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Molecular Detection of Asymptomatic Bacteriuria among Pregnant Women with Anemia in Iraq

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Abstract: The purpose of the research is the detection of the relationship between anemia in pregnant women in the second trimester of pregnancy and the Virulence of factors for bacterial infections of *Escherichia. Coli (E. Coli)* by PCR. 150 midstream urine samples were collected from pregnant women with age (16-45) years and also were taken blood samples. The percentage of bacterial Number which was isolated from 105 samples, 44,3 % (n=47) were *E. Coli* followed by other gram-negative bacteria, 11,3% (n=12), which include *Klebsiella pneumonia*, and 10,4% (n=11) *Pseudomonas aeruginosa*. Also was the Number and percentage of 50% (n=35) of the infection group women had anemia, while 100% (n=35) of the non-infected women and non-anemic. Further confirmation was done using by API 20E system and VITEK 2. This study proved the appearance of the cnf1 gene in the *E. Coli* bacteria, which is infect to pregnant women with anemia, and also showed the toxic role of these genes in tissue damage.(*cnf1,16SrRNA*).

Keywords: PCR, DNA, *E. Coli, VITEK, API, Asymptomatic, Pregnant, Urine, cnf1,16SrRNA.*

INTRODUCTION

Anemia is a condition that occurs in the human body when the quantity of circulating erythrocytes, or the count of red blood cells (RBCs), is low and inadequate. When compared to age-matched controls, it can be described as a low hemoglobin concentration or RBC mass. Normal value is a statistical phrase used to indicate a range within which 95 percent of the population's results fall in practically all human laboratory tests. Anemia is also defined as a hemoglobin level of less than 13 g/dL in adult males and less than 12 g/dL in non-pregnant adult women, according to the World Health Organization (WHO) (Anonymus, 2015).

Women who are anemic face considerable health risks. Severe anemia is a major risk for women during pregnancy. Severe anemia, which causes intrauterine growth retardation and premature birth, has been shown to increase perinatal morbidity and death. Adolescent females' physical labor capability and reproductive physiology are both affected by anemia. According to WHO, the global prevalance for pregnant women was 38,2% (Anonymus, 2015).

Preterm delivery for gestational age newborns are linked to anemia, which is a frequent pregnancy problem (Scanlon, *et al.*, 2000; Xiong, *et al.*, 2000). Over hydration at diagnosis, endotoxin-mediated hemolysis, renal erythropoietin suppression, and chronic illness anemia are all theories for the pathophysiology of pyelonephritis-associated anemia (Cavenee, *et al.*, 1994; Duff, 1984; Lucas and Cunningham, 1993). Despite the fact that pyelonephritis is known to cause anemia, there is little information on the consequences of anemia caused by pyelonephritis on pregnancy outcomes (Dotters-Katz, *et al.*, 2013).

Asymptomatic bacteriuria (AB) is the presence of rapidly proliferating bacteria in the urinary system, except in the distal urethra, in a patient who has no evident urine symptom. In the absence of urinary symptoms and indicators, the presence of colony forming units of single bacteriuria of two consecutive clean catch urine specimens or a single catheter specimen has been considered relevant in determining the diagnosis of silent bacteriuria. Asymptomatic bacteriuria is common in the general population, although it is more common during pregnancy due to physiological changes (Izuchukwu, *et al.*, 2017).

Asymptomatic bacteriuria is a infection of the urine that does not cause any of the normal symptoms of a urinary infection and affects 2% to 15% of pregnancies. Up to 30% of women will get acute pyelonephritis if they are not treated. Asymptomatic bacteriuria is associated with low birth weight and early birth (Smaill and Vazquez 2019).

There is discussion over the relationship between asymptomatic bacteriuria during pregnancy and unfavorable perinatal outcomes such as preterm delivery and low birth weight (LBW). In addition

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to, a meta-analysis of cohort studies showed that untreated asymptomatic bacteriuria during pregnancy significantly increased rates of LBW and preterm delivery. Moreover, analysis of randomised clinical trials showed that antibiotic treatment significantly reduced the risk of LBW. Accordingly, screening for and treatment of asymptomatic bacteriuria during pregnancy has become a standard of care (Sheiner, *et al.*, 2009).

