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Student's Section

Prevalence of asymptomatic bacteriuria and sterile pyuria in pregnant women attending antenatal clinic in a tertiary care center in Karnataka: A pilot study

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ABSTRACT

Background: Asymptomatic bacteriuria (ASB) is common in pregnancy; its prevalence varies between communities and different ethnicities and countries, and has been implicated in adverse pregnancy outcomes.

Objectives: Primary objective to assess the prevalence of ASB and sterile pyuria pregnant women attending the antenatal clinic.

Setting: Antenatal Clinic of Kasturba Medical College, Manipal.

Design: Prospective cohort study. Included were pregnant women between 21 and 35 years, in 2nd or 3rd trimester of pregnancy after obtaining written, informed consent. Excluded were those (a) who had documented intake of antibiotics in the last 72 h (b) known HIV positive (c) had urethral catheterization at the time of presentation or in the last 7 days (d) were incontinent and (e) diagnosed diabetics. Bacteriuria was defined as urine culture showing $\geq 10^5$ colonies and sterile pyuria was defined as ≥ 10 white blood cells/pus cells or/and ≥ 5 red blood cells/per high power field on microscopic examination of centrifuged urine sample.

Results: Out of a sample 59 patients, 5 (8.74%; 95% CI 3.27–18.75) had ASB with Gram-negative bacteria, 40% resistant to amoxicillin and co-trimoxazole and all sensitive to aminoglycosides. Sterile pyuria was found in 17 (28.81%; 95% CI: 18.77–41.45).

Conclusions: About one-tenth of pregnant women have ASB in KMC, Manipal. And therefore routine urine cultures must be done during pregnancy to detect and treat it. However since almost 28% women also have sterile pyuria further research is needed to find out the cause for this using newer molecular diagnostic technique.

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1. Introduction

Urinary tract infections (UTIs) are common in women, especially during pregnancy, because of hormonal and mechanical factors, which increase risk for urinary stasis and ureterovesical reflux. Progesterone levels rise during pregnancy, which causes relaxation of smooth muscles of the whole urinary tract resulting in dilatation of the pelvis, and ureter and vesico-ureteral reflux, stasis of urine and predispose to infection risk of UTIs in pregnant women. This risk is aggravated by a short urethra and difficulty with hygiene as a result of a distended, pregnant belly.¹

The term ASB is defined as significant bacterial colonization of the lower urinary tract without symptoms (I). Traditional diagnostic criteria of significant bacteriuria include culture of 10^5 colony forming units (CFUs)/ml of a single uropathogen on two consecutive clean catch urine specimens.² Sterile pyuria was defined as ≥ 10 white blood cells/pus cells or/and ≥ 5 red blood cells/per high power field on microscopic examination of centrifuged urine sample and the urine culture was sterile for commonly isolated organisms.³

Bacteriuria often develops in the first few months of pregnancy and is frequently associated with a reduction in concentrating ability, suggesting involvement of the kidney. The smooth muscle relaxation and subsequent ureteral dilatation that accompany pregnancy are thought to facilitate the ascent of bacteria from the bladder to the kidney. As a result, bacteriuria during pregnancy has a greater propensity to progress to pyelonephritis (up to 40%) than in non-pregnant women.¹

Bacteriuria has been associated with an increased risk of preterm birth, low birth weight, and perinatal mortality.^{2,4} Studies have also shown that treatment of bacteriuria during pregnancy reduces the incidence of these complications and lowers the long-term risk of sequelae following ASB.^{2,4}

A study conducted in the US⁵ reported 43% and a study conducted in Aligarh, India reported about 39%⁶ of ASB in pregnant women. The most common organism associated with bacteriuria in pregnancy is *Escherichia coli* and among the gram-positive cocci, coagulase negative species of *Staphylococci*.^{5,6} High resistance was reported by the gram-negative bacilli as well as gram-positive cocci to the β -lactam group of antimicrobials, fluoroquinolones and aminoglycosides in India in women with bacteriuria.^{2,6} However, there is dearth of literature on the prevalence of ASB, especially from Southern India. Since antimicrobial resistance (AMR) patterns vary from community to community, there is a need for continuous surveillance and generation of community specific data. Hence there is a need to assess the prevalence of ASB in south Indian setup.

