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Evaluation of screening tests for detection of asymptomatic bacteriuria in pregnant women

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Abstract

Asymptomatic Bacteriuria (ASB) as a condition characterized by absence of symptoms of acute Urinary Tract Infection (UTI) when true bacteriuria exists. The adverse effects of undiagnosed ASB on mother and child have made researchers to suggest routine culture screening for all pregnant women attending antenatal clinic. Screening tests for bacteriuria have their own place. With few pros and cons, they are of tremendous utility in earlier detection of bacteriuria and prompt treatment especially in the places where culture facilities are not within reach. This work was aimed at determining evaluation of various screening tests for detection of bacteriuria among pregnant women attending antenatal clinic in a Shri. Chatrapati Shivaji Maharaj Sarvopchar Rugnalaya, Solapur. Total 300 married pregnant (study group) and 300 married non-pregnant women (control group) were included in the study. Collection and transportation of urine was carried out by standard procedures. Screening tests like Wet film examination, Gram staining, Griess nitrate test, Triphenyl Tetrazolium Chloride (TTC) Test and Catalase test was carried out. Highest sensitivity was observed in the Gram staining method, while highest specificity was observed in the wet mount method. Lowest sensitivity was observed in the TTC method, while lowest specificity was observed in the catalase method. There are several ways to diagnose UTI, but urine culture still remains most reliable tool for its diagnosis [25]. Amongst five screening tests, Wet mount and Gram Stain of urine help in suggesting ASB and hence, being simple and rapid, these tests can be widely applied to screen the pregnant women on a routine basis.

Keywords: Asymptomatic bacteriuria, screening tests, pregnant women, wet mount, Griess nitrate test

1. Introduction

Asymptomatic bacteraemia (ASB) refers to the presence of bacteria in urine and is a condition in which urine culture reveals a significant growth of pathogens that is greater than $10^{5.1}$ bacteria/ml, but without the patient showing symptoms of urinary tract infection (UTI) [1]. This is common during pregnancy. It is quoted, ASB as a condition characterized by absence of symptoms of acute UTI when true bacteriuria exists [2]. Pregnancy enhances the progression from asymptomatic to symptomatic bacteriuria which could lead to pyelonephritis and adverse obstetric outcomes such as prematurity, low birth weight [3] and higher foetal mortality rates [4, 5]. The adverse effects of undiagnosed ASB on mother and child have made researchers to suggest routine culture screening for all pregnant women attending antenatal clinic [6] in order to prevent mother and child from any form of complication that may arise due to infection.

In many hospitals in developing countries including India, instead of routine urine culture test for antenatal patients various rapid tests are preferred, probably due to cost implication, heavy load of the patients and time factor for culture result, which usually takes 48 hours period. The true picture of such urine specimen cannot be fully assessed as the rapid tests cannot quantify the extent of infection in such a patient as well as provide antimicrobial therapy which is usually seen in the case of culture test. UTI by its timely diagnosis and therapy, one can frequently abort the progression from infection to disease and in doing so; implement the pinnacle of therapeutics - preventive medicine [7].

In spite of the documentation of sequel of ASB, serious attention, precisely to diagnose this condition is still not paid. Diligence in prompt, rapid or early diagnosis of UTI is lacking in both clinicians and microbiologists. Screening tests for bacteriuria have their own place. Nevertheless, with few pros and cons, they are of tremendous utility in earlier detection of bacteriuria and prompt treatment especially in the places where culture facilities are not

within reach. Also among the antenatal group of women who are rather more susceptible to develop UTI. The relatively high prevalence of ASB during pregnancy, the significant consequence for the pregnancy and ability to avoid sequel with treatment, justify screening pregnant women for bacteriuria^[7].

Against this background, this work is aimed at determining evaluation of various screening tests like Griess nitrate test, Triphenyl Tetrazolium Chloride Test, Catalase test, Wet mount and Gram stain for detection of bacteriuria among pregnant women attending antenatal clinic in a Shri. Chatrapati Shivaji Maharaj Sarvopchar Rughalaya, Solapur.

2. Material and methods

2.1 Place of work

Study was carried out over a period of one year, from January to December, 2005 after taking the permission from Institutional Ethics committee of Dr. V.M. Government Medical College (VMGMC), Solapur, in the Department of Microbiology in association with Department of Obstetrics and Gynecology. Part of the major study is presented in this paper.