MATERIAL AND METHOD

A total of one hundred and five samples of urine were collected from pregnant women with age (16-45) years during the period from 8 to June 2021 to 10th September 2021; at the same time, were taken blood samples in the form of venous blood for all examinations. Anemia was assessed by complete blood count (CBC); according to the classification of WHO, pregnant women with hemoglobin levels less than 11.0 g/dl in the first and third trimesters and less than 10.5 g/dl in the second trimester are considered anemic (Anonymus 2015). Attending to Al-Kut hospital for Gynecology obstetrics and health care clinics for pregnant women and pediatrics and private clinics in the Wasit province.

These samples were divided into three groups after being diagnosed by a specialist physician, and laboratory tests these groups include. First group include 35 female healthy pregnant without signs and asymptom of urinary tract infection and who don't have anemia disease (control). Second group consist of 35 pregnant female with an anemic with urinary tract infection infected with *E. coli.* Third group include 35 pregnant women with don't have anemia disease with urinary tract infection infected with *E. Coli.*

The bacterial isolates were collected into sterile screw-capped test tubes. Samples were brought to the laboratory under standard conditions and streaked immediately on MacConkey agar plates for bacterial isolation. At the same time, blood samples were taken from pregnant women, and a complete blood count test was done with the count complete blood count (CBC) device by drawing blood from the arm (a test that checks the concentration level of red blood cells as well as the level of hemoglobin in the blood). The isolated bacteria have been recognized based on morphological, biochemical tests, VITEK2, API 20Ekit, and Molecular method for detection genes (*16SrRNA*, *CNF1*) of *E. coli* isolates; the DNA Extraction for 35 isolates. DNA was extracted using Geneaid company (Presto™ Mini gDNA Bacteria Kit – Geneaid) for patients and controls. The nucleic acid concentration and purity ratio are automatic calculated by the NanoDrop electrophoresis. Polymerase chain reaction (PCR) mixtures were prepared according to the instructions supplied with the Promega kit (Promega Corporation, Madison, Wisconsin, USA), which contained 200 lM of deoxynucleoside triphosphate, 2.5 µ of 10 3 reaction buffer (100 mM Tris-HCl at pH 8.3, 500 mM KCl, 1.5 mM MgCl2,) a 0.1 lM concentration of the primers which are demonstrated in the Table (1) with 2.5 U of Taq DNA polymerase, and five ng of template DNA. The volume of this mix was adjusted to 25 µ with sterile water. The PCR reaction conditions were as follows: an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for one minute, and a final extension step at 72°C for10 minutes. PCR results gained were 919bp and 91bp for *16SrRNA*, and *cnf1*respectively has been visualized by electrophoresis and captured by gel documentation system to the observed bands.

Two primers with the sequences are listed in Table (1).

Cytotoxic necrotizing factor (*cnf1*)	91pb 55°c	Cnf1 F (TGCTGTTCTGTATGGCATAGCC) Cnf1 R (GAGCATCTCCAGTGTTCCGAC)	Reference HassanMomtaz, *et al.*, 2013
16SrRNA	919pb 55°c	16SrRNA F (AGAGTTTGATCMTGGCTAG 16SrRNA R (CCGTCAATTCATTTGAGTTT)	Johanna Bergh, *et al.*, 2006

STATİSTİCAL ANALYSİS

SPSS Version 26 SAS (2012) program is utilized to affect the different elements in the studied guidelines; the lowest significant differentiation LSD test (ANOVA) is utilized in a significant comparison means, and the Chi-Squre x2 test, which has been used to compare between percentages in this study.

