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MICROBIAL INFECTION AND IL-6 URINE LEVELS FOR PREGNANT WOMEN IN THI-QAR PROVINCE

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ABSTRACT

Urinary tract infection is a common health problem, affecting millions of women especially pregnant women each year. The present study aimed to shed light on microbial infection and IL-6 urine levels proinflammotory cytokine in pregnant women. Urine samples from 70 pregnant women and 20 urine samples from non- pregnant women with urinary tract infection as a (control) were included in this study**.** Urinary tract infections were studied for all groups by culturing urine samples using specific culture media. The incidence ratio of positive urine culture in pregnant women was 52 (74.3 %) while 18(25.7 %) was the incidence of negative urine culture. The frequency of the different microorganisms causing UTI in patient groups were studied, in which Gram-negative, particularly *Escherichia coli* showed the highest incidence among them (28.9%) . Also, the age group 20-29 years had the highest incidence of infection (82.8 %), and the urine levels of IL-6 were estimated by ELISA , the results showed significant different in IL-6 mean levels between trimester pregnancy groups and control group ($p \leq 0.01$). Results indicated microbial infection was high in pregnant women of Thi-qar province, so urine microbial screening should be done in routine checkups for pregnant women.

KEYWORDS: UTI, IL-6, pregnant women.

INTRODUCTION

Urinary tract infections (UTI), which are caused by the presence and growth of microorganisms in the urinary.[1] Women tend to have UTIs more often than men because bacteria can reach the bladder more easily in women. This is partially due to the short and

wider female urethra and its proximity to anus. Rectum bacteria can easily enter the urethra and cause infections.[2],[3],[4]

In pregnancy, UTI may involve the lower urinary tract or the bladder.[5] Symptomatic and asymptomatic urinary tract infection is a common phenomenon in pregnancy , pregnancy and sexual intercourse are the main factors predisposing to bacteriuria in married women.[6] Sexual activity increases the chances of bacterial contamination of female urethra. Having intercourse may also cause UTIs in women because bacteria can be pushed into the urethra .

This anatomical relationship of the female urethra to the vagina makes it liable to trauma during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during pregnancy/child birth.[4],[7] Using a diaphragm can also lead to UTIs because diaphragms push against the urethra and make it more difficult to completely empty the bladder.

The urine that stays in the bladder is more likely to allow growth of bacteria and cause infections.[3],[8]

Although the majority of infections in pregnancy are asymptomatic, the mother is placed at high risk for low birth weight, hypertension, maternal anemia, thrombosis, stillbirth and abortion.[9],[10] So pregnant women should be screened for asymptomatic bacteriuria in the first trimester and treated, if positive.

The hormonal effects in ureteric vasculature in pregnancy aided, perhaps, by mechanical pressure from the gravid uterus leads to urinary stasis and therefore, encourage bacterial growth in urine.[11] Reduced immune reactions that occur during pregnancy also contribute to increased incidence of urinary tract infection in pregnancy.[10] **C**ytokines, as critical immunoregulatory molecules, responsible for determining the nature of an immune response, have been shown to influence all steps of reproduction, playing a fundamental role in pregnancy outcome. During UTI, cytokines are released into the urine or into the systemic circulation. A considerable proportion of women with bacteriuria have increased urinary interleukin (IL)-6 levels, but serum IL-6 levels are only elevated in women with acute pyelonephritis. Elderly women and men with bacteriuria have also increased urinary IL-1α and IL-6.[12]

So, this study aims to evaluate the prevalence of urinary tract infection and the etiologic agents amongst pregnant women in Thi-Qar province, and estimated the urine levels of IL-6 in pregnant and non-pregnant women with UTI.

PATIENTS AND METHODS

In this study mid stream urine samples were collected from (70) pregnant women their mean age 29.3±4.8 range (20-42) attended to AL-Huda teaching hospital during the period from September 2014 to February 2015, 31 of them without any signs or symptoms of urinary tract infection , and 20 non-pregnant women had symptoms of urinary tract infection between the ages of 19 to 35 years as a control .The collected mid-stream specimens were transported to the laboratory within 30 minutes of the collection . After culture on a selective media The urine samples were mixed and aliquots centrifuged at 3000 rpm for 10 min. The supernatant was frozen at -20ºC until use to measure the urine level of IL-6 [13], and the deposits were used for general urine examination using both x10 and x40 objectives. A drop of the urine samples were applied to a glass microscope slides, allowed to air dry, stained with gram stain, and examined microscopically.[4],[14]

Culture plates with bacterial counts greater than or equal to 10^5 CFU/ml were taken as positive, thus indicative of UTI , motility test and classical biochemical tests according to Cheesbrough (2004).[15] The identification confirmed by using API 20 microtubes system (BioMerieux Company).

