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Original article

Bacteriuria Profile and Associated Risk Factors among Pregnant Women with and Without Diabetes at Al-Jala Hospital

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ABSTRACT

***Background and objectives**: This study was conducted to identify the pathogenic bacteriuria profile and associated risk factors in pregnant women with and without diabetes. **Methods**: Six hundred urine samples were examined from pregnant women in different age groups: 100 non-diabetics with asymptomatic bacteriuria (ABU), 100 non-diabetics with symptomatic bacteriuria (SBU), 200 diabetics with (ABU) and 200 diabetics with SBU. The culture media were prepared according to the manufacturer's instructions. **Results**: The number of positive cultures among asymptomatic UTI-tested women was 106 out of 300 (20%), that 60/200 cases (30%) in diabetic patients and 46/100 cases (46%) in non-diabetic patients. The number of positive cultures among symptomatic UTI-tested women was 195 of 300 (65%), 100/200 cases (50%) in diabetic patients and 95/ 100 (95%) in non-diabetic patients, with a higher percentage isolated from E. coli isolated from both non-diabetic pregnant women at the rate of (26.1%) and diabetic pregnant women at the rate of (56.7%) with asymptomatic bacteriuria as well as a higher percentage isolated at the rate of (30%) from non-diabetic pregnant women and at the rate of (34.7%) from diabetic pregnant women with symptomatic bacteriuria. Escherichia coli is the most important cause of UTIs, particularly in pregnant women with diabetes. **Conclusion:** This study has highlighted the need for greater awareness of urinary tract infection and the expansion of prevention and treatment services for pregnant women. Therefore, it is suggested that routine screening of patients and antimicrobial therapy should be guided by both in vitro susceptibility and clinical response.*

Keywords: Urinary tract infections, Bacteriuria, Diabetes, Pregnancy.

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المقدمة والهدف: تم إجراء هذه الدراسة لتحديد انواع بكتيريا البول الممرضة والعوامل المرتبطة بها في النساء الحوامل المصابات وغير مصابات بداء السكري. **طرق الدراسة:** تم فحص 600 عينة من البول من نساء حوامل في فئات عمرية مختلفة: 100 من النساء اللواتي لا يعانين من داء السكري ولا تظهر عليهم اعراض التهاب المسالك البولية و 100 لايعانون من اعراض السكري وتظهر عليهم اعراض التهاب المسالك البولية، 200 من النساء اللواتي يعانين من داء السكري ولا تظهر عليهم اعراض التهاب المسالك البولية ، و 200 من النساء اللواتي يعانين من داء السكري و تظهر عليهم اعراض التهاب المسالك البولية. تم تحضير وسائط الزرع وفقًا لتعليمات الشركة المصنعة. **النتائج:** اظهرت النتائج بان عدد المزارع الايجابية في النساء الحوامل اللواتي ليس لديهم اعراض التهاب المسالك البولية هو 106 \300 (20%)، منهم 60 حالة من عدد 200 من هن مصابات بمرض السكري، و 46 حالة من عدد 100 (46%) ممن هن غير مصابات بمرض السكري. كما كانت نتائج المزارع البكتيرية الايجابية من النساء الحوامل وتظهر عليهن اعراض المسالك البولية هو 195 من بين 300 (65%)، منهم 100حالة موجبة من عدد 200 أي (50%) ممن يعانون من مرض السكري و عدد 95 حالة موجبة من عدد 100 أي (95%) ممن لا يعانون من مرض السكري مع وجود نسبة عالية من عزلات الاشريكية القولونية في النساء الحوامل الذي لاتظهر عليهم اعراض بكتريا البول سواء المرضي بالسكري وبنسبة (56.7%)او الغير مرضى بالسكري وبالنسبة (26.1%). وكذلك نسبة عالية من هذه البكتريا في النساء التي تظهر عليهم اعراض المسالك ولذيهم السكري وبنسبة (34.7%) وممن ليس لديهم السكري وبنسبة (30%). **الاستنتاج:** نستنتج من هذه الدراسة ان الإشريكية القولونية من أهم أسباب الإصابة بالتهابات المسالك البولية، خاصة عند النساء الحوامل المصابات بداء السكري. وفي الخلاصة، فقد أبرزت هذه الدراسة الحاجة إلى زيادة الوعي بالتهاب المسالك البولية وتوسيع خدمات الوقاية والعلاج للنساء الحوامل. لذلك يُقترح إجراء فحص دوري للمرضى والعلاج بالمضاد للميكروبات بناًء على اختبار الحساسية في المختبر والاستجابة السريرية.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common problems encountered during pregnancy, with a significant number of patients requiring hospitalization [1]. Pregnancy increases the susceptibility of a pregnant woman to UTI infection due to the unique physiological changes that occur in the urinary tract [2, 3].