2. Methodology

2.1. Objectives

Primary objective was to assess the prevalence of ASB in pregnant women attending the antenatal clinic.

The secondary objectives were (a) to assess the prevalence of symptomatic bacteriuria and sterile pyuria in pregnant women attending the antenatal clinic, (b) to assess the common bacterial isolates in urine samples of pregnant women with asymptomatic and symptomatic bacteriuria, and (c) to assess the antimicrobial resistance in common bacterial isolates in urine samples of pregnant women with asymptomatic and symptomatic bacteriuria.

2.2. Setting

This study was conducted in the Antenatal Clinic, Department of Obstetrics and Gynecology as well as Department of Microbiology, Kasturba Medical College (KMC), Manipal. It was conducted after obtaining institutional ethical clearance. Thereafter, informed written consent was taken from the participants prior to recruitment.

2.3. Design

Prospective cross-sectional survey.

2.4. Participants

This study was conducted in immune-competent pregnant women, aged 21–35 years of age, in the 2nd or 3rd trimester of pregnancy coming for antenatal care in the department of Obstetrics and Gynecology. Excluded were those (a) who had documented intake of antibiotics in the last 72 h (b) were known HIV positive (c) had urethral catheterization at the time of presentation or in the last 7 days (d) were incontinent and (e) diagnosed diabetics.

2.5. Sample size

Initially we had assumed a conservative estimate of ASB of 50%, since it has been reported to be 43% from the US.⁵ Using the formula: $(1.96)^2 pq/d^2$, where $p = 0.5$, $q = 0.5$ and d , the margin of error is 0.1, 96 patients were to be recruited. However, sample size was recalculated after we had recruited 50 cases. We found a prevalence of bacteriuria in approximately 10% of pregnant women. To detect this with a margin of error of 8%, with a confidence level of 95% and a population size of 20,000, we required a minimum sample size of 54 using online sample size calculator.⁷ Therefore, we recruited 59 subjects.

2.6. Sampling technique

Consecutive pregnant women who fulfilled inclusion criteria and had no exclusion were invited to participate. Clinical data was abstracted from hospital records on preformed questionnaire. They were asked to collect midstream urine sample in sterile culture vial with all aseptic precautions. The urine was sent for culture to the Department of Microbiology.

2.7. Laboratory methods

Clean-catch midstream urine was collected from each patient into a sterile universal container. Samples were cultured on dried plates of blood agar and cysteine lactose electrolyte deficient agar (CLED), using a calibrated drop delivering 0.002 ml of urine. Plates were incubated aerobically of 37 °C overnight. Colony counts yielding bacterial growth of 10^5 /ml or more of pure isolates were regarded as significant for infection.

From each patient, 10 ml of urine was transferred into sterile centrifuge tubes and then centrifuged at 3000 rpm for 10–15 min. The supernatant was discarded and the deposit examined microscopically at high magnification for pus cells/white blood cells (WBC), red blood cells (RBC), epithelial cells, casts and crystals. Pus cells ≥ 10 or RBC > 5 per high power field were also considered as possible urinary tract infection.^{8,9}

The isolated organisms from culture plates were identified by standard laboratory techniques.¹⁰ Antimicrobial in-vitro susceptibility testing was performed using agar disc diffusion method. The National Committee for Clinical Laboratory Standard (NCCLS) operating procedure¹⁰ was followed.

2.8. Variables of data collection

Clinical data was collected on demographic variables like age, place of residence (urban versus rural), educational status of husband and wife and family income categories; obstetric variables like parity, gestational age, present medical history of diabetes and hypertension; and other clinical variables like past as well as current history of increased frequency and/or burning during micturition and pain in the loins.

2.9. Data management and analysis

Data was entered in MS Excel. Data was analyzed using IBM SPSS data analysis software version 21.0.0.¹¹ Descriptive analysis of all variables was done and has been reported. We are reporting the prevalence of ASB (primary outcome) as well as sterile pyuria, defined as $\geq$ WBC/pus cells or ≥ 5 RBCs per high power field.^{7,8} Symptomatic UTI was defined as presence of any of the following symptoms in a pregnant woman: increased frequency of micturition, burning/pain during micturition, and pain in loins. We also report proportion of patients having asymptomatic sterile pyuria as well as symptomatic sterile pyuria. Laboratory diagnosed UTI was defined as either isolation of a bacteria from urine culture with $>10^5$ colony count or centrifuged urine microscopic examination showing $\geq$ WBC/pus cells or ≥ 5 RBCs per high power field. We report 95% confidence interval of all proportions.