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RESULT

The cultural characteristics of 105 urine isolates on different media such as MacConkey agar (Pink color with precipitation of bile salt around colonies) and Eosin Methylene BlueSelective and differentialmedia (Growth with green metallic sheen colonize, which is a characteristic feature for E. coli from other gram-negative pathogens.

The results that there were no significant differences between the three study groups (16-25), (26-35), and (36-45) years was p=0.24, as shown in Table 2.

Table 1: Distribution of study groups according to age groups

Age Case		Case			Total	P-Value
		G1	G2	G3		
Age	16 - 25	No (15)	20	23	58	0.24
		41.7%	57.1%	65.7%	54.7%	
	26 - 35	No. (17)	11	11	39	
		47.2%	31.4%	31.4%	36.8%	
	36 - 45	No. (4)	4	1	9	
		11.1%	11.4%	2.9%	8.5%	
Total		35	35	35	105	
		100.0%	100.0%	100.0%	100.0%	

Table 2: Distribution of the infection group of women according to anemia

Anemia Infection		Infection		Total	P-Value
		WithoutG1	With G2		
	Anemia	0	35	35	0.00
		0.0%	50.0%	33.0%	
	Non anemic	35	35	70	
		100.0%	50.0%	67.0%	
Total		35	70	105	
		100.0%	100.0%	100.0%	
*Means significance differences (P ≤ 0.05) **means high significance differences (P ≤ 0.001)					

Table 3: Distribution of the women infected with *E. coli* according to anemia

Anemia *E. coli*	*E. coli*		Total	P-Value
	No infected	Infected		
Anemia	0	35	35	0.00
	0.0%	74.5%	50.0%	
Non anemic	23	12	35	
	100.0%	25.5%	50.0%	
Total	23	47	70	
	100.0%	100.0%	100.0%	

In Table 5, the results show that 0 (0%) of women infected with other bacteria, 74,5% (n=35) were infected with *E. coli*, and 0 (0%) of the controls were anemic, while 100% (n=23) of women infected with other bacteria, and 25,5% (n=12) infected with *E. coli*, and 100% (n=35) of the controls were non-anemic, with a highly significant difference (p=0.000).

Table 4: Infection of the study groups with other bacteria (*Pseudomonas* and *Klebsiella*)

Anemia *E. coli* Other bacteria (group3) Pseudomonas; klebseilla	*E. coli*			Total	P-Value
	Other bacteria	With *E. coli*	control		
Anemia	0	35	0	35	0.00
	0.0%	74.5%	0.0%	33.0%	
Non anemic	23	12	35	70	
	100.0%	25.5%	100.0%	67.0%	

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Total	23	47	35	105	
	100.0%	100.0%	100.0%	100.0%	

Table 5: Distribution of the infection group according to the three pregnancy trimesters

Pregnant stage Infection		**Infection**			**Total**	**P-Value**
		Other bacteria (*pseudomonas* and *klebsiella*	***E. coli***	**Control**		
Pregnant stage	**First trimester**	3	5	5	13	0.924
		13.0%	10.6%	13.9%	12.3%	
	Second trimester	14	32	21	67	
		60.9%	68.1%	58.3%	63.2%	
	Third trimester	6	10	10	26	
		26.1%	21.3%	27.8%	24.5%	
Total		23	47	35	105	
		100.0%	100.0%	100.0%	100.0%	

Distribution of diseases in the infection group of pregnant women in table 7 showed that 95,4% (n=45) of women infected with *E. coli* had no other diseases, (n= 22) 95,7% of women with other infections had no other diseases, and 34 (94,4%) of women without infection had no other diseases. While 4,3% (n=2) of women infected with *E. coli* had toxoplasmosis, 4,3% (n=1) of other infections had toxoplasmosis, and 2.8% (n=1) of those without infection had toxoplasmosis, whereas no women 0 (0%) of women infected with *E. coli* or other infections had hypertension, but 100% (n=35) of women without infections had hypertension, with non-significant differences (p= 0,719).

