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Evaluation of The Relationship between Estrogen and some other risk factors In The Pregnancy of UTI

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Abstract

Background : During pregnancy, many women develop a urinary tract infection (UTI), which is a natural reaction against microorganisms that cause inflammation, and it affects different age stages, and due to the many physiological and hormonal changes in pregnant women, these factors can increase the incidence of UTI, and because of the importance of this topic and the lack of studies that show the impact of several factors, including the location of housing, age and other factors, in addition to the lack of studies on understanding the relationship between increasing estrogen and infection, according to the above our study was conducted to evaiuate these factors and their relationship to UTI.

Methods: Urine samples were collected from pregnant women with UTI, where the number of samples was 120 samples collected from women attending medical clinics and Samarra General Hospital with ages ranging from 18 to 40 years. 40 years old, culture media were prepared and bacterial isolates were grown on different media, including MacConkey agar medium and blood agar medium, and diagnosed according to phenotypic and microscopic characteristics, and the relationship of UTI to some factors was studied by making a special questionnaire for factors, and the relationship of estrogen to UTI was studied by the ELISA technique, and the results were analyzed.

Results: Culture and diagnostic results from 120 samples from pregnant women with UTI revealed that only 73 (60.8%) of the samples had bacterial growth, while the rest of the samples did not show growth despite the appearance of pus cells, According to phenotypic and microscopic characteristics and biochemical tests as these bacterial isolates were diagnosed into seven different types as follows: *Escherichia coli,Staphylococcus aureus , Klebsiella pneumonia, Enterobacter cloacae, Staphylococcus epidermidis, Pseudomonas aeuroginosa*, and *Leuconostoc mesenteroides*.*E. coli* ranked first with 48 isolates and a percentage of 65.75%, while L. mesenteroides showed the lowest number of isolates, amounting to one isolate and a percentage of 1.36%.The results also showed that UTI is affected by several factors, including housing location, age, number of sexual intercourse, use of immunosuppressants, and infection with the COVID-19. The study also proved that increased concentrations of estrogen hormone play a role in increasing UTI.

Conclusion: This study revealed the high prevalence of UTI among pregnant women, and the most common pathogen causing UTI was *Escherichia coli*, The results also showed that UTI is affected by several factors, including housing location, age, number of sexual intercourse, use of immunosuppressants, and infection with the COVID-19, The study also proved that increased concentrations of estrogen hormone play a role in increasing UTI.

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Key words: - pregnant, UTIs, *E. coli*, Estrogen Hormone.

Introductions

The urinary system consists of the kidneys, ureters, bladder and urethra, and its main function is to filter the blood by removing waste and excess water. The urinary system also plays a major role in removing metabolic wastes from the bloodstream, and another important function performed by the system is to normalize the concentration of ions and dissolved substances [1], where some microorganisms may be present without resulting in infection, and there are several reasons for this, such as the close proximity between the urethra and the anus and direct contact with the external environment through the urethra in the case of women and also for anatomical and hormonal reasons, the likelihood of developing urinary infections is much higher than men and it is estimated that they may experience at least one urinary infection throughout their lives [2],UTIs in women, both pregnant and non-pregnant women as well as those who use contraceptives, are caused by many naturally occurring bacteria in the body [3].

The most common bacteria associated with UTI are primarily *Escherichia coli*, *Pseudomonas* spp. and *Klebsiella* spp. The most common Gram-negative bacteria, And *Staphylococcus saprophyticus, Staphylococcus aureus, Enterococcus faecalis*, and others such as *Streptococcus* spp. and *Acinetobacter* spp. The most common Gram-positive bacteria [4,5].

