JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH

How to cite this article:
GAYATHREE L, SHETTY S, DESHPANDE S R, VENKATESHA D T. SCREENING FOR ASYMPTOMATIC BACTERIURIA IN PREGNANCY: AN EVALUATION OF VARIOUS SCREENING TESTS AT THE HASSAN DISTRICT HOSPITAL, INDIA. Journal of Clinical and Diagnostic Research [serial online] 2010 August [cited: 2010 August 15]; 4:2702-2706.

Available from
http://www.jcdr.net/back_issues.asp?issn=0973-709x&year=2010 &month= August &volume=4&issue=4&page=2702-2706 &id=1129

ORIGINAL ARTICLE

Screening For Asymptomatic Bacteriuria In Pregnancy: An Evaluation Of Various Screening Tests At The Hassan District Hospital, India

GAYATHREE L , SHETTY S, DESHPANDE S R, VENKATESHA D T

ABSTRACT

Objective: To study the prevalence of asymptomatic bacteriuria (ASB) in pregnant women who attended the Hassan District hospital, Hassan.

Method/Design*:* The group A- study group subjects were 900 pregnant women of any gestational age who attended the Obstetrics Department for antenatal care. The Group B (control group) consisted of 50 non pregnant women of the fertile age group. Midstream clean catch urine was used to screen for asymptomatic bacteriuria.

Results: Asymptomatic bacteriuria was prevalent in 6.2% of the 900 women who were evaluated in our study. Urine culture was the gold standard for the detection of asymptomatic bacteriuria. Gram's stain of uncentrifuged urine was found to be the best among the screening tests which were evaluated. There was a higher prevalence of asymptomatic bacteriuria in the IIIrd trimester (61.77%) than in the IInd trimester (32.35%) and the Ist trimester (5.88%).

Conclusions: Screening for asymptomatic bacteriuria in all the three trimesters is necessary to prevent the dangerous complications which are associated with asymptomatic bacteriuria in pregnancy.

Key Words And Phrases: Asymptomatic bacteriuria in pregnancy, urine culture, Gram's stain, *Escherichia coli.*

Dr Gayathree L MD., Assistant Professor in Microbiology, Hassan institute of Medical Sciences, Hassan 573201 India. Mobile: 91-9480158773 Email: gaya3doc@yahoo.com
Mr.Sharathkumar Shetty MSc., Assistant Professor in Microbiology, Hassan institute of Medical Sciences, Hassan 573201.Mobile: 91-9449970001.
*Dr Srinivas R Deshpande MD., Associate Professor in Biochemistry, Hassan institute of Medical Sciences, Hassan 573201 Mobile: 91-9480158773
Dr D.T Venkatesha MD., Professor & HOD Microbiology, Hassan institute of Medical Sciences, Hassan 573201 Mobile: 91-9448262118
*corresponding author

Introduction

Asymptomatic Bacteriuria is a microbiological diagnosis based on the isolation of a specified quantitative count of bacteria in a properly collected specimen of urine from persons without signs or symptoms, who are referable for urinary tract infection [1]. The term asymptomatic bacteriuria (ASB) is used when a bacterial count of the same species over 10^5/ml in mid-stream clean catch urine on two occasions is detected without symptoms of urinary tract infection. The apparent reduction in immunity of pregnant women appears to encourage the growth of both commensal and non-commensal microorganisms [2]. Global prevalence of asymptomatic bacteriuria varies widely and in pregnancy, it is 1.9-9.5% [2].

It is well known that asymptomatic bacteriuria (ASB) indicates the active multiplication of bacteria in the urinary tract and 25% of the affected women are likely to develop acute pyelonephritis in the third trimester, if left

untreated. Postpartum investigation is indicated when the urinary tract infection is recurrent [2], [3]. The incidence of ASB varies from 2-10%, depending on the socioeconomic status of the patients [4], [5]. In one antenatal study [6], in which 9.9% of women took part in at least one screening, the risk of onset of bacteriuria was highest between the 9^{th} and 17^{th} weeks of gestation. The 16^{th} week is the optimal time for a single screen for bacteriuria, which has been calculated, based on the numbers of bacteriuria free gestational weeks gained by the treatment [6].

