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A comparative study of various screening tests of Asymptomatic Bacteriuria in pregnant women attending Antenatal Outpatient Department'

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Introduction: Urinary tract infections are the most common bacterial infections in pregnancy. Asymptomatic bacteriuria (ASB) refers to the presence of bacteria in the urine of an individual without symptoms of urinary tract infection. ASB which occurs in 2-11% of pregnancies is a major predisposition to the development of pyelonephritis. **Aims and objectives:** The aims and objectives of the study were to: Study the effectiveness of various screening tests: urine microscopy, gram stain, catalase test, leukocyte esterase test and nitrite test and to compare their sensitivity, specificity, positive predictive value and negative predictive value. **Materials and Methods:** The study included 500 pregnant women attending the outpatient department over 18 months. The urine samples collected in sterile containers were screened for urine microscopy, gram stain, catalase test, leukocyte esterase test and nitrite test. The samples were processed on CLED (Cysteine lactose electrolyte deficient) agar as the standard against which other screening tests are identified. **Results:** Gram's stain (89.34%) was the most sensitive of all and the least reliable test was the catalase test among the methods of screening tests. **Conclusion:** Urine culture is considered to be the gold standard in detecting ASB in pregnant women. Gram's stain of urine is a good screening test when compared to other screening methods. Screening for bacteria in all trimesters is necessary to prevent the dangerous complications associated with ASB.

Keywords: Asymptomatic bacteriuria, Pregnancy, Gram stain, Culture

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Introduction

Urinary tract infections are the most common bacterial infections in pregnancy [1]. Asymptomatic bacteriuria (ASB) is used when a bacterial count of the same species over 105/ml in midstream clean catch specimen of urine on two occasions is detected without symptoms of urinary infection. Significant bacteriuria, the criteria to determine ASB, is usually determined by urine culture, refers to the finding of ≥105 colonies of a single organism from 1ml of uncentrifuged urine sample cultured [2]. Pregnancy causes numerous mechanical and hormonal changes in the body [3]. ASB occurring in 2-11% of pregnancies is a major predisposition to the development of pyelonephritis [1-3]. Among pregnant women with asymptomatic bacteriuria, 40% develop symptomatic infections and approximately 30% of these develop acute pyelonephritis when left untreated. ASB can produce complications which include premature deliveries, low birth weight babies, postpartum urinary tract infections, postpartum endometritis, hypertension, anaemia and at times perinatal deaths [4]. The ideal screening test should be inexpensive, simple and rapid and should have high sensitivity and specificity. The confirmatory test should be done by the gold standard culture [2]. The objective is to conduct a cross-sectional study and identify the most sensitive screening test in pregnant women.

Aims & objectives: 1. To study the effectiveness of various screening tests: urine microscopy, gram stain, catalase test, leukocyte esterase test and nitrite test and to compare their sensitivity, specificity, positive predictive value and negative predictive value.

Materials and Methods

Source of data: The study was carried in the Department of Microbiology and Department of Obstetrics and Gynecology, in a tertiary care centre, South India.

Duration of study: The study was conducted over 18 months.

Type of study: This is a cross-sectional study wherein subjects were selected based on purposive sampling.

Study population: All the pregnant women attending antenatal OPD in the tertiary care hospital.

Inclusion criteria: All pregnant women without any signs and symptoms of urinary tract infection attending OPD in our hospital.

Exclusion criteria:

- Pregnant women on current antibiotic therapy or in the past 2 weeks due to any reason.
- Pregnant women presenting with clinical features of UTI.
- Pregnant women who have congenital urinary tract anomalies.
- Pregnant women with gestational diabetes mellitus and hypertension.

Ethical consideration: Clearance for the study was obtained from Institutional Ethical Committee. All the pregnant women have explained the purpose of the study and the procedures involved. Urine samples were collected after obtaining consent for them. The patients were studied as per the proforma formulated. Specimen collection: Clean catch midstream urine (MSU) samples were collected in a sterile container.

For the proper collection of midstream and to avoid contamination, every patient was instructed to clean the periurethral area and perineum with soap and water followed by a rinse with sterile saline or water. With labia held apart, midstream urine was collected in a sterile container [5]. Samples thus collected were transported to Microbiology Department within 1 hour. In case of delay, samples were refrigerated at 40C as long as 24 hours [6].

Processing of specimens:

The specimens were processed immediately in the laboratory after collection.