Table 6: Distribution of the infection group according to other diseases

Diseases Infection		**Infection**			**Total**	**P-Value**
		Other bacteria	***E. coli***	**Control**		
Diseases	**No**	22	45	34	101	0.719
		95.7%	95.7%	94.4%	95.3%	
	Toxoplasma	1	2	1	4	
		4.3%	4.3%	2.8%	3.8%	
	Hypertension	0	0	1	1	
		0.0%	0.0%	2.8%	0.9%	
Total		23	47	35	105	
		100.0%	100.0%	100.0%	100.0%	

Table 7: Distribution of the infection group according to preanemia

Preanemia Infection	**Infection**			**Total**	**P-Value**
	Other bacteria	***E. coli***	**Control**		
Without Preanemia	16	16	31	63	0.00
	69.6%	34.0%	86.1%	59.4%	
With Preanemia	7	31	5	43	
	30.4%	66.0%	13.9%	40.6%	
Total	23	47	35	105	
	100.0%	100.0%	100.0%	100.0%	

Table 8: Distribution of the infection group according to the number of children

		Infection			**Total**	**P-Value**
		Other bacteria	***E. coli***	**Control**		
Number of Children	0	10	13	11	34	0.9
		43.5%	27.7%	30.6%	32.1%	
	1	6	11	8	25	
		26.1%	23.4%	22.2%	23.6%	
	2	4	7	7	18	
		17.4%	14.9%	19.4%	17.0%	

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	3	2	9	7	18	
		8.7%	19.1%	19.4%	17.0%	
	4	1	5	2	8	
		4.3%	10.6%	5.6%	7.5%	
	5	0	2	1	3	
		0.0%	4.3%	2.8%	2.8%	
Total		23	47	35	105	
		100.0%	100.0%	100.0%	100.0%	

Table 9: Distribution of anemic women by the pregnancy trimesters

		anemia	Non anemic	Total	P-Value
Pregnant stage	**First trimester**	5	8	13	0.227
		14.3%	11.3%	12.3%	
	Second trimester	25	42	67	
		71.4%	59.2%	63.2%	
	Third trimester	5	21	26	
		14.3%	29.6%	24.5%	
Total		35	70	105	
		100.0%	100.0%	100.0%	

Distribution of anemic women by other diseases demonstrated that 87,1% (n=34) anemic and 94,4% (n=67) nonanemic women were free of other diseases, while 2,9% (n=1) anemic and 4,2% (n=3) nonanemic women had toxoplasmosis, and 0 (0%) anemic, and 1,4% (n=1) nonanemic women were hypertensive, with non-significant differences (p=0.73), as shown in Table 11.

Table 10: Distribution of anemic women by other diseases

		anemia	Non anemic	Total	P-Value
Diseases	No	34	67	101	0.73
		97.1%	94.4%	95.3%	
	Toxoplasma	1	3	4	
		2.9%	4.2%	3.8%	
	Hypertension	0	1	1	
		0.0%	1.4%	0.9%	
Total		35	70	105	
		100.0%	100.0%	100.0%	

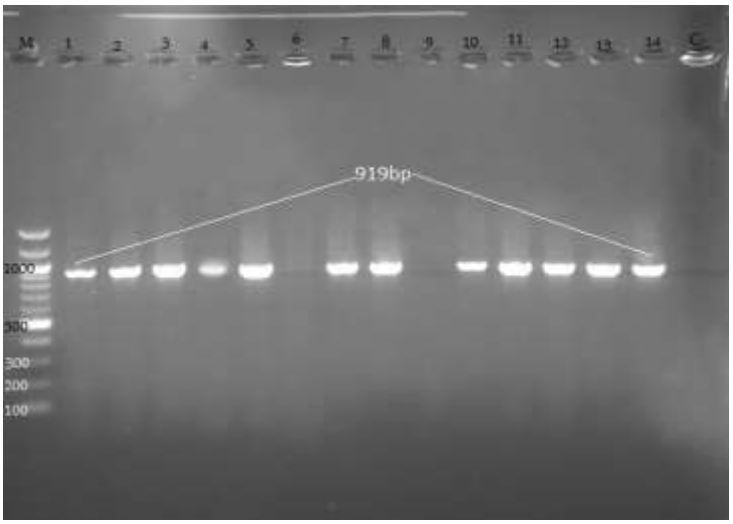

Figure 1: Gel electrophoresis of the PCR product of (*16SrRNA* gene 919 bp) by (1.5%) agarose at (1h/90v). Lane M (100bp) DNA marker. Lane C-: negative control, Lanes (1-14) are samples