From a physiological point of view, pregnancy can contribute to an increase in urinary tract infections. In fact, urinary tract infections are the most common among pregnant women and can be associated with an increased risk of maternal and fetal mortality or morbidity, and are of great concern due to their negative effects on mother and child [6] ,Among the various explanations associated with UTI in pregnant women are uterine dilatation and hormonal component that leads to decreased urine flow and increased urine retention thus increasing the likelihood of bacteria [7],One of the variable components in urine is sex hormones. Hormones such as estradiol, testosterone and progesterone reach their peak production in puberty and gradually decrease with age [8], studies have emphasized the influence of these hormones on infectious diseases [9]. A study on the role of sex hormones on clinical outcomes in females with cystic fibrosis found that estradiol stimulates mucus in *P. aeruginosa* and this phenotype is associated with chronic infection and disease progression was observed in females during the follicular phase when estradiol is highest in serum [10], Female hormones can also affect urinary tract infections (UTIs) by changing the nature of the organisms naturally found in the urinary tract[11], Due to the prevalence of UTI in pregnant women and the lack of studies regarding the impact of certain factors that can increase UTI such as age, location of residence, taking

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medications, etc. as well as the relationship of estrogen to UTI in pregnant women, according to the above our study was conducted to evaiuate these factors and their relationship to UTI.

Material and methods

Collecting samples

For the period from February 15, 2023 to March 10, 2024, 120 samples were collected from pregnant women with UTI who visited medical clinics as well as those who visited Samarra General Hospital, and the age limit for sample collection was between 18-40 years old, and 120 samples were collected from healthy pregnant women who did not have any symptoms of UTI.

Clinical examination of urine samples

Urine samples were collected from pregnant women according to the method described by Cheesbrought [12]. Urine samples were cultured on culture media prepared in advance according to the manufacturer's instructions and the agar plates were cultured on MacConkey agar medium and blood agar medium.

Diagnosis of bacteria

Bacterial isolates were characterized according to phenotypic characteristics such as colony shape and texture, colony edges, texture, odor, in addition to the pigments they produce on different culture media such as MacConkey agar and blood agar medium, and microscopic characteristics were studied after staining with Cram's stain [13].

Biochemical tests

The biochemical characteristics adopted in the diagnosis of bacterial isolates such as catalase test, oxidase test, methyl red test, indole and stearate test, mannitol fermentation test and hemolysin production were studied according to de la Maza *et al.* [14].

Study of the relationship of certain factors with UTI in pregnant women

For this study, a special questionnaire was designed for this study, which included information regarding age, location of residence, number of sexual intercourse, history of previous infection and use of immunotherapy (immunosuppressants).

Determination of Estradiol

The estrogen concentration of the 120 samples of pregnant women was estimated by using the ready-made kit from the American company 1 Monobind by following the steps attached to the kits, and was measured using the ELISA technique. After completing the preparation and detection processes, calculations were performed, as the average values of the optical absorbance values were calculated for each group.

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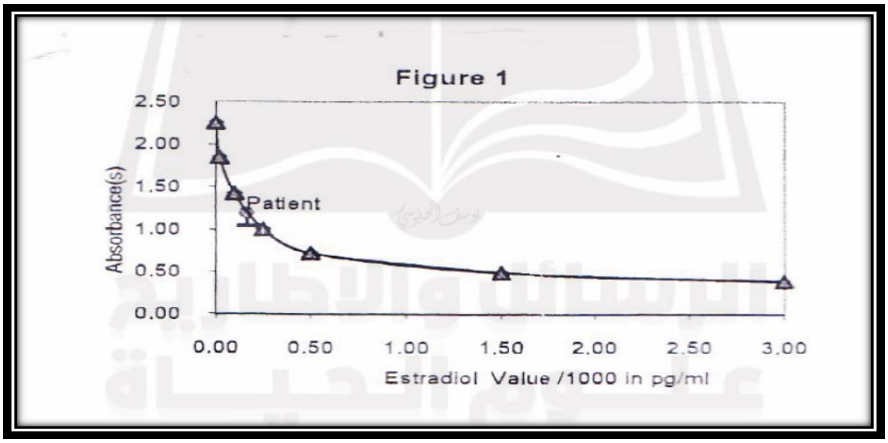

Figure 1: The standard curve of the estrogen hormone Estrogen in the ELISA technique

Statistical analysis

In order to analyze the results statistically, the analysis of variance ANOVA test was used for the randomized complete design CRD and the arithmetic means were compared by Duncan's polynomial tests under the 0.05% probability level [15].