UTIs in pregnancy are of two forms, Asymptomatic bacteriuria (ASB) in which urine culture have positive result (bacteria ≥1 × 10^5 colony-forming units per millilitre of urine) without clinical manifestations and symptomatic bacteriuria (SBU). ASB occurs in 2–10% of pregnant women [4] if untreated, it can cause serious complications such as pyelonephritis, preterm birth, and low birth weight [5]. Diabetes, including gestational diabetes mellitus (GDM) is believed to be an additional risk factor for UTI [6]. GDM is a condition in which women with no history of diabetes have high blood glucose levels during pregnancy. Women with GDM experience disturbances in the regulation of iron storage and transport in the blood, and reduced kidney function through reduced glomerular filtration rate [7].

Diabetic women are much more likely to develop asymptomatic bacteriuria (ASB) compared with non-diabetic women [8]. In addition to a higher risk of UTI, women with diabetes are at increased risk developing complications for both mother and fetus, including low birth weight, preterm labor, premature birth, preeclampsia, hypertension [9,10], emphysematous cystitis, pyelonephritis [11]. The cause of UTIs in women is usually colonization of the vagina and then the urethra with bacteria from the intestinal tract. Commensal colonic gram-negative aerobic bacteria cause most bacterial UTIs [12]. E. coli with specific attachment factors for transitional epithelium of the bladder and ureters are the most frequent causes [13]. The remaining gram-negative urinary pathogens are other *enterobacteria*, especially *Klebsiella, Proteus mirabilis,* and *Pseudomonas aeruginosa. Enterococci* (group D *streptococci*) and coagulase-negative *staphylococci* (e.g., *Staphylococcus saprophytic*) are the most frequently implicated gram-positive organisms [14].

The aim of the present work was to investigate the bacterial profile and antibiogram pattern of bacteriuria in pregnant women in Libya and associated risk factor as well as to assess whether diabetes in pregnancy have influenced the UTIs.

METHODS

Study Subjects

Urine samples were randomly collected from a total of 600 diabetic and non-diabetic pregnant women attending the Obstetrics and Gynecology Clinic at Al-jala Hospital in Libya in 6 months' time (1ST December 2010 to the 29th May 2011). Analysis of the urine was critical in determining the likelihood of infection.

Following, explanation the purpose of study to the patients, consent for participant were taken and samples were collected. Clean catch mid-stream voided urine was collected from each patient. All the samples were collected in clean sterile containers, and labeled according to each patient's information. The samples were labeled and transported immediately on iced pack for analysis within 30 min to 1 h of collection to the Medical Microbiology laboratory of Al-jala Hospital. The media used in this study were Nutrient agar (NA) from Biotec Limited, MacConkey agar (MCA), Blood Agar (BA) and Cystein Lactose Electrolyte Deficient (CLED) Agar medium were supplied by Oxoid Limited and prepared according to the manufacturer's instructions.

Culturing of bacteria from urine samples was carried out as described by Whiting P *et al.* (2006) [15], 0.01 ml of un-centrifuged, mid-stream urine specimen were inoculated onto each of NA, MCA, BA and CLED) plates using a sterile swab or inoculating loop and incubated overnight at 37°C. The pure colonies of isolates bacteria were transferred to nutrient agar slants and stored in refrigerator at 4°C for use in further tests.

Characterization of isolated bacteria was achieved using Gram's staining reactions, culture characteristic

and API-20E test. Complete urine analysis was performed using reagent Multistix Pro 10 LS urine reagent test strips (Bayer) according to the manufacturer's instructions. The urine was centrifuged and examined microscopically for White blood cell (WBC), Red blood cell (RBC), crystals, casts, bacteria and yeast.

Statistical analysis

The collected data and the results of laboratory were entered a computerized database and statistically analysis by SPSS presenting the frequencies and percent of the outcomes. To evaluate the relationship between the bacterial positive culture and related risk factors (age groups distribution, and proportion of UTIs and the type of bacteria within non-diabetic and diabetic pregnant women), ANOVA test had been used at 95% level of significance [16].