We have calculated sensitivity, specificity, predictive value positive as well as negative for urine microscopic findings of $\geq$ WBC/pus cells or ≥ 5 RBCs per high power field for the diagnosis of ASB, taking bacterial culture positivity as the gold standard. We also report the AMR of the bacterial isolates from the urine culture of pregnant women.

3. Results

This study was conducted from 5th May to 8th August 2012 in the Department of Obstetrics and Gynecology, KMC Manipal. Patients attending the antenatal clinics on Wednesday and Saturday who fulfilled inclusion criteria and had no exclusion criteria were invited for participation and were recruited after obtaining informed written consent. In the study duration 59 pregnant women were recruited. Baseline characteristics of enrolled women are given in Table 1.

Data on clinical characteristics of the women was noted and is reported in Table 2. There were only 4 (6.78%) women who had any symptom of suspected UTI and among these two each were in 2nd and 2 in 3rd trimester of pregnancy, respectively. Two (3.3%) women had history of pregnancy-induced hypertension and 3 (5.08%) had history of pain in abdomen. There were no patients with essential hypertension, chronic medical disease, previous UTI, and none of the patients were subjected to internal examination during the current pregnancy.

On urine culture examination were only 5/59 (8.5%) patients had ASB, which was the primary objective of this study (Table 4). There were no cases of symptomatic bacteriuria. However, on urine microscopic examination, sterile pyuria (≥ 10 WBCs/pus cells or ≥ 5 RBCs per high power field) was found in 17/59 (28.81%), and only 1 pregnant woman in this group was symptomatic (Table 3). Laboratory diagnosed UTI, defined as either positive bacterial urine culture or presence of sterile pyuria, was found in 22/59 (37.29%).

Table 1 – Baseline characteristics of enrolled subject (n = 59).

	Variable	Mean, SD
1	Age in years	27.78, SD = 3.86
2	Parity	1.51, 0.68
3	Gestational age in weeks	25.72, 6.92
	Variable	n, %
4	Family income in rupees	
	<Rs. 5000	4, 6.8%
	>Rs. 5000–<Rs. 10,000	24, 40.7%
	>Rs. 10,000–<Rs. 30,000	24, 40.7%
	>Rs. 30,000–<Rs. 50,000	2, 3.4%
	>Rs. 50,000	5, 8.5%
5	Primiparous	34, 57.6%
6	2nd Trimester	32, 54.2%
7	3rd Trimester	27, 45.8%
8	Education of woman	
	Uneducated	0
	Up to Class X	8, 13.6%
	Up to Class XII	12, 20.3%
	Graduation	30, 50.8%
	Post-graduation	9, 15.3%
9	Education of husband	
	Uneducated	1, 1.7%
	Up to Class X	11, 18.6%
	Up to Class XII	18, 29.5%
	Graduation	23, 39.0%
	Post-graduation	6, 10.2%
10	Rural residence	47, 79.7%

Table 2 – Clinical characteristics of enrolled subjects (categorical variables).

	Variable	n, %
1	History of pregnancy-induced hypertension	2, 3.3%
2	H/o gestational diabetes	0, 0%
3	H/o unexplained fever	0, 0%
4	H/o abdominal pain	3, 5.08%
5	H/o suspected symptomatic urinary tract infection (any symptom: increased frequency of micturition, burning/pain during micturition, pain in loins)	4, 6.78%

We have computed the diagnostic test characteristics of sterile pyuria for the diagnosis of UTI taking bacterial culture positivity as the gold standard in Table 4.

The values given in Table 4 show that if the urine microscopic test results are positive (that is ≥ 10 , WBCs/pus cells or ≥ 5 RBCs per high power field) there is only 10.69% chances of a patient having UTI which can be diagnosed by routinely used culture techniques, leaving 89.31% chances of the result being false positive.

Our secondary objective was to assess the AMR in common bacterial isolates in urine samples of pregnant women with asymptomatic and symptomatic bacteriuria. Since there were no cases of symptomatic bacteriuria this has only been reported for women with ASB in Table 5.