Results

The results of the udder culture test taken from 120 udder samples from pregnant women with UTI, aged between 18-40 years, whom visited Samarra General Hospital and local medical clinics, which were cultured on Blood agar medium, MacConkey agar medium, Eosine Methylene Blue Agar (EMB) medium and Mannitol agar medium, revealed that only 73 samples, or 60.8%, had bacterial growth, while the rest did not give growth despite the appearance of pyogenic cells.Table 1.

Table 1: Urine sample test results for UTIs in pregnant women.

Total number	UTI positive	UTI Negative
120	73	47
Percentage	60.8%	39.16%

The results in Table 2 revealed the results of staining the bacterial isolates with Cram's Grams Stain that 14 bacterial isolates were positive for the stain (19.17%), while 59 isolates were negative for Cram's Grams Stain (80.8%).

Table 2: Percentage of Gram's stain positive and negative isolated from pregnant women with UTI.

Bacterial isolates	Number of isolates	Percentage%	P value
Gram positive	14	19.17%	$P \leq 0.001$
Gram negative	59	80.08%	
Total Number	73	100%	

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The 73 bacterial isolates isolated from pregnant women with UTI were diagnosed based on their culture characteristics and based on the phenotypic characteristics discussed in the previous paragraph in addition to the chemistry tests, as these bacterial isolates were diagnosed into seven different types as follows: *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Enterobacter cloacae, Staphylococcus epidermidis, Pseudomonas aeuroginosa,* and *Leuconostoc mesenteroides.*

E. coli ranked first with 48 isolates and a percentage of 65.75%, while *L. mesenteroides* showed the lowest number of isolates, amounting to one isolate and a percentage of 1.36%, as shown in Figure 2 and Table 3.

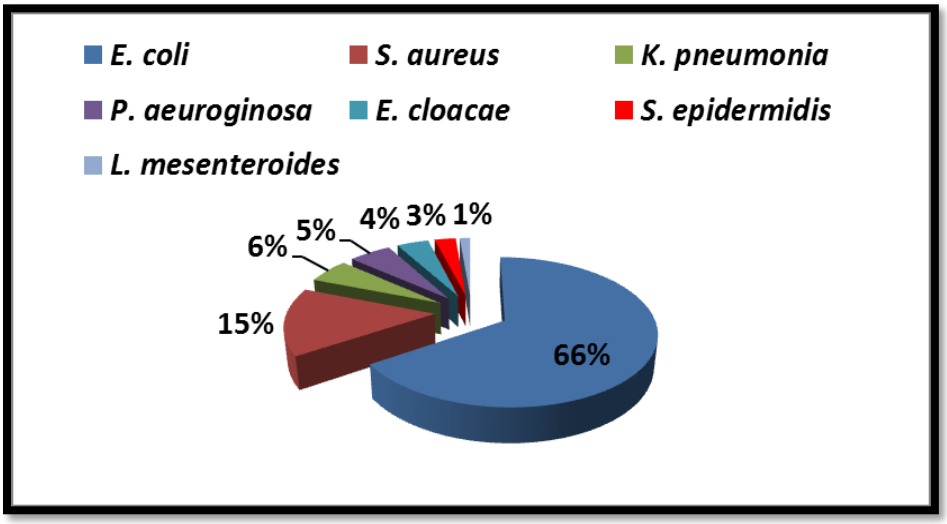

Figure 2: Types of bacteria that cause UTI in pregnant women.

Table 3: Percentages of bacteria determined from pregnant with UTIs

	Type of Bacteria	Number of isolates	Percentage%	P value
Pregnant women with UTI	*E. coli*	**48**	**65.75%**	**P ≤ 0.001**
	St. aureus	**11**	**15.06%**	
	K. pneumonia	**4**	**5.47%**	
	E. cloacae	**4**	**5.47%**	
	S.epidermidis	**3**	**4.10%**	
	P. aeuroginosa	**2**	**2.73%**	
	L. mesenteroides	**1**	**1.36%**	
total number		**73**	**100%**	

Table 4 and figure 3 indicates the phenotypic characteristics of bacterial isolates isolated from pregnant women with UTI on different culture media

Table 4 : Results of phenotypic screening of bacterial isolates on different culture media.