2.2 Participants

Patients from Gynecology department, attending ANC OPD, at Shri. Chatrapati Shivaji Maharaj Sarvopchar Rughalaya, Solapur which were recruited for bacteriologic evidence of ASB were included in the study. 300 married pregnant (study group) and 300 non pregnant women who belonged

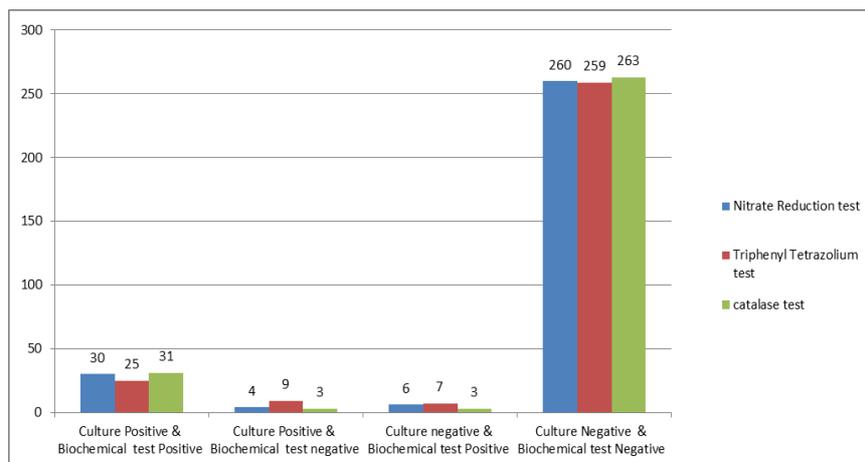
to the age group of 15-35 years, from a neighboring slum area (control group) were included. Counseling of subjects for enrollment procedure was done. Subjects showing symptoms of UTI, suffering from diabetes, under antibiotic or steroids treatment in past, were excluded from the study. Counseling of subjects for enrollment procedure was done. Detailed data from the patients were recorded in a specially formulated structured proforma. Continuous follow-up of subjects was done up to delivery.

2.3 Collection and microbiological analysis

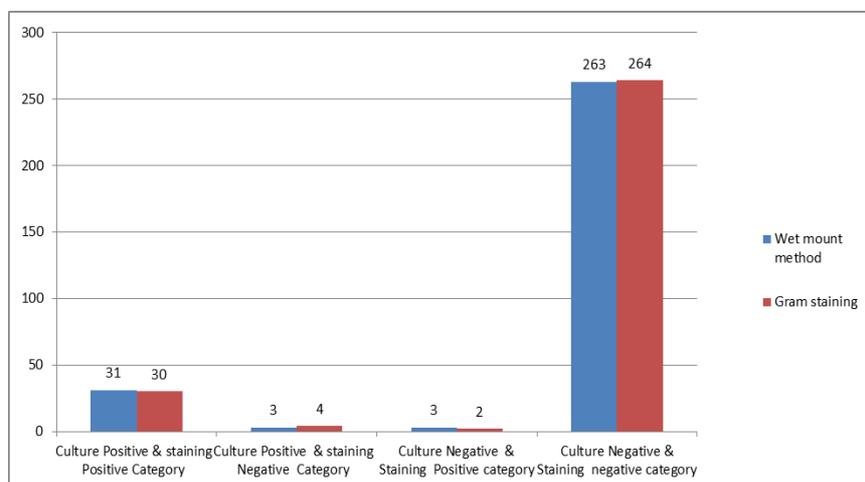
Collection and transportation of urine was carried out by standard procedures⁸. Urine was collected in a sterile tube and capped tightly with cotton. Each patient was instructed to clean her vulva and perineum with soap and water. Urine was collected as midstream sample. Urine specimens were transported to the laboratory within 2 hours of collection. Further work was carried out in the Department of Microbiology, VMGMC Solapur.

Microscopic examination was done by Wet film examination^[9] and Gram staining^[10]. Biochemicals namely, Griess nitrate test^[11], Triphenyl Tetrazolium Chloride Test^[11] and Catalase test^[11] was carried out for detection of bacteriuria among pregnant women. Further, Identification of isolates was done on basis of morphological, cultural characteristics and biochemical tests^[12].

