ORIGINAL RESEARCH

Study On Epidemiological Profile Of Asymptomatic Bacteriuria In Pregnancy And Its Outcome: A Pilot Study

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Received date: 06 February, 2024 Acceptance date: 14 March, 2024

ABSTRACT

Background: To study the prevalence of asymptomatic bacteriuria in pregnancy, for its early screening, diagnosis, and appropriate treatment. **Materials & Methods:**The present study was conducted on antenatal patients in the Department of Obstetrics and Gynecology and Microbiology of Dr. B.L. Kapur Memorial Hospital, Pusa Road, New Delhi.Methods for diagnosing ASB include midstream urine culture (the gold standard), Gram stain and urine dipstick tests. A urine culture can take up to two days to get a result, with the threshold for diagnosis usually defined as the presence of 10^5 colony-forming units (CFU)/mL of a single organism. Data collected during the study was tabulated in Microsoft Excel. Statistical analysis wasconducted with the statistical package for the social science software version (SPSS 20.0). The quantitative data were processed by Student t test and ANOVA and qualitative measurements by Chi-square test. For all statistical tests, a p value less than 0.05 was taken to indicate a significant difference/association. **Results:**Overall prevalence of asymptomatic bacteriuria was observed to be 6.21% (9/145) among the pregnant females.In present study, urine samples were collected in all the three trimesters for screening from all the subjects. Means and standard deviations of gestational periods (POG) at the time of screening were 10.7±1.16, 20.7±2.27 and 32.2±2.17 weeks in first, second and third trimester respectively. A subject showing pus cells ≥10/HPF in any trimester during her pregnancy was considered as having a positive urine routine microscopy test. Of the 145 total subjects, 68 subjects (46.90%) were positive for this test. **Conclusion:**The most common uropathological isolate found in our study was *Escherichia coli* (88.9%) followed by *Klebsiella* (11.1%).Complications related to asymptomatic bacteriuria occur more profoundly if left untreated to reach the stage of pyelonephritis. All the sequelae of ASB during pregnancy can be reduced by early detectionand appropriate antimicrobial treatment. Hence, it should be made mandatory to screen every antenatal women in each trimester, to save bigger complications at lesser cost.

Keywords: Asymptomatic bacteriuria, Pregnancy, Treatment.

INTRODUCTION

Urinary tract infections are the most common bacterial infection in pregnancy. It refers to both colonization of the urine and tissue invasion of any structure of urinary tract. Its incidence ranges from 2 to 10 % in pregnancy and varies a little from non-pregnant state.[1, 2, 3]

Significant bacteriuria, as introduced by Kass is presence of at least 1,00,000 colony forming units (CFU) of the same species of microorganism per ml of urine on culture of a carefully collected urine sample.Asymptomatic bacteriuria is the persistent presence of bacteria within urinary tract in absence of clinical features which include- lower abdominal pain, burning micturition, fever, dysuria, frequency, urgency, supra pubic discomfort, offensive smelling urine, strangury, urge incontinence and nocturia.Acute cystitis is significant bacteriuria with associated bladder mucosal invasion and often the urethra is also infected. It is distinguished from asymptomatic bacteriuria by the presence of symptoms in afebrile women with no evidence of systemic illness. [4,5] Most mothers may not be aware that they are having the infection because urgency and frequency are common symptoms in a normal pregnancy.

Maternal complications which are associated with asymptomatic bacteriuria are hypertension, pre-eclampsia, maternal anemia, chorioamnionitis, symptomatic acute cystitis and pyelonephritis. Neonatal complications associated are intrauterine growth restriction, low birth weight and pre-term premature rupture of membrane.[6,7,8]It is hypothesized that the mechanism of preterm labor is associated with microorganism producing phospholipase A2 and subsequent prostaglandin activation. These complications are commonly associated with