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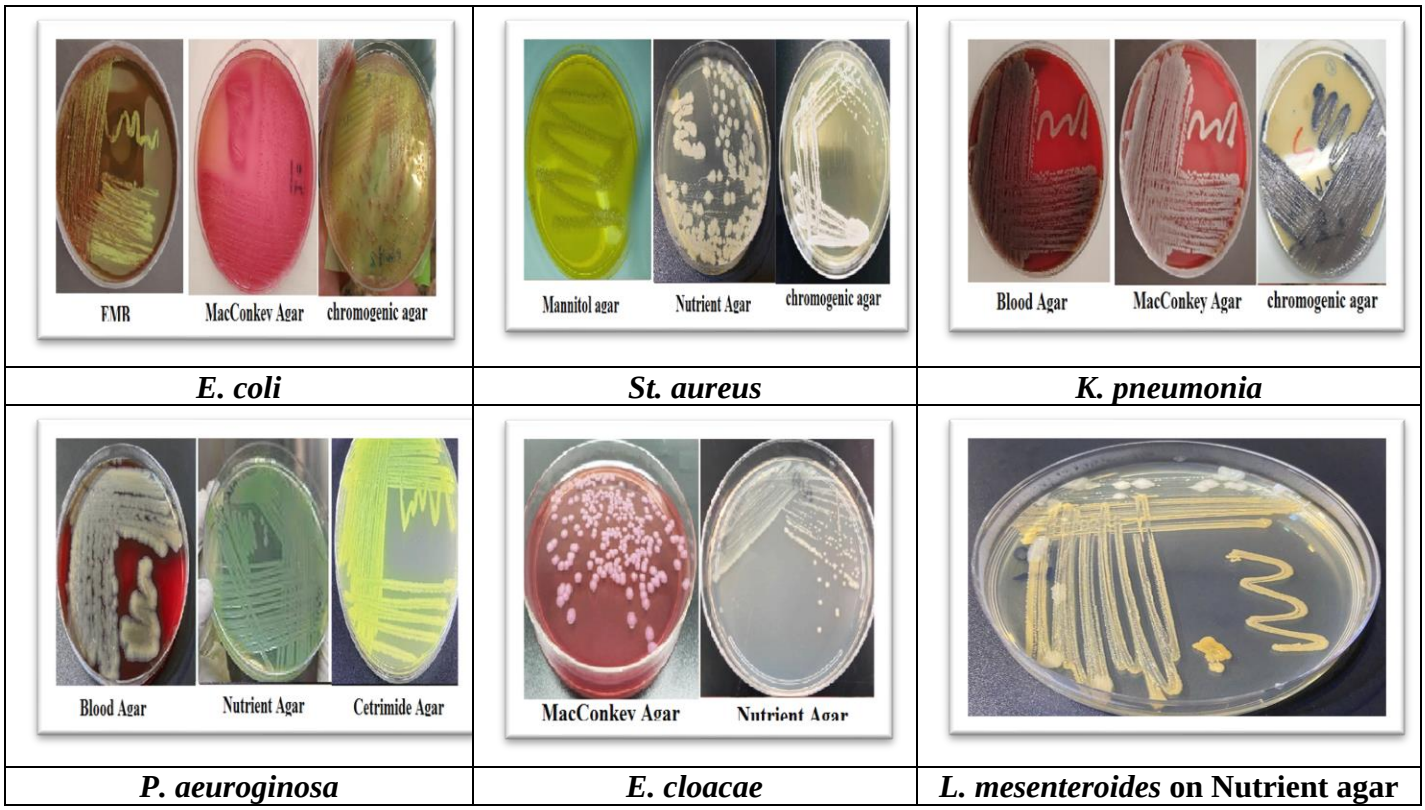
Type of bacteria	Chromogenic agar	MacConkey agar	Blood agar	EMB	Mannitol Salt
E. coli	Pink	round, small, pink-coloured, dry colonies	Greyish-white and moist	Green metallic sheen	-
S. aureus	White or cream	No growth	No growth	No growth	Yellow
K. pneumonia	Dark Blue	Pink, mucous and large colonies	white Greyish or	Purple colonies and mucus	-
E. cloacae	pink to red	Large Pink Colonie	No growth	No growth	-
S. epidermidis	cream colonies	No growth	No growth	No growth	Pink
P. aeuroginosa	Yellow	Pale in colour	Creamy colonies surrounded by a transparent area	No growth	-
L. mesenteroides	Pink	No growth	No growth	No growth	-