RESULTS

The prevalence ABU and SBU in pregnant women with or without diabetes

As shown in table 1, overall prevalence of bacteriuria regardless ABU or SBU, diabetic or non- diabetic is 50% (301 out of 600). The percentage of positive culture among asymptomatic UTI tested women were 35% (106 out of 300), from that 60 out of 200 cases (30%) recorded in diabetic patients and 46 out 100 cases (46%) in non-diabetic. The number of positive cultures among symptomatic women tested for UTI were (65%), from that 47% of cases reported in diabetic patients and 100 % in non-diabetic patients.

Table 1: The prevalence ABU and SBU in pregnant women with or without diabetes

Prevalence of bacteriuria	Non-diabetic	Diabetic	Total
Asymptomatic bacteriuria (ABU)	46 out of 100 **(46%)**	60 out of 200 **(30%)**	106 out of 300 **(35%)**
Symptomatic Bacteriuria (SBU)	100 out of 100 **(100%)**	95 out of 200 **(47%)**	195 out of 300 **(65%)**
Total	146 out of 200 **(73%)**	155 out of 400 **(38.8)**	301 out of 600 **(50%)**

Frequency of UTI according to the age

As shown in table 2, according to the age group distributions among 200 non-diabetic, the age of 31-35 years had the highest incidence of infection (n=52) (26%). The age group with the second highest incidence of infection was the 21-25 years' group (24%) (n= 48), while the lowest frequency was in the 20-year-old patients (7%) (n=14), and the above 40 years' age group (4.5%) (n= 9). Regarding, age group distributions among 400 diabetic patients, the highest incidence of infection was 24.3% in the 31-35years age group (n=92), followed by age group 36-40 years old patients 23% (n=92) and age group 26-30-year-old patients with 21.2% (n=85), while the lowest incidence of infection was seen among patients above 40 years old with 8.5% (n=34), and in less than 20-year-old patients with 7% (n= 28).

Table 2: Age group distributions among non-diabetic and diabetic pregnant women

Age Groups (years old)	Non-diabetic Pregnant Women		Diabetic Pregnant Women	
	No.	%	*No.*	%
15-20	14	7	28	7
21-25	48	24	64	16
26-30	45	22.5	85	21.2
31-35	52	26	97	24.3
36-40	32	16	92	23
41-45	9	4.5	34	8.5
Total	200	100	400	100

Distribution of Type of diabetes in pregnant women with UTIs

Of all pregnant women with diabetes, the majority were diagnosed with type II diabetes (48%), followed with GDM (30%) while type I diabetes accounted for 21% of diabetic pregnant women (Table 3).

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Table 3: Types and number and incidence percentages of different types of diabetes mellitus in pregnant women.

Diabetes mellitus types	Number of positive samples	
	No.	%
Type I	85	21.2
Type II	195	48.8
Gestational diabetes	120	30
Total	400	100

Distribution of the UTIs according to pregnancy trimesters

The results showed a highest incidence of bacterial infection among diabetic pregnant women was during the third trimester, with 77 out of 155 diabetic patients (49.7%). Infection during the first trimester and second trimester was low with 38 patients (24.5%) and 40 patients (25.8%) respectively. Similarly, the highest incidence of bacterial infection among non-diabetic pregnant women was during the third trimester, with 69 out of 146 non-diabetic patients (47.2%). Infection during the first trimester and second trimester was low with 35patients (24. %) and 42 patients (28.8%) respectively. There was no significant difference of the incidence of infection between non-diabetic and diabetic patients in the three trimesters (Table 4).

Table 4: Relationship between trimester of pregnancy to incidence of UTIs in non-diabetic and diabetic pregnant women.

Pregnancy period	**Positive culture of non-diabetic pregnant women**		**Positive of diabetic pregnant women**	
	No.	**%**	**No.**	**%**
First trimester	35	24.0	38	24.5
Second trimester	42	28.8	40	25.8
Third trimester	69	47.2	77	49.7
Total	146	100	155	100

Level of pus cells /ml, and bacteriuria in urine among diabetic and non-diabetic pregnant women

Urinalysis Reagent Test Strips by Medi-test Combi used to analyze urine samples for glucose, protein, ketones, and specific gravity. level of bacteria in routine urine examination obtained from non-diabetic and diabetic pregnant women having UTIs were measured. As shown in table 5, colony counts reveal significant bacterial growth (3+ colonies or $\geq 10^5$CFU/ml) in just above half of analyzed samples in diabetic patients and 43.8% of non-diabetic patients. levels of sugar in routine urine examination taken from diabetic pregnant women are shown in Table 5. Out of all studied participants' samples, 70% of non-diabetic pregnant women have more than 12 pus cell/ ml in their urine samples and 28% had 8-12 pus cells/ ml. while urinalysis of diabetic pregnant women reveals nearly half of them (46.2%) have 8-12 pus cells/ ml and the second have (47.8%) have more than 12 pus cells/ ml, only 2% of non-diabetic women and 6% of diabetic women have few pus cells (6-8 pus cells/ml) shed in the urine.