Interleukine-6 Determination

The urine level of IL-6 was quantitatively determined in urine of UTI pregnant women patients and UTI non-pregnant women as a control subjects by mean of ELISA (Enzyme Linked Immunosorbent Assay) using ready kit for human IL-6 manufactured by USBiological company (USA). The urine samples were obtained by informed consent of the women used for this study and the permission to that effect was obtained from the ethical committee of the hospitals.

RESULTS AND DISCUSSION

General urine microscopy of pregnant women revealed pus cells in 15 (21.4%) of the urine samples collected while yeast cells was only found in 3 (4.3%) of the samples , also two of sample have red blood cells , and some of it contain type of casts , crystals , also appeared the presence of bacteria. Of the 70 urine samples of pregnant examined in this study,

52(74.3 %) were found to contain heavy and appreciable bacterial growth (significant bacteriuria), while 18 (25.7 %) had no appreciable bacterial growth (Table -1), 13 patients (18.6%) were identified to have asymptomatic bacteriuria. This high incidence of microbial infection reported in this study may be attributed to many factors such as poor housing, poor drainage systems, lack of proper personal and environmental hygiene, genuine population susceptibility since it is that factors such as low socio-economic status.[4] The physiological increase in plasma volume during pregnancy decreases urine concentration and up to 70% pregnant women develop glucosuria, which encourages bacterial growth in the urine.

Asymptomatic bacteriuria is a condition characterized by bacteriuria without classical symptoms attributable to the urinary tract. Women with asymptomatic bacteriuria during pregnancy are more likely to deliver premature or low-birth-weight infants and have a 20- to 30-fold increased risk of developing pyelonephritis during pregnancy compared with women without bacteriuria. Frequency of asymptomatic urinary tract infection in pregnant women in this study higher than reported by similar studies, Turpin *et al* found the incidence of asymptomatic urinary tract infection in pregnant women was 7.3%[16], while Tadesse *et al* found the prevalence of asymptomatic bacteriuria in pregnant women was 9.8%.[17] The high frequency of a symptomatic bacteriuria may be due to bad level of personal hygiene in pregnant women. Also these differences in prevalence of asymptomatic urinary tract infection in pregnant women in these studies perhaps due to climate in developed and developing countries.

Five types of bacterial species were isolated from the urine samples as *Escherichia coli, Klebsiella, Proteus, Staphylococci*, *Pseudomonas*, and *Candida* (Table 1).

E. coli was the most frequently isolated pathogen (28.9%), followed by *Staphylococcus aurous* (13.3%), *Klebsiella pneumonia* (12.2%), *Proteus mirabilis*(11.1%) and *Pseudomonas aeroginosa* (10%) While *Candida albicans* was found in only 4 (4.4%) of urine samples.

E coli was the most common bacteria isolated from the urine of 19 pregnant women (27.1 %), The result agree with Turpin *et al*. (2007) who found that dominant bacteria isolates were *E. coli* (37%)[16], *E. coli* remains the predominant organism implicated in urinary tract infection in pregnancy, though recent reports show change in pattern of the infection.[10] The studies in Nigeria show an increasing involvement of *Klebsiella Spp. Staphylococcus aureus, Proteus spp*., and *Pseudomonas spp* in urinary tract infection in pregnancy.[18]

Table (1): Microbial species isolated from the urine samples.

Culture results	Isolated numbers & percentage				Total N0. & %
	Pregnant	%	Non-pregnant	%	
E. coli	19	27.1	7	35	26(28.9%)
K. pneumonia	9	12.9	2	10	11(12.2%)
P. mirabilis	7	10	3	15	10 (11.1%)
P. aeruginosa	6	8.6	3	15	9 (10%)
Staphylococcus aureus	8	11.4	4	20	12(13.3%)
Candida albicans	3	4.3	1	5	4(4.4 %)
*NG****	18	25.7	0	0	18(20%)
Total number	70	100	20	100	90(100%)

NG:No growth*

Twenty-five pregnant women in first-trimester pregnancy, 29 second-trimester pregnancy, 16 third-trimester pregnancy who presented in the Department of Obstetrics and Gynecology, AL-Huda teaching Hospital, were enrolled in the study. The gestational age of all women, based on ultrasound measurements.