E. coli | ***St. aureus*** | ***K. pneumonia***

P. aeuroginosa | ***E. cloacae*** | ***L. mesenteroides*** **on Nutrient agar**

Figure 4: Phenotypic characteristics of bacterial isolates isolated from pregnant women with UTI on different culture media

The results shown in Table 4 about the prevalence of UTI among pregnant women and its relationship with some factors, including housing, revealed that pregnant women who live in the countryside are more likely to be infected than women who live in the city, with a percentage of 54.79% for the countryside and 45.20% for the city. The results of the effect of age showed that the age group 21-25 years was the highest percentage of infection among pregnant women at 36.98%, and the lowest percentage of infection was in the age group 36-40 years at 4. The results showed that there were significant differences with the increase in the number of sexual

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intercourse in pregnant women during one week, as the relationship was direct, as the more sexual intercourse, the higher the incidence of UTI in pregnant women, as the incidence rate for less than three times per week reached 26.02%, while the incidence rate with more than three times per week amounted to 73.97%. As for the factor of previous history of UTI, the results showed that 37 pregnant women with UTI had a previous UTI (50.68%), while 36 pregnant women with UTI had no previous UTI (49.31%). Pregnant women with Psoriasis and Rheumatoid Arthritis who were taking immunosuppressants had an incidence of 16.4%, while pregnant women who were not taking immunosuppressants had an incidence of 82.19%. As for the relationship between UTI and COVID-19, the results showed that 31 women with COVID-19 (42.46%) had UTI, while 42 pregnant women had UTI but did not have COVID-19 (57.53%).

Table 6: The relationship of UTI in pregnant women to some of the variables

Variable	Frequency from 73 Women with UTI	Percentage%	P value
Residential Area			
Rural	40	54.79%	
Urban	33	45.20%	
Age			
<20	11	15.06 %	
21-25	27	36.98 %	
26-30	18	25.65 %	
31-35	14	19.17 %	
36-40	3	4.10 %	
frequency of intercourse			**P ≤ 0.001**
Less than three times	19	26.02%	
Three or more times	54	73.97 %	
History of UTI			
Yes	37	50.68%	
No	36	49.31%	
Immunosuppressants			
Yes	13	16.4%	
No	60	82.19%	
COVID-19			
Yes	31	42.46%	
No	42	57.53%	

As for the study of the relationship between estrogen and UTI in pregnant women, our results shown in Table 5 showed that there were significant differences under the probability level of P ≤ 0.001, a significant increase in estrogen concentration was observed with the presence of infection, whether with or without bacterial growth, indicating an association between estrogen

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and UTI in pregnant women, as the average hormone concentration in women with UTI with bacterial growth was 2716. 9+3.012 (pg/ml) and 1312.8+2.407 (pg/ml) compared to 128.25+0.866 (pg/ml) in healthy women.

Table 7: of estrogen levels in pregnant women with UTI

Groups	Estrogen concentration (pg/ml) + standard error	Percentage%	P value
Healthy women	128.25+0.866	14.28%	P ≤ 0.001
Women with UTI With bacterial growth	2716.9+3.012	85.71%	
Women with UTI with no bacterial growth	1312.8 +2.407	100%	

Discussion

The study of UTI has been the focus of several recent studies, including Kazmi *et al*.[16] and Al-Shahrani and Belali [17] whose study dealt with the isolation and diagnosis of bacteria from UTI in pregnant women, as they diagnosed bacterial growth in diuresis samples taken from pregnant women with UTI, and the results of this study were close to the findings of Kitagawa *et al*., [18] but our study results did not agree with what they found Basu *et al*, [19] who found that 30.35% and 46%, respectively, of pregnant women with UTI had bacterial growth. The reason for the dominance of *E. coli* bacteria is due to their virulence factors such as toxins, adhesins, and pili factors that allow them to adhere to the epithelial tissues lining the urinary tract, which protect the bacteria during urinary elimination and allow the bacteria to multiply and invade the tissues of the urinary epithelium [20].

