



ORIGINAL RESEARCH ARTICLE

DETECTION OF ASYMPTOMATIC BACTERIURIA IN PREGNANCY

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ABSTRACT

Background – Pregnant women are at higher risk of urinary tract infections. Urinary tract infections are most commonly occurring infections in pregnancy leading to multiple complications. Asymptomatic bacteriuria is commonly occurring condition in pregnancy.

Material and Methods - A total of 200 antenatal women in their first, second or third trimesters were screened over a period of 1 year, for asymptomatic bacteriuria by collecting 20 ml of clean caught mid stream urine samples. The samples were processed in microbiology laboratory as per standard methods and antibiotic sensitivity was done as per CLSI guidelines. 4 screening tests – Gram staining, pus cell counting, catalase test and Greiss nitrite test were done and the sample that gave a positive result was subjected to standard loop method (semi quantitative) and pour plate method (quantitative), for confirmation.

Results - Out of 200 cases, 29 were culture positive. A control group of 50 non-pregnant women, both married and unmarried were screened simultaneously. The control group yielded an overall positivity of 6% (8% in married non-pregnant women and 4% in unmarried women). Primigravida had the highest percent culture positivity of 55.1%. *E.coli* was the most common organism isolated. The organisms were in general sensitive to norfloxacin and cephalixin.

Conclusion – Standard loop method was found to be best semi-quantitative method for diagnosis of asymptomatic bacteremia. There was satisfactory agreement between standard loop method and pour plate method. Primigravida had highest incidence of significant bacteriuria.

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INTRODUCTION

Even in present 21st century, in the field of Medicine, when we try to find out cause or causes for a particular disease or syndrome, even the recent most books on the subjects in many cases say 'Exact cause is not known'!! Many hypothesis and thesis are thought of and rather than cause the effect is treated. Infection of urinary tract is one of such disease. Pregnant women are at higher risk of UTI. From the 6th week of pregnancy up to full term, approximately 90% of women develop ureteral dilatation, which will remain until delivery (hydronephrosis of pregnancy). Increased bladder volume and decreased bladder tone, along with decreased ureteral tone, contribute to increased urinary stasis and uretero-vesical reflux. The hormonal changes in pregnancy and pressure of gravid uterus also facilitate infection to ascend. So in pregnant women, if this silent entity is not treated, leads to adverse perinatal outcome and maternal morbidity. So this study is

thought of to explore different aspects of UTI during pregnancy, from the Microbiologist point of view. The definition of asymptomatic bacteriuria requires absence of symptoms, but urine showing positive culture. As the asymptomatic bacteriuria is detectable and more so treatable, screening pregnant women is definitely justified. To trace the magnitude of the problem, this study is thought of. The screening of antenatal women will provide an excellent opportunity towards safe motherhood.

MATERIALS AND METHODS

This study was undertaken after obtaining Institutional Ethical Committees approval. About 20 ml of clean catch midstream urine samples were obtained from 200 pregnant women attending the obstetric outpatient Dept. of Dr.D.Y.Patil Medical College, Hospital and Research Institute and RCSM Govt.Medical College, Kolhapur. Samples thus collected were immediately transported to the Microbiology laboratory and processed within 1 hour. In case of delay, the samples were refrigerated at 4C. The urine samples were first screened by 4

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screening tests and were then confirmed by semi quantitative & quantitative methods. Gram staining: A drop of uncentrifuged well mixed urine was taken on a clean slide and stained by Gram's Method of staining and examined under oil immersion objective of the microscope. The presence of 1 or more bacteria in most oil immersion fields is indicative of over 1 lakh organisms per ml of urine. Pus cell counting: By wet mount method using a Neubauer's chamber. In this method 7 pus cells / HPF will be considered as significant bacteriuria. Catalase test: Presence of the enzyme Catalase, evidenced by frothing, after addition of few drops of hydrogen peroxide to a 2ml sample of urine, was taken as positive.

Griess nitrite test: Principle is the reduction of nitrates to nitrites. In presence of nitrites, there will be immediate appearance of intensive red colour which will be a positive test and will be taken as significant bacteriuria.

Culture

Standard loop method: This is a semi quantitative method in which a sterilized loop with an inner diameter of 4 mm, delivering 2 ml is used. Streaking on Blood agar and McConkeys agar was done. After incubating aerobically for 24 hours at 37C, colony forming unit (CFU) per ml of urine was described.