4. Discussion

This study done on 59 pregnant women between the ages of 21–35 years in a tertiary care hospital in Manipal, Karnataka found that 5 (8.47%) had ASB. There were only 4 (6.78%) cases of symptomatic UTI and none had bacteriuria. However, a significant proportion 17 (28.81%) had sterile pyuria in urine microscopic examination.

Predisposing factors to ASB include low socioeconomic status, increasing age, multiparity, sexual behavior, and a history of childhood UTIs (with or without scarring).² The prevalence of ASB also is increased markedly in certain pre-existing medical conditions, such as diabetes mellitus, sickle cell disease, immunocompromised states (e.g., AIDS), urinary tract anatomic anomalies, and spinal cord injuries. UTI before

Table 4 – Sensitivity and specificity of microscopic urine examination for diagnosis of urinary tract infection in asymptomatic pregnant women (n = 55), taking urine culture for bacteria as gold standard.

Bacterial isolates	Bacteria present N	Bacteria absent n
≥ 10 , WBCs/pus cells or ≥ 5 RBCs per high power field	2	16
< 10 WBCs/pus cells or < 5 RBCs per high power field	3	34
Sensitivity = 2/5 (40%; 95% CI: 11.6–77.1), Specificity = 34/50 (68%; 54.13–79.30), Positive predictive value (PPV) = 10.69%, Negative predictive value (NPV) = 92.21%.		

pregnancy is a predictor of the diagnosis of ASB at the first prenatal visit.²

The presence of ASB in pregnancy places patients at increased risk for the development of cystitis and pyelonephritis with their respective morbidities.^{2,4} A critical meta-analysis by Romero et al¹² showed the relationship between ASB alone and preterm delivery and low birth weight infants. The risk of preterm delivery in women who had ASB during gestation was two-fold greater than those who never were affected. With adequate treatment of ASB, the relative risk of low birth weight infants was 0.56 compared with an untreated group.⁴

Several theories have been suggested to explain the mechanism by which uncomplicated UTI triggers preterm labor and delivery. Bacterial endotoxin release is believed to provoke labor directly or through a prostaglandin-mediated cascade.² Alternatively, it is believed that UTI predisposes women to amnionitis, and thus, preterm labor. Although previously suggested, ASB does not seem to be related to pre-eclampsia or anemia. There also has been a suggestion that UTI during pregnancy is associated with developmental delay and mental retardation in the neonate.

Urinary tract infections during pregnancy are common and have been reported by various workers from India. In a study conducted in 2006 in Andhra Pradesh, in a total of 500 antenatal women in their first or second trimesters over a period of 2 years for ASB, 8.4% (n = 42) were culture positive. This is similar to our findings of prevalence of ASB. They reported that primigravida had highest percent culture positivity of 66.6%, and those < 20 years age group of 71.42%.¹³

Unlike our prevalence of ASB in pregnancy, a study conducted on 4290 pregnant women in Aligarh, India reported that 51.2% had bacteriuria and among them almost 3/4th were asymptomatic, giving a prevalence of 39% for ASB.⁶

In another study conducted in Jhalawar, Rajasthan, India, out of the 250 samples screened, a total of 60 (24%) samples of urine from pregnant females, in different stages of pregnancy were found to be positive on culture. The Enterobacteriaceae accounted for nearly two-thirds of the isolates and *E. coli* alone accounted for 63% of the urinary isolates followed by *Klebsiella pneumonia* 8%.¹⁴

In study conducted in Chandigarh a total of 161 pregnant ladies visiting a primary care clinic were screened for bacteriuria. Prevalence of symptomatic and ASB was found to be

Table 3 – Assessment of prevalence of asymptomatic bacteriuria, sterile pyuria, symptomatic urinary tract infection and laboratory diagnosed urinary tract infection in pregnant women in ANC clinics of KMC, Manipal.

S. no	Condition	N, %; (95% CI)
1	Asymptomatic bacteriuria	5, 8.47%; (3.27–18.75)
2	Sterile pyuria	17, 28.81%; (18.77–41.45)
3	Asymptomatic sterile pyuria	16, 27.12%; (17.35–39.68)
4	Symptomatic sterile pyuria	1, 1.69%; (0.01–9.85)
5	Lab diagnosed UTI	22, 37.29%; (26.06–50.07)

Table 5 – Antimicrobial resistance patterns of gram-negative bacterial isolates from pregnant women with asymptomatic bacteriuria.