Importance Of Diagnosis Of ASB

Bacteria originate from the large bowel and colonize in the urinary tract transperineally. The most common infecting organism is *Escherichia coli*, which is responsible for 75-90% of bacteriuria during pregnancy. Other organisms that have been isolated are Klebsiella, Proteus, Coagulase Negative Staphylococcus and Pseudomonas [7].It is important to identify and treat the infected group, as 40% of the ASBs develop acute symptomatic UTI [8]. A positive history of previous UTI may be almost as effective as screening, in predicting UTI in pregnancy [9]. Also, there is a good evidence of an association between any type of UTI in pregnancy and sudden unexpected infant death [10].Relapse of UTI is the recurrence of bacteriuria caused by the same organism, usually within 6 weeks of the initial infection. Reinfection is the recurrence of bacteriuria with a different strain of bacteria, after successful eradication of the initial infection [11]. Approximately 15% of the patients will have a recurrence during pregnancy and a second course of treatment should be given, based on repeat culture with sensitivity testing.

Materials And Methods

This study was conducted from April 2007 to April 2008 in the Microbiology Department, Hassan District Hospital, which is attached to the Hassan Institute of Medical Sciences ; a tertiary care referral centre. Out patients attending the Obstetrics Department were recruited for the yearlong study. Institutional approval and approval from the Institutional Ethics Committee was taken prior to the study. Informed consent was taken from all the patients participating in the study after explaining the study details in the patient's mother tongue.

Methods

The group A- study group subjects were 900 pregnant women of any gestational age who attended the Obstetrics Department. Only women who fulfilled the criteria of apparently normal health, without any signs or symptoms of UTI, were included in the study. The group B- control group subjects were 50 non-pregnant females of the age group of 18-45 years, without any symptoms or signs of UTI. Certain patients were excluded as per the exclusion criteria described below.

Exclusion Criteria

1) History of UTI symptoms (dysuria, frequency and urgency, etc).
2) Pregnancy induced Diabetes Mellitus/ Hypertension.
3) History of antibiotic therapy in the previous two weeks.
4) Pyrexia.
5) Known congenital anomalies of the urinary tract.

The study group was interviewed and the data was recorded in the approved proforma. The patient's demographics included age, gestational age, education, socioeconomic status, occupation and parity.

Sample Collection And Processing

About 30ml of clean catch mid-stream urine samples were collected in 100ml sterile wide mouth containers with lids, after giving instructions to the patients regarding the sample collection. The samples were immediately transported to the laboratory and were processed within one hour. In case of delay, the samples were refrigerated at $4^{o}C$. The specimens were first processed in the laboratory for culture by the semi quantitative calibrated loop technique and then, other screening methods were performed, which were compared with the culture.

Culture Of The Specimen

The urine was cultured on blood agar, Mac Conkey's agar and CLED agar. A loopful of well-mixed uncentrifuged urine was streaked onto the surface of the culture plates. Incubation was done

aerobically at 35 °C for 18-24hrs. A minimum of 24 hours is necessary to detect uropathogens [12].Pure growth of $\geq 1\times10^5$CFU/ml of one organism was considered to be suggestive of significant bacteriuria. Pure growth between $>1\times10^3$ and 1×10^5 CFU/ml was taken as doubtful significance and the culture was repeated, while pure growth of 1×10^3 CFU/ml was taken as insignificant bacteriuria. Mixed growth of two or more organisms was considered to be contamination [13]. Significant bacterial isolates were identified by standard procedures and were subjected to antibiotic susceptibility by the Kirby Bauer's disc diffusion method.

Gram's Staining Of Uncentrifuged Urine

A loopful of uncentrifuged, well mixed urine was placed on a grease free slide and it was air dried. Then, the smear was stained by Gram's stain and was observed under oil immersion. The presence of ≥1 bacteria/Oil immersion field in 20 fields correlated with the diagnosis of significant bacteriuria of $\geq10^5$ CFU/ml of urine [14].

Leukocyte Esterase Test And Nitrite Test

Evidence of a host response to infection is the presence of polymorphonuclear leucocytes in the urine. Because inflammatory cells produce Leukocyte esterase, a simple and rapid method that measures this enzyme has been developed. The nitrite reductase test is a screening procedure that looks for the presence of urinary nitrite, an indicator of UTI. Nitrite reducing enzymes that are produced by the most common urinary tract pathogens reduce nitrate to nitrite.[13]Uncentrifuged urine specimens were tested by the Colorimetric Combur-10 multireagent test (Boehringer Mannheim & Co.) for the presence of nitrite and leukocyte esterase activity. The manufacturer's instructions were followed.