pyelonephritis which occurs because of undiagnosed or inadequately treated infections of the urinary tract [4, 5, 9,10]. Maternal UTI has few direct fetal sequelae because fetal bloodstream infection is rare; however, uterine hypoperfusion due to maternal dehydration, maternal anemia, and direct bacterial endotoxin damage to the placental vasculature may cause fetal cerebral hypoperfusion.Asymptomatic bacteriuria should be treated with antimicrobials even though the mother has no clinical symptoms. Various studies have proven that early treatment of asymptomatic bacteriuria in pregnancy reduces the incidence of acute pyelonephritis and decreases the incidence of pre-term delivery and low birth weight infants.[11,12,13] Early recognition and treatment of asymptomatic bacteriuria can reduce up to 70% of acute symptomatic UTIs. [14]Treatment of asymptomatic bacteriuria according to NICE guidelines is a choice between cefalexin, amoxicillin or nitrofurantoin based on recent culture and susceptibility results. Hence, this study was conducted to evaluate the prevalence of asymptomatic bacteriuria in pregnancy, for its early screening, diagnosis and appropriate treatment.

MATERIALS & METHODS

The present study was conducted on antenatal patients in the Department of Obstetrics and Gynecology and Microbiology of Dr. B.L. Kapur Memorial Hospital, Pusa Road, New Delhi.Methods for diagnosing ASB include midstream urine culture (the gold standard), Gram stain and urine dipstick tests. A urine culture can take up to two days to get a result, with the threshold for diagnosis usually defined as the presence of 105 colony-forming units (CFU)/mL of a single organism. The Gram stain test uses colour stains (crystal violet and safranin O) to exaggerate and distinguish between Gram positive (purple) and Gram-negative (red) organisms on a prepared glass slide. Urine dipstick tests for nitrites (not found in normal urine) and leucocytes, which are identified by a reaction with leucocyte esterase, to identify the presence of bacteria and pus in the urine, respectively.Data collected during the study was tabulated in Microsoft Excel. Statistical analysis wasconducted with the statistical package for the social science software version (SPSS 20.0). Continuous variables were presented as Mean±SD. Categorical variables were expressed as frequencies and percentages. Nominal categorical data between the groups were compared using Chi-square test. The quantitative data were processed by Student t test and ANOVA and qualitative measurements by Chi-square test. For all statistical tests, a p value less than 0.05 was taken to indicate a significant difference/association.

RESULTS

The age distribution of all the subjects 31.72% (46/145) subjects were from 23-27 years age group; 59.31% (86/145) were from 28-32 years age group and 8.96% (13/145) from 33-37% age group.Urine routine microscopy was repeated in each trimester on each subject and percentage positivity was computed for each trimester. Positivity rate was observed to be maximum in the first trimester results, i.e., 30.34% (44/145) followed by second and third trimester as 28.27% (41/145) and 17.93% (26/145) respectively.

Table 1: Age distribution among the subjects under study

Age group (in years)	Number of subjects (N=145)	%
23-27	46	31.72
28-32	86	59.31
33-37	13	8.96
TOTAL	**145**	**100**

Table 2: Trimester wise results of urine routine microscopy among the subjects under study

Trimester	Total number of urine samples collected (N)	Number of subjects found positive for pus cells (≥10) on routine microscopy		% Positivity (N=145)
		Positive	Negative	
I	145	**44**	101	**30.34**
II	145	**41**	104	**28.27**
III	145	**26**	119	**17.93**

Table 3: Statistical analysis of Pus cells positivity on urine routine microscopy in all three trimesters

Trimester	Samples found positive for pus cells (≥10) on urine routine microscopy		TOTAL (N)
	Yes	No	
I (N=145)	44 (30.34%)	101	145
II (N=145)	41 (28.28%)	104	145
III (N=145)	26 (17.93%)	119	145
TOTAL (N=435)	**111 (25.52%)**	**324**	**435**

Chi-square = 6.749 with 2 degrees of freedom; P = 0.034 (Significant)

A total 435 samples were tested for urine routine microscopy collected from totally asymptomatic pregnant females throughout their pregnancy (one from each trimester), out of which 25.52% (111/435) samples showed positive results, i.e., pus cells ≥10. All these results necessitated further urine culture test. When compared by chi-square test, correlation between urine routine microscopy and trimester was observed to statistically significant with p value of 0.034 (i.e., <0.05). Based on the result of the test of significance, it can be stated that probability of finding a positive urine routine microscopy test result is maximum in the first trimester.