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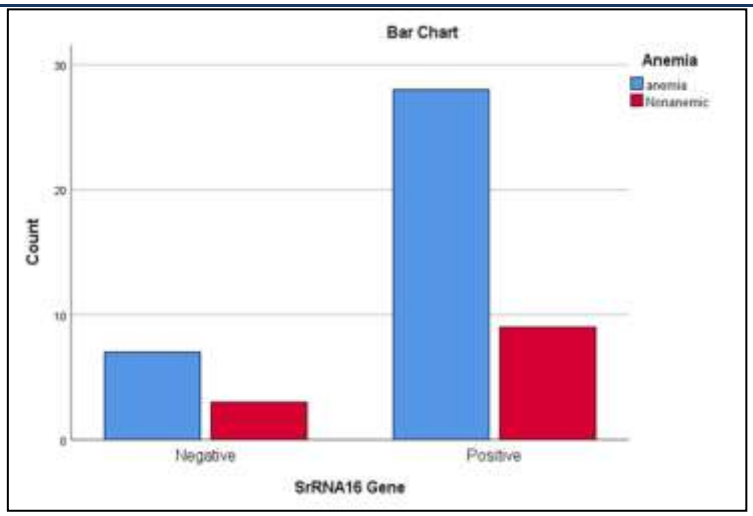

Figure 2: Distribution of anemic women according to positive and negative *16 SrRNA* gene

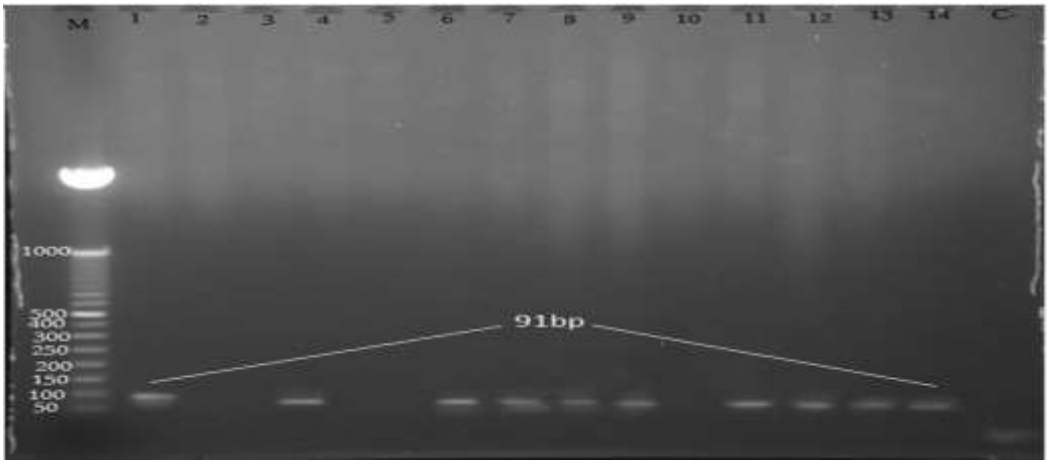

Figure 3: Gel electrophoresis of the PCR product of *cnf1* gene (91 bp) by (1.5%) agarose at (1h/90v). Lane M (100bp) DNA marker. Lane C-: negative control. Lanes (1-14) are samples

Table 11: Distribution of anemic women according to positive and negative cnf1 gene

		Anemia	Non anemic	Total	*R. R*	*OR*
cnf1	**Negative**	10	5	15	1.171	1.7857
		28.6%	41.7%	31.9%		
	Positive	25	7	32		
		71.4%	58.3%	68.1%		
Total		35	12	47		
		100.0%	100.0%	100.0%		

DISCUSSION

This study included 105 pregnant women who were divided into three groups: (1) the healthy control group, including those without anemia and not infected with *E. coli* (2) the patient group, including anemic women who were infected with *E. coli*, and (3) the infection group including women without anemia but infected with *E. coli*.