Statistical Analysis

P values were derived from standard statistical tables and t-values. T-values were calculated by the Student's "t" test formula for means ± standard deviations of ages. Chi- square test (x2) was applied for t- value derivation, for comparison of the findings in the two groups.

Results

Age-wise distribution of the subjects in Group A and Group B is represented in Table I. There were 690 subjects from Group A in the range of 18 -25 years, whereas there were 24 controls in Group B, with mean ages of 21.59 ± 2.30 and 21.16± 4.24, respectively. In the age ranges of 26 -35 years and 36 -45 years, the mean of the ages and the number of subjects are also shown for both groups A and B in the [Table/Fig 1] (Table 1). There is statistical significance in the mean of ages of the subjects in both groups A and B, between the ages of 18-35 years. (P value< 0.05).

(Table/Fig 1) Age-wise distribution of Pregnant Women (Group A) and control (Group B)

Age Group (Years)	Mean of age Group A (900)	Mean of age Group B (50)	P Value	Inference
18 -25	21.59 ± 2.30 (690)	21.16± 4.24 (24)	< 0.05	Significant
26 -35	28.93 ± 2.85 (189)	29.14 ± 2.44 (21)	<0.05	Significant
36 -45	41.1 ±2.028 (21)	38.25 ± 1.34 (05)	>0.05	Not significant

(P-Value > 0.05 Non significant-Value < 0.05 Significant- Value < 0.01 Highly Significant).

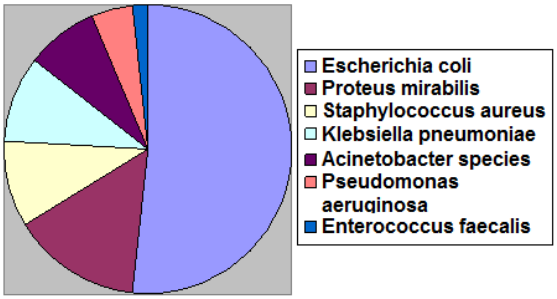
(Table/Fig 2) Bacterial Growth Isolated (ASB) from urine of Pregnant Women (Group A) and Non-Pregnant Women Controls (Group B).

Groups	No. of. Cases tested	No. of .Positive Cultures	X^2	P -Value	Inference
A	900	62(6.8%)	1.67	> 0.05	Not significant
B	50	1(2%)			

Out of the urine samples from 900 pregnant women, 62 samples of urine (6.8%) and only 1 (2%) out of the 50 controls were positive for culture and had significant bacteriuria (ASB). The number of positive ASB cases in pregnant and non-pregnant women did not show any statistical difference (P value > 0.05) as per the results in [Table/Fig 4], which is shown above. Only 1 of the controls (2%) had significant ASB which was statistically insignificant. *Escherichia coli* emerged as the most frequent ASB with 32 cases (51.61%), followed by *Proteus mirabilis* with 9 cases (14.51%), *Staphylococcus aureus* and *Klebsiella pneumoniae* with 6 cases (9.67%) each, *Acinetobacter spp.,* with 5 cases (8.05%), *Pseudomonas aeruginosa* with 3 cases (4.83%) and *Enterococcus faecalis* with 1 case (1.61%), as enumerated in [Table/Fig 3] and [Table/Fig 4]. The control group showed only growth in 1 (2%) sample with *Escherichia coli.*

(Table/Fig 3) Organisms isolated in percentages for asymptomatic bacteruria in pregnancy

Organisms Isolated	No.Of. Positive Cultures	% Of Total
Escherichia coli	32	51.61%
Proteus mirabilis	9	14.51%
Staphylococcus aureus	6	9.67%
Klebsiella pneumoniae	6	9.67%
Acinetobacter species	5	8.06%
Pseudomonas aeruginosa	3	4.83%
Enterococcus faecalis	1	1.61%



(Table/Fig 4) Spectrum of bacterial isolates from urine samples of Pregnant Women (Group A) for asymptomatic bacteruria in pregnancy.

Urine culture was taken as the gold standard, against which the comparison of various screening tests was done. Statistical formulas were applied and thus sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. Gram's stain of uncentrifuged urine showed a maximum number of true positives (56/62) and a high sensitivity of (90.32%). A minimum number of true positives were seen with the leukocyte esterase test (38/62), with a low sensitivity of 61.29%. The leukocyte esterase test showed maximum false positives (60/62) and a lower number of false positives were seen with the nitrite test (6/62), thereby decreasing and increasing the specificity of the leukocyte esterase test (92.84%) and the nitrite test (99.28%), respectively. Combined nitrite and leukocyte esterase tests gave a low sensitivity of 53.22%, but specificity and positive predictive value were 100%, since no false positives were wrongly identified. The above values are shown in [Table/Fig 5] Table IV and [Table/fig 6] V respectively.