Table 4: Urine examination reports: Routine microscopy and culture reports

Urine routine microscopy	Urine culture report	No. of reports generated in			Total No. of final reports (N=435)
		I Trimester (N=145)	II Trimester (N=145)	III Trimester (N=145)	
Pus cells ≥10	*E.coli*	6	1	1	8(1.84%)
	Klebsiella	1	0	0	1 (0.23%)
	Sterile	37	40	25	102 (23.45%)
	Sub-total	44	41	26	111 (25.52%)
Pus cells <10	CND	101	104	119	324 (74.48%)
	TOTAL	**145**	**145**	**145**	**435 (100%)**

[CND = Culture Not Done (Because Pus cells on urine routine microscopy <10)]

Urine culture was not performed among the subjects when their urine sample was found to be negative on urine routine microscopy (324/435; 74.48%) while following the positive urine routine microscopy, further urine culture was performed (111/435; 25.52%).On urine culture, 1.84% (8/435) samples were detected positive for E.coli; 0.23% (1/435) samples were found positive for Klebsiella and 23.45% (102/435) samples were found to be sterile among the asymptomatic pregnant females enrolled under the present study.Out of total 9 culture positive cases, 8 were E. coli, constituting the maximum number (88.9%) of organisms isolated. Only 1 case of Klebsiella (11.1%) was found.

Table 5: Overall prevalence of asymptomatic bacteriuria among the pregnant females under study

Total number of asymptomatic pregnant females under study (N)	Number of subjects found positive for bacteriuria on urine culture	% Prevalence (N=145)
145	**9**	**6.21**

Overall prevalence of asymptomatic bacteriuria was observed to be 6.21% (9/145) among the pregnant females.

Table 6: Trimester wise prevalence of asymptomatic bacteriuria among the pregnant females under study

Trimester	Total number of asymptomatic pregnant females	Number of subjects found positive for bacteriuria on urine culture	% Prevalence
I	**145**	**7**	**4.83**
II	**145**	**1**	**0.69**
III	**145**	**1**	**0.69**

Trimester wise prevalence of asymptomatic bacteriuria has been depicted in Table 11. It was observed to be 4.83% (7/145) in first trimester, 0.69% (1/145) in second trimester and 0.69% (1/145) in third trimester

Table 7: Statistical analysis of urine routine microscopy versus preterm delivery

(Pus cells ≥10 on urine routine microscopy)	Preterm delivery		TOTAL
	Yes	No	
Yes	5	63	68
No	0	77	77
TOTAL	**5**	**140**	**145**

Chi-square = 3.863 with 1 degree of freedom; P = 0.049 (Significant)

A comparison between occurrence of pre-term delivery and positive urine routine microscopy. On applying chi-square test, the association was found to be significant with a p value of 0.049, i.e., less than 0.05.

Table 8: Statistical analysis of urine culture reports versus low birth weight

Urine culture report	LBW delivery		TOTAL
	Yes	No	
E. coli (N=8)	3 (37.5%)	5	8
Klebsiella (N=1)	0 (0.00%)	1	1
Sterile (N=102)	4 (3.92%)	98	102
TOTAL (N=111)	**7 (6.31%)**	**104**	**111**

Chi-square = 14.224 with 2 degrees of freedom; P = 0.0001 (Highly significant)
Among the total 8 E.coli cases, 3 (37.5%) had LBW delivery. Among the 102 cases, who showed positive urine routine microscopy tests but revealed sterile culture on the urine culture tests, 4 cases (3.92%) observed to have LBW delivery.