Pour Plate method: A quantitative method done for confirmation and also significant bacteriuria may be differentiated from contamination. The significant isolates were identified by standard procedures and subjected to antibiotic susceptibility by disc diffusion method.

RESULTS

Out of the 200 antenatal mothers screened, 29 (14.5%) were culture positive. 16(55.1%) were primigravida, 10 (34.4%) were II nd gravida and 3 (10.3%) were III rd gravida. Out of 29 culture positive women, 20(68.9%) were in their Ist trimester, 6 (20.6%) were in II nd trimester and 3 (10.3%) were in their III rd trimester. Incidence of culture positivity was 1/25 (4%) in unmarried women and 2/25 (8%) in married non-pregnant women. *Escherichia coli* was the predominant organism isolated (41.37%), followed by *Klebsiella pneumoniae* (20.68%), *Pseudomonas aeruginosa* (13.79%), *Proteus mirabilis* (10.34%), *Coagulase negative Staphylococci* (10.34%) and *Citerobacter diversus* (3.44%). *Escherichia coli* was the only organism isolated in the control group. Out of 29 cases, 18 (62%) were positive by Gram stain. 4 (2%) cases out of 200 were false positive.

The age wise distribution was as follows:

Age in Years	Total screened	Cases with Bacteriuria
18 – 21	18	02 (11.1%)
22 – 25	51	10 (19.6%)
26 – 30	91	13 (14.2%)
31 – 35	32	01 (3.12%)
36 and above	8	03 (37.5%)
TOTAL	200	29 (14.5%)

DISCUSSION

Ever since Kass (1955) drew attention of the medical world to 'asymptomatic bacteriuria during pregnancy' and its effects on

to mother and the foetus, the study of bacteriuria during pregnancy received an impetus (Kass, 1960). Since then it has been the subject of excellent studies and reviews (Beard and Roberts, 1968; Lavanya and Jogalakshmi, 2002; Little, 1966; Whalley *et al.*, 1965; Ganguli, 1970; Roy *et al.*, 1974). Many laboratory tests have been described for determining whether a sample of urine contains over 1, 00,000 organisms per ml which is crucial in the diagnosis of UTI (Kass, 1960; Gillespie *et al.*, 1978). Efficacy of various screening tests for early detection of significant bacteriuria as an aid to treatment has been evaluated by laboratory workers for last 50 years. For this study, the collection and processing of samples was carried out for the period of one year. The samples were transported to Microbiology laboratory in a vaccine carrier. The samples that were positive by screening tests were also simultaneously assessed by semi quantitative method, using the accurate pour plate method, as a reference standard.

The incidence of asymptomatic bacteriuria in some of the important studies was as follows:

Author	Year	Incidence
Grace Turner	1961	7.5%
Kaitz and Hodder	1961	12.8%
Truck <i>et al</i>	1962	6.5%
P. J. Whalley	1965	6.9%
William J. D.	1965	5.3%
Kincaid-Smith Bullen	1965	6.0%
Leela Gunguli	1970	6.1%
S. K. Roy	1974	12.4%
Kakoty	1974	5.0%
Mitra	1977	10.3%
Yashodhara	1987	10.5%

In the present study, the incidence of asymptomatic bacteriuria was 14.5%, which compares favorably well with incidence of other workers mentioned above (Table No.1). The incidence in control group of asymptomatic non-pregnant women and unmarried women is 8% and 4% respectively (Table No.2) Thus; the incidence during pregnancy was noted to be higher. Unlike socio-economic factors, the effects of other host factors such as age and parity in pregnancy are less clear. Williams (1965) has claimed that neither age nor parity influences the prevalence of bacteriuria, while others state that increasing age or parity is associated with a higher frequency of significant bacteriuria (Leigh and Williams, 1964; Whalley *et al.*, 1965; Kincaid-Smith and Bullen, 1965). In present study no increase was found in the incidence of bacteriuria with increasing age and parity (Table No.3) In the present study, primi gravida showed 55.1% positivity while second and third gravida were 34.4% and 10.3% positive, respectively. Primi gravida had the highest incidence of significant bacteriuria, followed by second and third gravida (Table No.4) Zhanel *et al.* (1990) has reported higher incidence of significant bacteriuria in primi gravida which correlates well with our present study. Garingalo (2000) has reported an overall incidence of 62.3% positive significant bacteriuric cases amongst primi gravida in his study and found it to be highest. All the women chosen were from first, second and third trimesters. Extensive physiological changes occur in the female body during pregnancy. This accommodative response of the body during pregnancy, also involves some risk. The laxity in the tone of ureters is an important change, which predisposes to establishment of UTI in pregnant women more frequently as compared to the normal healthy female populations (Andriole and Patterson, 1991).