In this study, women in their 2nd and 3rd trimester were found to have the higher incidence of UTI; 89.7.4 and 87.5 % respectively (Table-2). While women were in their first trimester, they showed lower specific bacteria growth (48%). the frequency of urinary tract infection was higher in the third trimester compared to the second trimester. This is in agreement with Onuh *et al*, (2006) who reported a higher prevalence of urinary tract infection in the second trimester compared to the third trimester. This difference may be as a result of either change in urinary stasis by mechanical pressure from the gravid uterus on the bladder, preventing the complete emptying of urine. This stagnant urine leads to encourage bacterial growth in urine and vesicoureteral reflux or decrease in urinary progestines and oestrogens in the various trimester of pregnancy. Urinary incontinence symptoms increase with gestational age although majority remain tolerable.[10],[11] Thus, pregnant women should be screened for bacteriuria by urine culture at 12 to 16 weeks of gestation.

Table (2): Trimester (period of 3- three-months)

Trimester period	No. tested	% positive
First trimester (1st 3months)	25	12(48%)
Second trimester (2nd 3 months)	29	26(89.7%)
Third trimester (3rd 3 months)	16	14 (87.5%)
Total	70	52(74.3%)

The prevalence of infection for pregnant women in relation to age are shown in (Table-3), individuals of the age group 20-29 years had the highest incidence of infection (82.8 %). Followed by age group 30-39 years (73.5%) . Comparatively, the age group 40-49 years had the lowest incidence of infection (42.9/%) . Tturpin *et al.*(2007) indicated the highest age-specific prevalence was found in the 35-39 year-olds (13%) and the lowest in the 15-19 year-olds(0.0%).[16]

Table (3): The prevalence of infection for pregnant women in relation to age groups

Age groups(years)	Number examined	Positive number	% positive
20-29	29	24	82.8
30-39	34	25	73.5
40-49	7	3	42.9

Interleukine-6

The mean levels of IL-6 in urine were different among pregnant women groups and control group ($p \leq 0.01$). The mean value ± SEM for IL-6 was (51.55 ± 1.5) pg/ml in first trimester, (54.44 ± 1.04) pg/ml in second trimester (59.03 ± 1.9) pg/ml in third trimester and (66.09 ± 1.2) pg /ml in control women (Table- 3) . There was significant difference among the mean levels of IL-6 in urine of trimester pregnant women groups compare to the mean levels of IL-6 in control (non-pregnant women) with urinary tract infection ($P \leq 0.01$) , while there was no significant difference between the mean levels of IL-6 in first trimester and second trimester , second trimester and third trimester ($P > 0.05$) (Table-4) .The results indicated IL-6 urine mean levels were decreased in pregnant women compare to control (non-pregnant women) . The results of this study was compatible with Denney *et al.*(2011) who found that IL-6 was decreased in the pregnant women having UTI, compared with non-pregnant women.[19] The systemic T helper 1/T helper 2 (Th1/Th2) cytokine balance during normal human pregnancy is controversial, and observations about the balance in the postpartum period have only been reported for up to 3 months.[20] So decreased cytokine and immunoglobulin production during pregnancy may explain why pregnant women are more prone to develop UTI.

Table 3: The Mean Levels of IL-6 in Urine of Pregnant Women and Control .

Groups	No.	IL-6 Mean± S.E. pg/ml	Sig. between groups	P value
1st trimester*	25	51.55 ± 1.5	1st-2nd	0.108
2nd trimester **	29	54.44 ± 1.04	1st- 3thrid	0.001
3 third trimester ***	16	59.03 ± 1.9	1st-con	0.0
Control	20	66.09 ± 1.2	2nd-3 third	0.27
Total	90	57.04 ± 0.88	2nd-Con.	0.0
			3third- Con.	0.002

* First-trimester pregnancy **Second-trimester pregnancy***Third-trimester pregnancy

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