With regard to the relationship of UTI with some factors, which were explained in the table 6 that these factors have a clear impact on UTI, and these results are close to what Swadi [21]found in his results for the factor of housing location, that the percentage of women infected with UTI from rural residents was 53%, while the percentage was 47% in the city. The reason for this can be explained by the difference in the cultural level of health care during pregnancy and the accompanying negligence and lack of care for personal hygiene, especially defecation areas, which leads to the arrival of pathogenic organisms (bacteria) to the urinary tract to the bladder and then the urinary system [22]. Regarding the age factor, it was consistent with Vicar *et al.* [23] and Shakoor [24], and the reason for the high incidence rates between the age group of 20-25 years and 26-30 years is due to what Basu *et al,* [19], stating that young women aged 20-30 years are highly sexually active, as well as these ages are more likely to have children, which puts them at risk of UTI, as well as the complete maturation of the reproductive organs and the pressure of the urinary bladder with the enlarged uterus so that the urinary bladder cannot empty

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completely and this leads to bacterial growth when urine is retained in the urinary bladder. As for the factor of number of sexual intercourse, the reason is that sexual activity increases the chance of contamination of the urethra during intercourse, which increases the risk of bacteria entering the urethra, as well as the proximity of the urethra to the outlet [25]. The effect of the factor of previous history of inflammation, Barnawi *et al.* [26] found that pregnant women with a previous history of inflammation are more likely to have an infection by 58%, and Sacristán *et al.* [27] indicated that taking immunomodulators is associated with UTI. As for the relationship between UTI and COVID-19 infection, it is clear from the above results that the percentage of women with UTI increases with COVID-19 infection, and this is consistent with what Hateet [28] stated that COVID-19 has a relationship with UTI in pregnant women, and the same researcher explained the reason for the relationship between UTI and COVID-19 infection to the fact that patients with UTI may have something to do with the side effects of the COVID-19 pandemic, or it is a risk factor for COVID-19 infection. 19 pandemic or that it is a risk factor for UTI.

Regarding the study of the relationship between estrogen and UTI in pregnant women, a local study in Iraq by Hussein and Razzaq, [29]. indicated that the effect of sex hormones, including estrogen, on UTI in pregnant women found that an increase in estrogen levels increases the incidence of UTI, which is consistent with the results of our study. Konapala *et al*. [30] also indicated that lower estrogen levels and higher progesterone levels increase the risk of UTI, which is not consistent with the results of our study in which higher estrogen levels were found. Studies have shown that during pregnancy it is developmentally beneficial to decrease inflammatory immune responses that may lead to fetal rejection and increase anti-inflammatory responses that promote the transfer of maternal antibodies to the fetus. Sex hormones play a role in this as hormones modulate the immune shift that occurs during pregnancy and this explains why estrogen increases during pregnancy [31].Studies have confirmed that during pregnancy, some physiological changes occur in women under the influence of sex hormones, such as ureteral dilatation, increased bladder capacity and decreased bladder tone in the urinary system, and these changes may cause urinary stasis, ureteral and vesicular reflux and bladder emptying dysfunction, and thus appear in about 70% of pregnant women, reduce the resistance of the urinary system and cause bacterial growth in addition to adding insufficient genital hygiene behaviors [32,33].

Conclusion:

This study revealed the high prevalence of UTI among pregnant women, and the most common pathogen causing UTI was Escherichia coli. The results also showed that UTI is affected by several factors, including housing location, age, number of sexual intercourse, use of immunosuppressants, and infection with the Corona virus. The study also proved that increased concentrations of estrogen hormone play a role in increasing UTI.

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