Table 9: Antibiotic sensitivity pattern among the culture positive subjects

Causative Organisms	Antibiotic Drugs				
	AM	**AC**	**NIT**	**CEF**	**FOF**
E. coli(N=8)	37.5% (3)	62.5% (5)	75% (6)	87.5% (7)	87.5% (7)
Klebsiella (N=1)	0% (0)	0% (0)	100% (1)	100% (1)	100% (1)
Total (N=9)	**33.3% (3)**	**66.7% (5)**	**77.8% (7)**	**88.9% (8)**	**88.9% (8)**

(AM-Amoxicillin, AC-Amoxicillin-Clavulanic acid, NIT-Nitrofurantoin, CEF-Cefuroxime, FOF-Fosfomycin)
Maximum sensitivity for FOF and CEF, both accounting for 88.9% out of total 9 culture positive cases. E. coli had 87.5% sensitivity (7 sensitive cases) while Klebsiella showed 100% sensitivity. AC and NIT followed the sensitivity of 66.7% and 77.8% respectively. Maximum resistance was found with AM (62.5%) with only 33.3% overall sensitivity.

DISCUSSION

Relapse and reinfections are frequently seen in pregnancy. Relapse is the occurrence of infection by the same strain of organism within six weeks of initial infection whereas reinfection is the recurrence of bacteria with a different strain of bacteria after successful eradication of initial infection. Recurrent pyelonephritis has been implicated as a cause of intra uterine growth restriction and fetal death. The overall incidence of recurrence is about 2-3% and it can recur during the same pregnancy. [14] Hence, this study was conducted to evaluate the prevalence of asymptomatic bacteriuria in pregnancy, for its early screening, diagnosis and appropriate treatment.
In the present study, the age distribution of all the subjects 31.72% (46/145) subjects were from 23-27 years age group; 59.31% (86/145) were from 28-32 years age group and 8.96% (13/145) from 33-37% age group.Urine routine microscopy was repeated in each trimester on each subject and percentage positivity was computed for each trimester. Positivity rate was observed to be maximum in the first trimester results, i.e., 30.34% (44/145) followed by second and third trimester as 28.27% (41/145) and 17.93% (26/145) respectively. A total 435 samples were tested for urine routine microscopy collected from totally asymptomatic pregnant females throughout their pregnancy (one from each trimester), out of which 25.52% (111/435) samples showed positive results, i.e., pus cells ≥10. All these results necessitated further urine culture test. When compared by chi-square test, correlation between urine routine microscopy and trimester was observed to statistically significant with p value of 0.034 (i.e., <0.05). Based on the result of the test of significance, it can be stated that probability of finding a positive urine routine microscopy test result is maximum in the first trimester.Azami et al. (2019) [15] found that the prevalence of ASB in pregnant Iranian women based on trimester of pregnancy shows that the highest prevalence occurs in the first trimester of pregnancy (11.7% [95% CI: 7.9–16.9]). Elzayat et al. (2017) [16] and Onuh et al. (2006) [17]reported a higher prevalence of urinary tract infection in the second trimester compared to the third trimester. Contrary to these, many studies have suggested that the incidence and the risk of acquiring bacteriuria enhanced along with the progression of pregnancy from 0.8% at the end of first trimester to 2% at the end of pregnancy.[18-20] Kant et al. (2017) [21] reported that the proportion of pregnant women with UTI was maximum in the third trimester.
In the present study, urine culture was not performed among the subjects when their urine sample was found to be negative on urine routine microscopy (324/435; 74.48%) while following the positive urine routine microscopy, further urine culture was performed (111/435; 25.52%).On urine culture, 1.84% (8/435) samples were detected positive for E.coli; 0.23% (1/435) samples were found positive for Klebsiella and 23.45% (102/435) samples were found to be sterile among the asymptomatic pregnant females enrolled under the present study.Out of total 9 culture positive cases, 8 were E. coli, constituting the maximum number (88.9%) of organisms isolated. Only 1 case of Klebsiella (11.1%) was found. Overall prevalence of asymptomatic bacteriuria was observed to be 6.21% (9/145) among the pregnant females. A comparison between occurrence of pre-term delivery and positive urine routine microscopy. On applying chi-square test, the association was found to be significant with a p value of 0.049, i.e., less than 0.05.Among the total 8 E.coli cases, 3 (37.5%) had LBW delivery. Among the 102 cases, who showed positive urine routine microscopy tests but revealed sterile culture on the urine culture tests, 4 cases (3.92%) observed to have LBW delivery. Maximum sensitivity for FOF and CEF, both accounting for 88.9% out of total 9 culture positive cases. E. coli had 87.5% sensitivity (7 sensitive cases) while Klebsiella showed 100% sensitivity. AC and NIT followed the sensitivity of 66.7% and 77.8% respectively. Maximum resistance was found with AM (62.5%) with only 33.3% overall sensitivity.The frequencies of virulence associated determinants are lower in E. coli associated with asymptomatic bacteriuria compared

