profound appreciation to the Muthanna Health Department in Iraq for providing the necessary facilities, which were crucial for the effective completion of this project.

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The data available are supportive, and the data and materials are available.

Declarations

Conflict of Interest

There are no conflicts of interest.

Human and Animal Rights

The authors assert that the current study seeks to obtain consent from human subjects. The authors further argue that this publication is original work that has yet to be previously presented in any form or language.

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