01. Macroscopic appearance: The appearance of the urine was described for its colour and turbidity.

02. Microscopic examination of uncentrifuged urine:

    - *Wet film examination:* A drop of well-mixed urine was placed on the slide, covered with a coverslip and observed under low and high power objectives of microscope for the presence of pus cells, RBCs, microorganisms, casts, etc [7].
    - *Gram stain examination:* A drop of well mixed urine was smeared on the slide, allowed to dry, heat-fixed, stained and observed under oil immersion.

The presence of at least one organism per field (examining 20 fields) correlates with significant bacteriuria [7].

03. Griess nitrite test: Nitrate reducing enzymes that are produced by most common urinary tract pathogens reduce nitrate to nitrites. This is done by the dipstick paper strip method manufactured by DIRUI Industrial Co., Ltd., China. The nitrites react with amines impregnated on the dipstick pad to form a diazonium compound resulting in a pink colour reaction within 60sec [8].

A dipstick strip test that determines 10 different parameters including the Griess nitrite test was used. The strip was dipped in well-mixed uncentrifuged urine for no longer than a second. After one minute the color change in the strip compared with the color scales provided with the kit. The pink color produced was considered positive. Any change in color after 2 minutes had no significance [9].

04. Leucocyte esterase (LE) test: LE is an enzyme produced by inflammatory cells. LE reacts with the chloroacetate stain impregnated in a dipstick pad resulting in iodoxo-moiety that is oxidized by room air and produced a calorimeter change in 1-2 min [8].

A dipstick strip test that determines 10 different parameters was used [3,8].

The strip was dipped in well-mixed uncentrifuged urine for no longer than a second. After one minute the color change in the strip compared with the color scales provided with the kit. The pink color produced was considered positive. Any change in color after 2 minutes had no significance [3,8].

Urine culture

A semi-quantitative method was adopted for the primary isolation of organisms using a calibrated loop, which delivers 0.01ml of urine. A calibrated loop was flamed and allowed to cool without touching any surface. The loop was inserted vertically into the urine to allow urine to adhere to the loop. The specimen containing in the loop were cultured on dried plates of Cysteine Lactose Electrolyte Deficient agar, MacConkey's agar and on 5% sheep Blood agar and incubated at 370C providing 5-10% CO2 for 18-24 hours. The number of CFUs were multiplied by 100 to determine the number of microorganisms per ml in the urine specimen.

Urine specimens with ≥105 CFU/ml were considered as significant bacteriuria [7].

Results

Table 1: Distribution of positive screening methods in pregnant women

Screening methods	Number	Percentage (%)
Wet mount	112	22.4
Gram stain	154	30.8
Catalase test	16	3.2
Leucocyte esterase	93	18.6
Nitrite test	99	19.8

Among the 5 screening tests done, the table shows that gram stain detects more positive cases in women suffering from ASB.

154 of them with 30.8% of them were able to detect ASB as compared to other screening methods.

Table 2: Distribution of statistical values of various screening tests in pregnant women

Test	True positive	True negative	False positive	False negative	Total
Wet mount	46	388	31	35	500
Gram's stain	109	346	32	13	500
Catalase test	34	437	14	15	500
Leucocyte esterase test	34	407	48	11	500
Nitrite test	46	401	32	21	500

The above table shows that true positives i.e. the patients who are having ASB are better detected in gram stain and true negatives i.e. the patients who are not having ASB are better detected by catalase test.

Table 3: Statistical analysis of various screening tests in pregnant women

Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Wet Mount	56.8	92.6	59.7	91.7
Gram's Stain	89.34	91.05	76.47	96.3
Catalase Test	69.38	96.8	70.83	96.68
Leucocyte Esterase Test	79.06	89.45	41.46	97.36
Nitrite Test	68.7	92.6	58.9	95.02

The table shows that gram stain has the highest sensitivity with 89.34% and catalase test has the highest specificity with 96.8%.

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 [3]. The relatively high prevalence of ASB during pregnancy, its harmful consequences for both the mother and the fetus, and the ability to avoid unwanted results with proper treatment, justify their screening in this period of life of the woman [10]. Hence, in the present study, an attempt was made to evaluate four rapid screening methods compared to standard semiquantitative urine culture in detection of ASB in antenatal women to know the frequency of ASB, to identify a better screening test, isolate and identify the organism and to study the antibiotic susceptibility pattern.