with pyelonephritis. Only 22% of strains of E.coli isolated from women with asymptomatic bacteriuria had the capacity to adhere to uroepithelial cells compared with 75% in the group of women who developed acute pyelonephritis. Adherence is the single marker most frequently associated with progression to pyelonephritis.[22]

Schieve and associates [23] conducted a study involving 25,746 pregnant women and found that the presence of UTI was associated with premature labor (labor onset before 37 weeks of gestation), hypertensive disorders of pregnancy (such as pregnancy-induced hypertension and preeclampsia), anaemia (hematocrit level less than 30 percent) and amnionitis .While this does not prove a cause and effect relationship, randomized trials have demonstrated that antibiotic treatment decreases the incidence of preterm birth and low-birth-weight infants.[24]A risk of urosepsis and chronic pyelonephritis was also found.[25]In addition, acute pyelonephritis has been associated with anaemia.[26]Pyelonephritis in pregnancy has been associated with many perinatal complications including bacteraemia, respiratory insufficiency, anaemia, renal disease, hypertension, preterm labour and low birth weight. J. Schnarr and F. Smaill (2008) [27] concluded the two strongest predictors of bacteriuria at prenatal care initiation were an antepartum urinary tract infection prior to prenatal care initiation [for whites, adjusted prevalence odds ratio (POR) 2.5, 95% confidence interval (CI) 0.6–9.8; for blacks, POR 8.8, 95% CI 3.8–20.3] and a pre-pregnancy history of urinary tract infection (POR 2.1, 95% CI 1.4–3.2). A similar study in the same population to identify predictors of symptomatic urinary tract infection after 20 weeks gestation also concluded the strongest predictor of pyelonephritis was prior antenatal urinary tract infections (adjusted incidence odds ratio 5•3, 95% CI 2•6–11•0).[28]Guinto et al. (2010) [29] assessed the effectiveness of different antibiotics for the treatment of asymptomatic bacteriuria in pregnant women. There was no significant difference between fosfomycin and cefuroxime in reducing the incidence of persistent infection, or in the number of women who required a change of antibiotic (very low quality evidence). Similarly, there was no significant difference between pivmecillinam and ampicillin in the number of women with persistent infection after treatment or in the incidence of recurrent infection (very low quality evidence).ŞevkiÇelen et al. found that E. coli, the most frequent isolate, was 99.2% sensitive to fosfomycin while Klebsiella pneumonia, the second most common microorganism isolated in this study was also 88% sensitive to fosfomycin. Culture results for ampicillin and amoxicillin/clavulanate yielded 57.2% and 68% sensitivities to these agents, respectively. Sensitivity for cefuroxime was 86%, which was comparable to that of cefazolin (87%), cefepime (91.6%), and ceftriaxone (90%).[30]

CONCLUSION

All the sequelae of ASB during pregnancy can be reduced by early detection and appropriate antimicrobial treatment. Hence, regular and repeated trimester wise screening needs to be incorporated as a routine antenatal care for an integrated approach for safe motherhood and newborn health. The common pathogens involved are of fecal origin (E.coli. etc.). Because of complications associated with asymptomatic bacteriuria in pregnancy, it should be made mandatory to screen every antenatal women in early pregnancy for the same. Timely management of asymptomatic bacteriuria will prevent the adverse maternal and perinatal outcome associated with urinary tract infections.

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