Table 5: Counts of pus cells/ml and Level of bacteria in routine urine examination taken from non-diabetic and diabetic pregnant women.

Urine / Cases	**Number of pus cell/ml**						
	6-8 pus cell/ml		8-12 pus cell/ml		> 12 pus cell/ml		Total
	No.	%	No.	%	No.	%	
Non-diabetes pregnant women	4	2	56	28	140	70	200
Diabetic pregnant women	24	6	185	46.2	191	47.8	400
Urine / Cases	**Level of bacteriuria**						
	Low (+)		Medium (++)		High (+++)		Total
	No.	%	No.	%	No.	%	
Non-diabetes pregnant women	21	14.4	61	41.8	64	43.8	146
Diabetic pregnant women	33	21.3	43	27.7	79	51	155

Medium (++)= 10^3 * High (+++) =105

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Frequency and bacteriuria profile among diabetic and non-diabetic pregnant women in both ABU and SBU cases.

The results in Table 6 showed that the highest percentage of pathogen was isolated from diabetic patients with ABU was *Escherichia coli* (34 /60;56.7%). *Escherichia coli* was also the predominant pathogen from non-diabetic with ABU patients (12 /46 or 26%), and non-diabetic with SBU patients (30/100 ;30%). *Escherichia coli was* the second most predominant pathogens after *Pseudomonas spp* in diabetic SBU patients (36.8% and 34.4% respectively). Interestingly, *E. coli, K. aeroginosa, and S. aureus* were the most pathogens isolated from pregnant women in both asymptomatic and non-asymptomatic in non-diabetic cases. While, *E. coli, K. pneumonia* and *Pseudomonas spp were* the most pathogens isolated from pregnant women in both asymptomatic and non-asymptomatic in diabetic cases.

Table 6: Frequency of urinary pathogens cases.

Type of symptom	Diabetic	No	%	Non-diabetic	No	%
Asymptomatic	*E. coli*	34	56.7	*E. coli*	12	26.1
	Pseudomonos spp	10	16.7	*Klebsiella aeroginosa*	10	21.7
	Klebsiella pneumonia	8	13.3	*S.aureus*	10	21.7
	S.aureus	8	13.3	*Enterococcus spp*	8	17.4
				Proteus spp	6	13.1
	Total	**60**	**100**	**Total**	**46**	**100**
Symptomatic	*E.coli*	33	34.7	*E.coli*	30	30
	Pseudomonos spp	35	36.8	*S.aureus*	22	22
	Klebsiella pneumonia	21	22.2	*Klebsiella aeroginosa*	21	21
	Enterococcus spp	6	6.3	*Pseudomonos aeroginosa*	15	15
				Proteus	7	7
				Mixed klebisella & staphylococcus	5	5
	Total	**95**	**100**	**Total**	**10**	**100**
					0	
	Total diabetics	**155**		**Total non-diabetics**	**145**	

DISSCUSION

Urinary Tract Infections (UTIs) are one of the most frequently encountered problems during pregnancy, leading to hospitalization and serious complications if left untreated [17]. Diabetes, including gestational diabetes, is considered an additional risk factor for both symptomatic and non-symptomatic urinary tract infections [6]. These relations remain uncertain because of the scarcity of studies discussing these factors. our study compared the rates of ABU and SBU in pregnant women with and without diabetes in one of the main and largest Obstetrics and Gynecology Clinic in Libya that we believe it continued to be the first study in Libya to discuss these factors. Despite the delay in publishing our results (due to political instability following the data collected) these data are still of value, particularly with the increasing number of low birth weight and premature and infected births in Libya.