Sensitivity and specificity of urine wet mount: Lavanya SV et al in their study have revealed a sensitivity and specificity of 52.3% and 96.5% respectively [11]. Chongsomochoi et al in their study have shown a varying sensitivity and specificity of 18.4% and 96.5% respectively [12]. In the present study, urine wet mount for pus cells showed a sensitivity and specificity of 56.8% and 96.2% respectively. The lower sensitivity observed with urine wet mount may be due to insufficient inflammatory response in ASB and hence no symptoms of UTI.

Comparison of gram stain: The study of Sweet RL et al has reported a sensitivity and specificity of 90% and 88% respectively [13]. Jayalaksmi J et al in their study have revealed a sensitivity and specificity of 85.1% and 98.8% respectively. Gram's stain of uncentrifuged urine to have good sensitivity (90.30%), specificity (99.04%), and negative predictive value (98.28%) than other screening tests vis-a-vis urine culture [5,3].

The present study showed gram stain sensitivity of the uncentrifuged urine smear showed a sensitivity of 89.34% and specificity of 91.05%. Other studies show that gram stain correlates well with significant bacteriuria. Gram stain provides information about stain characteristics and organism morphology. It is also the least expensive rapid method to screen ASB. The limitation of Gram stain is that methodically reviewing the smears is too labor intensive and may have technical variations [5].

Comparison of catalase test: The study conducted by Berger SA et al concluded that one-Plus (+) reaction was recorded for 47 of 62 (75.8%) false-positive catalase tests (insignificant cell and bacterial concentrations) and only 91 of 229 (39.7%) true-positive catalase tests [14]. The study conducted by Thomas et al concluded that the specificity for wholly cell-free urines was 73% (255/347). The sensitivity was 98% (62/63) for urines with more than 20 combined red and white cells per mm3, 95% (119/125) for 10 or more cells and 82% (149/182) for any cells visible [15]. Specificity co-relates with other studies but sensitivity is low when compared to these studie[14]. The sensitivity and the specificity of the catalase test in our study were found to be 69.3% and 96.8%.

Comparison of leukocyte esterase test: Gayathree et al in their study concluded that a minimum number of true positives were seen with the leukocyte esterase test (38/62), with a low sensitivity of 61.29%. The LE 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 LE test (92.84%) and the nitrite test (99.28%), respectively. Combined nitrite and LE tests gave a low sensitivity of 53.22%, but the specificity and positive predictive value were 100% [3]. Ajayi AB et al in their study reported high false positives with the LE test thereby decreasing the specificity of the LE test. The sensitivities of Multistix® nitrite and LE were each 14% and the specificity 100% and 96% respectively. Multistix® nitrite and LE are not sufficiently sensitive to be used as a screening tool for ASB [16]. Taneja N et al in their study reported a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and the diagnostic odds ratio (DOR) of the dip-stick LET were 73.5%, 58.5%, 33.0%, 88.8%, and 3.9 respectively [17]. The sensitivity and specificity of the LE test in this study were found to be 79.06% and 89.45% respectively and correlated with other studies.

Comparison of the nitrite test: Our study correlated well with Gayathree L et al and Berger SA et al showing similar conclusions. Gayathree et al showed a sensitivity and specificity of 70.96% and 99.28% [3]. Berger SA et al showed sensitivity and specificity of 41.5% and 92.3% [14]. The present study showed a nitrite test with sensitivity and specificity of 68.7% and 92.6% respectively.

Strength of the study: The key strength of this study involves a large population for a long period.

One of the other strengths of this study is that it represents a comprehensive examination of the screening tests that were measured against the gold standard method that gives us accurate interpretations. The equal contributions made by the other author in the form of guiding through the workflow process and in editing and correcting the manuscript was immense for the timely completion of the work.

Conclusion

To conclude, it is suggested that all pregnant women should be screened for ASB. The ideal screening test should correctly identify the negative samples, the ones with high sensitivity and with reasonably good specificity. Gram's stain of urine is a better screening test when compared to other methods. Screening for bacteriuria in all trimesters is necessary to prevent the dangerous complications associated with ASB. Urine culture is considered to be the gold standard in diagnosing ASB in antenatal women.

Adding to the existing knowledge

In the present study, an attempt was made to evaluate four rapid screening methods compared to standard semiquantitative urine culture in the detection of ASB in antenatal women to identify a better screening test. We conclude that culture remains the gold standard and none of the screening tests can be considered on par with culture. We have observed in our study that gram stain is the most reliable screening test that can be done for detecting ASB on an emergency basis. However, culture remains the standard diagnosis for ASB.

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