Kass (1962) has noted that maternal bacteriuria is usually present by the second month of gestation. It was originally

thought that because bacteriuria was detected in 5% to 6% of pregnant women at the time of the first hospital visit, usually at the end of first trimester, infection became established within the first two months of pregnancy (Kass, 1960; Savage *et al.*, 1967). Beard and Roberts concluded that it is during the first trimester that most susceptible women acquire bacteriuria (Beard and Roberts, 1968). A variety of tests available and opinions have differed considerably for their usefulness (LeBlanc and McGanity, 1963). In the present study, women in their Ist Trimester showed highest incidence (68.9%) of asymptomatic significant bacteriuria, followed by IInd (20.6%) and IIIrd Trimester (10.3%) (Table No.5) A battery of four tests has been applied in the present study with the idea of providing uniform conditions of evaluations to different tests. Described here is a comparative study of these methods using the accurate, pour plate method as a reference standard for enumerating the number of bacteria in a urine sample. The results of screening tests are 22 (75.8%) positive by Gram staining, 13 (44.8%) positive by Griess nitrite test, 15 (51.7%) positive by Catalase test and 20 (68.9%) positive by Pus cell counting (Table No.6) Although quantitative culture method would seem to be the best, the chemical screening tests cannot be discredited for lack of correlation obtained in a minor proportion of cases only. Even though it is difficult to explain the lack of correlation in these cases, this defect could be circumvented by a simultaneous semi-quantitative culture technique of simple nature or even a qualitative routine streaking. The time consuming quantitative technique could therefore be dispensed with, in favor of a chemical screening test for quantitative assessment and routine culture for the isolation, identification and testing antibiotic susceptibility of the urinary pathogen concerned. In a hugely populated country like India, if all the pregnant women are to be subjected to frequent bacteriological examination of urine samples then logistics involved will be of unimaginable proportions. To subject all these urine samples to frequent culture examination is almost impossible. Therefore search of rapid, quick, reliable, reproducible, cheap, simple and easily available tests for accurate diagnosis of UTI has continued.

Conclusion: The microscopy by Gram stain was a reasonable screening test because of less numbers of false positive and false negative results. All the 29 cases were positive by standard loop method. It was found to be the best semi-quantitative method for diagnosis of asymptomatic bacteriuria. The Gram negative bacilli were found to be predominant in causing asymptomatic significant bacteriuria. The standard loop method shows good general agreement with pour plate method. The identification and antibiogram of culture were essential in every case as it was necessary to know the causative organism of asymptomatic bacteriuria and its sensitivity pattern. The extra labor involved in setting up pour plate was only for accurate diagnosis of asymptomatic bacteriuria which may not be justified in routine antenatal screening. Gram stain of uncentrifuged urine was easy to perform, rapid, economic and could be therefore recommended for use as a screening test in laboratory practice. The Griess nitrite, Catalase and other tests gave both false positive and false negative results, indicating that culture and pour plate remain the Gold standards for detecting significant bacteriuria. In control group of 25 married non-pregnant women, the incidence of culture positivity and significant bacteriuria was found to be 2/25 i.e. 8%. In the second group of 25 unmarried women, the incidence was found to be 01/25 i.e. 4%. Antibiotic sensitivity was tested as per Kirby-Bauer's

technique. Majority of gram negative organisms were seen sensitive to Ceftriaxone (75.8%) and 82.7% resistance was seen to Cloxacillin. Unpredictable antibiotic sensitivity in individual cases seems to be the feature of Gram negative bacillary infections. Therefore a method, which provides bacterial counts, species identification and antibiogram, should be the method of choice for screening urine samples in the future. Based on these criteria standard loop test is the most suitable screening test for asymptomatic bacteriuria. As most of the tests evaluated in this work are far from satisfactory in providing dependable information which will be useful in the diagnosis and treatment, it is tempting to recommend that these tests should not be evaluated here after for their efficacy. Further studies of this nature should evaluate newly discovered tests against the classical pour plate method and standard loop method.

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