(Table/Fig 5) Statistical analysis of various screening tests for asymptomatic bacteruria in pregnancy as compared to urine culture.

Tests	True Positive	True Negatives	False Positive	False Negatives
Gram's stain	56	830	8	6
Leukocyte esterase test	38	778	60	24
Nitrite test	44	832	6	18
Combined Leukocy esterase & Nitrite test	33	838	0	29

(Table/Fig 6) Sensitivity, Specificity and Predictive values of various screening tests for asymptomatic bacteruria in pregnancy compared to Urine culture.

Tests	Sensitivity	Specificity	Positive Predictive value	Negative Predictive value
Gram's stain uncentrifuged urine	90.32%	99.04%	87.5%	98.28%
Leukocyte esterase test	61.29%	92.84%	38.77%	97.0%
Nitrite test	70.96%	99.28%	88.0%	97.88%
Combined Leukocyte esterase&Nitrite test	53.22%	100%	100%	96.65%
Urine Culture	100%	100%	100%	100%

Discussion

Urinary tract infection (UTI) is one of the most common health problems in pregnancy because of the increase in the sex hormones and anatomical and physiological changes during pregnancy.[15-17] The global prevalence of UTI in pregnancy is found to range from 1.9-9.1% as per literature. In our study, we found a prevalence of 6.8%, which was similar to a study in Iran (6.1%)[15] .Studies at Pakistan have showed a prevalence of 4.8% [16] , while Jayalaxmi et al in India showed a prevalence of 7.4% [17].We also found a higher (79.42%) prevalence of asymptomatic bacteriuria in lower socioeconomic groups, as in other studies [15],[16],[17]. We found a higher prevalence of asymptomatic bacteriuria in the IIIrd trimester (61.77%) than in the IInd trimester (32.35%) and in the Ist trimester (5.88%) of pregnancy. Hence, we would like to recommend a routine screening for asymptomatic bacteriuria in all the three trimesters of pregnancy as an important measure, in order to avoid the complications of asymptomatic bacteriuria, as observed by Mc Isaac et al [18] , than a single occasion ASB screening in between the 9 and 16th weeks of gestation [6].

Escherichia coli was the most predominant organism in our study 32(51.61%), as reported in various other studies [16],[17]. Numerous previous studies have established that the gold standard method for the diagnosis of UTI, as well as ASB, is the urine culture of midstream catch urine

[13],[17],[19],[20]. It is well known that various other routine screening tests can only poorly detect all culture positive bacteriuria cases in pregnant women [17],[19],[20],[21]. In our evaluation of the screening tests like Gram's stain of uncentrifuged urine, the Leukocyte esterase test and the Nitrite test, we found Gram's stain of uncentrifuged urine to have a good sensitivity (90.30%), specificity (99.04%), and negative predictive value (98.28%) than other screening tests vis-a -vis urine culture [20],[23],[24]. Though the nitrite test alone showed a good specificity (99.28%), it was less sensitive (70.96%) than Gram's stain (90.32%). Combined Leukocyte esterase and Nitrite tests showed a good specificity (100%) than Gram's stain (99.04%). Among the screening tests evaluated, we observed that Gram's stain of uncentrifuged urine was the best screening method for ASB, as in other studies [17].Also, in our opinion, the Dipstick test for Leukocyte esterase and Nitrites can also serve as a rapid screening method for asymptomatic bacteriuria, as its sensitivity and specificity is nearer to that of Gram's stain and the urine culture.

Conclusion

Asymptomatic bacteriuria was prevalent in 6.2% of the 900 women who were evaluated in our study. Urine culture remained the gold standard for the detection of asymptomatic bacteriuria. Gram's stain of uncentrifuged urine was observed to be the best among the screening tests which were evaluated. Screening for asymptomatic bacteriuria in all three trimesters is necessary to prevent the dangerous complications which are associated with ASB.

Acknowledgements

We thank the patients who co-operated with us and the staff of the Obstetrics and Microbiology Departments of HIMS hospital. We are grateful to the Director, HIMS for encouraging our research.

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