The polymerase chain reaction (PCR) technology was used to detect the16 SrRNA and cnf1 genes in the *E. coli* bacteria isolated from pregnant women infected with UTI.

Among the three study groups based on age, no big differences showen in infection rates, but in the younger age group (16-25) years highest number of infections were observed, which contradicted many studies such as Colgan, *et al.*, (2006), which stated that age is one of the risk factors for bacteriuria and that the prevalence of bacteriuria increases with age. According to Eriksson, (2011) Urinary tract infection (UTI) is common in women of all ages, but the incidence and prevalence rise with age.

However, our findings matched those of (Baranda-Nájera, *et al.*, 2014) in terms of risk of maternal

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age, which included elderly pregnant women, while pregnant adolescent women ≥ 35 years of age as defined by the International Federation of Gynecology and Obstetrics System (FIGO) (Ríos and Vera, 2015).

The current study showed that the majority of an infected group of pregnant women were anemic in comparison with non-infected women. Anemia during pregnancy in impoverished nations is caused by a variety of factors, including micronutrient deficiencies in iron, folate, and vitamins A and B12, as well as anemia caused by parasite illnesses as well as chronic infections.

Anaemia during pregnancy is a commen health issue and is linked to negative pregnancy outcomes. According to global data, anemia affects 56 percent of pregnant women. Fatigue, low job capability, diminished immunological function, higher risk of heart illnesses, and death are all severe health impacts for the mother (Black, *et al.,* 2013)

It was observed that the major bacteria causing UTI in our study was *E. coli* in comparison with other bacteria such as *Klebsiella* and *Pseudomonas*. This result agreed with Tugrul, *et al.,* (2005), who found that 77,8 % of the samples were infected with E. coli.

Distribution of UTI infection according to gestation period revealed that the highest incidence of infection was in the second trimester of pregnance when compared with the first and third trimesters.

The findings of this study matched those of earlier studies, which found that many of mothers suffers with UTI in their second trimester. The studies roported that at the 22nd-44th week of pregnancy showed a high risk of UTI starting from the 6th week (Delzell Jr and Lefevre, 2000). There is a lot of factors, such as fast physiologic changes, that can be causes the increased frequency of UTIs in the second trimester (Onyango, *et al.,* 2018)

Moreover, it was observed from our results that anemia cases were higher among pregnant women with recurrent infections when compared with those without recurrent infections. Women with a history of UTIs are more likely to develop UTIs during pregnancy for a variety of reasons, one of which is anemia (Nowicki, 2002). It was reported by Smaill and Vazquez, (2019) that acute renal damage, anemia, and other problems may develop in some women with pyelonephritis and bacteremia.

CONCLUSION AND RECOMMENDATION

E. coli isolate constituted the highest frequency than other isolates in pregnant women with UTI, by 67%. The results showed that the infection of UTI before pregnancy and the lack of treatment has a relationship with the continuation of infections during pregnancy due to the lack of pregnant immunity, having anemia before pregnancy and not treating it has a relationship with its continuation during pregnancy, the virulence factor CNF1 gene found in *E. coli* has a relationship with anemia in pregnant women by 71,4%.

It is recommended to taking more samples to include a larger group of people, studying more genes and knowing their effect on patients, study other types of bacteria and know which types have a harmful effect, spreading a healthy and personal culture among the community to limit the spread of UTIs, encourage proper nutrition and focus on foods that contain large amounts of iron and folic acid, more research on virulence factors present in genes to understand the relationship between anemia and UTI.

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