The overall incidence rate of bacteriuria in our study was 50% (301/600). We found that the prevalence of ABU was lower in pregnant women with diabetes than in their nondiabetic counterparts (30% vs. 46%). This result contrasts with a study by Geeerling *et al.*, who suggested that ABU is more prominent in pregnant women with diabetes than in those without diabetes [18]. Similarly, the rate of symptomatic UTI (SBU) among patients with diabetes was significantly lower than patients without diabetic. These results agree with those of a study by Rizk *et al.* (2001), which suggested that gestational diabetes was not associated with increased urinary tract infection [19]. The overall percentages of bacteriuria in diabetic versus non-diabetic patients for both ABU and SBU were 38.8% and 73%, respectively. However, considering the acceptable threshold of the number of bacteria per high-power field (equivalent to 10^5CFU = +++ in our urinalysis; Table 3), the rate of bacteriuria in both ABU and SBU will be reduced

from 38.8% in diabetic patients to 19.8% (79/400) and from 73% in non-diabetic patients to 32% (64/200).

Regarding the age group distribution among 400 diabetic patients, the highest prevalence of infection was recorded in age group 31-35 years (24.3%), followed by age group 36-40 (23%). The lowest incidence of infection was observed among age groups above 40 years (n=34) (8.5%) and in less than 20-year-old patients (n= 28) (7%). The relatively low infection rate in their over-40s and under-20s in this study may be due to the nature of the study population, which was in the most sexually active period of their lives (21-40 years).

Our study found that diabetes was more likely to increase the UTI rate among female aged between (36-45 years old). However, age group 20-25 years old shows non-diabetics pregnant women are more likely to be infected than pregnant women with diabetes. These observations are in agreement with a previous study suggesting that asymptomatic bacteriuria occurs more frequently in older diabetic females than in non-diabetic females [20].

Our survey showed that the prevalence of type 2 diabetes in pregnant women was higher than that of type 1 and Gestational Diabetes Mellitus (GDM). These results are in agreement with the findings of Rizk *et al.* (2001) that GDM is not associated with the risk of UTI [19]. The results of these studies reported that the highest rate of UTI occurred during the third trimester in both diabetic (49.7% [77 out of 155]) and non-diabetic pregnant women (47.2% [69 out of 146]). Following the second trimester, 28.8% and 25.8% of non-diabetic and diabetic pregnant women had diabetes respectively. In agreement with our results, Karlowsky *et al.* (2006) reported that women in the second and third trimesters were more likely to have UTI (41.4 and 55.1 %, respectively). They recommended that pregnant women should be screened for bacteriuria by urine culture between the 12th and 16th week of pregnancy [21]. However, the systemic review study commences by USPSTF (US Preventive Services Task Force) concluded and recommended screening asymptomatic pregnant women with gestational diabetes at 24 weeks and above [10]. This recommendation goes well with our results that the highest rate of UTI occurs at third trimesters.

Escherichia coli is the predominant Gram-negative bacteria, isolated from pregnant women. The rate of asymptomatic bacteriuria in non-diabetic pregnant women was (26.1%) whereas that in asymptomatic pregnant women with diabetes was (56.7%). *Escherichia coli* was also the most commonly isolated bacterium at a rate of (30%) from non-diabetic pregnant women and at the rate of (34.7%) of diabetic pregnant women with symptomatic bacteriuria. These results are in agreement with other studies in which *Escherichia coli* was the most commonly isolated organism [22, 23]. This could be attributed to the fact that *Escherichia coli* is a commensal of the gut and that infection occurs mainly through fecal contamination [22]. Other frequently isolated bacterial pathogens included *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa, Proteus spp., and Enterococcus spp.* at different rates (Table 4).

Interestingly, we found that the predominant pathogens in both diabetic pregnant women were *Escherichia coli* and *Pseudomonas aeruginosa*, while the most common pathogens in non-diabetic pregnant women were *Escherichia coli* and *Klebsiella aeruginosa*. We could not determine an explanation for these results. *Klebsiella aeruginosa* is a hospital acquired organism while our samples were taken from outpatients. However, the rate of infection was reduced from 38.8% in diabetic patients into 19.8% (79/400) and from 73% in non-diabetic patients into 32% (64/200) when interpreting the results guided by the result of urinalysis and the laboratory culture. Therefore, there could have been an improper collection of the midstream urine sample or a delay in the transport of the sample to the laboratory, which could have resulted in a false positive culture report [24].

CONCLUSION

In conclusion, this study highlights the need for greater awareness of tract infection and the expansion of prevention and treatment services for

pregnant women. Therefore, routine screening of patients and antimicrobial therapy should be guided by both in vitro susceptibility and clinical responses. Asymptomatic bacteriuria exceeding 105 CFU/ml with the full picture of urinalysis and the level of pus cells are also indicators. In both cases, a 7-10 day course of the appropriate drug should eliminate the infection, although this must be confirmed by repeat cultures [25].

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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