Antibiotic sensitivity pattern ^a										
Bacteria isolated	No. of isolates	Ampicillin/amoxicillin n (%)	Co-trimoxazole	Ciprofloxacin	Ofloxacin	Levofloxacin	Coamoxiclav	Cefotaxime/ceftriaxone	Cefuroxime	
<i>Enterococcus</i> species	2	2S	2R	1R 1S	1R 1S	1R 1S	2S	2S	2S	
<i>Escherichia coli</i>	1	R	S	S	S	S	R	R	R	
<i>Klebsiella</i> species	1	R	S	S	S	S	S	S	S	
<i>Enterobacter</i> species	1	S	S	S	S	S	S	S	S	
Total resistant n (%)		2 (40)	2 (40)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	1 (20)	

S = Sensitive; R = Resistant.

^a All isolates were sensitive to gentamicin, amikacin and netilmicin.

19.87% and 4.34% respectively. Prevalence of bacteriuria was significantly high among those who had intercourse more frequently and those with less water intake.¹⁵

Similar to our findings, in a study conducted in 2008 in Coimbatore, Tamil Nadu, forty-four out of 630 consecutive pregnant women (7.4%) had urine samples culture positive, with *E. coli* as the predominant organism isolated (57.4%).¹⁶

Sterile pyuria is the presence of elevated numbers of white cells (≥ 10 white cells/mm³) in urine, which appears sterile using standard culture techniques. We have found sterile pyuria to have a low sensitivity as well as specificity for the diagnosis of ASB (Table 4). If we obtain a negative urine microscopic results, then there are 92.21% chances that the patient does not have the disease, leaving a 7.79% chance of being false negative. Hence urine microscopic examination is of use for excluding UTI in asymptomatic patients, rather than using it for diagnosing it, as reported by others.^{13,15}

Sterile pyuria is not an uncommon laboratory finding and we found it in 28.81% of pregnant women at our setting. If sterile pyuria is found in female patients with symptoms of urinary tract infection it may be due to standard laboratory culture conditions may not be optimal for growth of atypical organisms or the laboratory may not report significant growth either because it was not a single organism or recognized urinary pathogen. Conversely, sterile pyuria is an asymptomatic pregnant woman may be due to recently (within last 2 weeks) treated UTI or inadequately treated UTI or UTI with 'fastidious' organism (an organism that grows only in specially fortified artificial culture media under specific culture conditions), e.g. *Neisseria gonorrhoeae* or renal tract tuberculosis.³ In our settings there is good antenatal care, hence there is a possibility that some of the cases may have been treated for UTI in the preceding 2–3 weeks. Thus there is a need to do further in-depth research into the causes leading to sterile pyuria in pregnant women in our setting so that we get a true pattern of bacterial isolates found here.

In all 5 cases of bacteriuria, we isolated gram-negative bacteria. Various studies from India have reported *E. coli* to be the commonest bacterial isolate^{6,16} and Enterobacteriaceae,¹⁴ as found by us. The number of isolates in our study was too small to comment on the AMR patterns and further work is needed with much larger sample size to monitor AMR patterns.

This was a prospective study to find out the prevalence of ASB. We also did urine microscopic examination very carefully and have reported sterile pyurias as well as laboratory diagnosed UTI. Since this was a pilot project we did not have sufficient sample size to get cases of symptomatic bacteriuria. Also only routine organisms were cultured in the urine and there is a possibility of missing atypical and uncommon bacteria in urine cultures. Only one urine culture was done per subject. Further research is needed to quality additional utility of identifying ASB by second urine culture.

We conclude that about one-tenth of pregnant women have ASB in KMC, Manipal. Therefore routine urine cultures must be done during pregnancy to detect and treat it. However, since almost 28% women also have sterile pyuria further research is needed to find out the cause for this using newer molecular diagnostic technique.

5. Implications

ASB is common in Manipal, Karnataka but prevalence is lower than that reported in North India. However, exact prevalence can only be ascertained by routine follow-up of pregnant women and by using newer molecular techniques.

Conflicts of interest

All authors have none to declare.

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