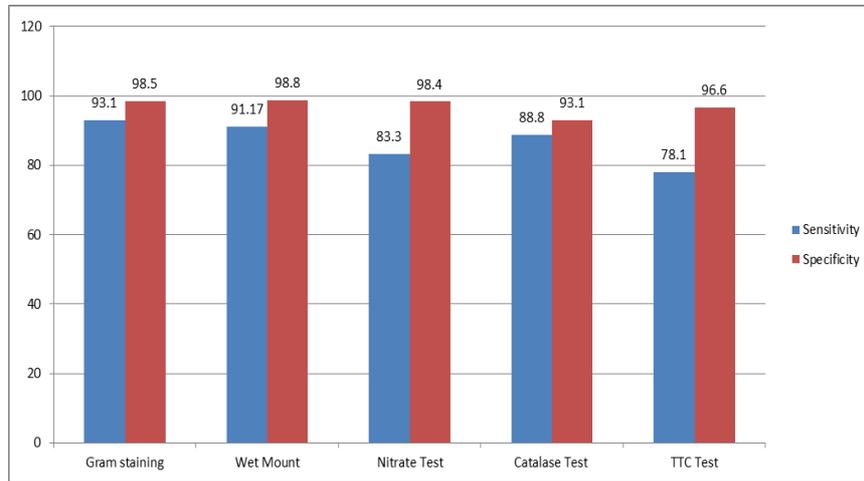
3. Results



Graph 1: Co-relation of Biochemical tests and culture. n=300



Graph 2: Co-relation of staining methods and culture. n=300



Graph 3: Comparison of Sensitivity (%) and Specificity (%) of various screening tests

Highest sensitivity was observed by Gram staining and wet mount method followed by catalase and nitrate test an test. While least sensitivity was of TTC test. Highest specificity was observed in the wet mount method, followed by Gram staining, while lowest specificity was observed in the catalase method.

4. Discussion

Screening urine cultures should be advised for all women, early in pregnancy particularly in sixteenth gestational week [7]. Urine cultures are the most reliable and cost-effective methods of establishing the diagnosis of UTI. However it should be noted that sources of error in the quantitative bacteriological study of urine specimen are frequent, though, that a single positive culture in an asymptomatic patient does not constitute sufficient criteria for starting antimicrobial therapy. Similarly a single negative culture with symptoms of UTI should not be interpreted as final evidence of absence of infection; at least two consecutive cultures are required to come to a conclusion. Many screening methods have been proposed to detect bacteria in urine on the day of collection, both to shorten the delay inherent in the culture system and to decrease the time spent in the laboratory separating negative specimens from those considered to have significant bacteriuria. Although the sensitivity of these screening tests is variable, it may still serve as a useful adjunct to culturing and other urine processing systems in the microbiology laboratory.

Griess nitrate test used to detect the presence of nitrate reducing enzymes produced by the common urinary pathogens [11]. The sensitivity of test ranges between 92% - 100%. False positive and false negative results should be kept in mind and it is not a wholesome criteria for deciding significant bacteriuria [11]. The present study revealed 36 positive Nitrate tests, of which 30 showed culture positivity, 6 were false positive. The sensitivity found out was 83.3% and specificity was 98.4%. Study done by Bandhopadhyay *et al.* [13] suggested that Griess nitrate test is better parameter for detecting bacteriuria. Millar *et al.* [14] showed Nitrate reduction to be 45% sensitive and 97% specific. Williams *et al.* [15] showed 40% positivity of this test with coliform bacteriuria. Kacmaz *et al.* [16] showed 60% sensitivity and 99.2% specificity. Turner *et al.* [17] reported 80% correlativity with positive culture, while Kincaid-Smith *et al.* [18] reported only 50% culture correlativity. This test could be negative on one occasion, positive on another. It seemed

that the test depends upon the concentration of the substrate and time required for the coliform to convert into nitrite. It is definitely a better modality for diagnosis where bacteriological set up is lagging severely [15].

Triphenyl Tetrazolium Chloride Test (TTC) detects urinary pathogens which are capable of reducing colorless soluble 2-3-5 triphenyl tetrazolium chloride to pink or red color of triphenyl formazan by metabolic activity of actively respiring bacteria, though this should not accepted as a sole criteria for detecting significant bacteriuria [11]. Present study showed, Sensitivity 78.1% and specificity 96.6% of TTC. 25 out of 32 positive TTC test results, showed correlation with culture. Lavanya *et al.* [19] noted 76.1% sensitivity and 85.1% specificity. William *et al.* [15] evaluated TTC for culture positivity and found to be 78%. He also stated that TTC does not require skilled personnel, can be carried out within 4 hours and enables detection of almost 85% gram negative bacilluria. Simmon *et al.* [20] quoted that screening by TTC was more simple, quick, cheap and sensitive. However drawback of this test is that it could give false positive results because of presence of lactobacilli, gram negative anaerobes which could be present in scanty amounts in urine with resultant culture negativity. Where health care facilities are limited, TTC serves a better job in early detection of significant bacteriuria.

Catalase test indicates presence of catalase producing organisms [11]. This test is done to detect to activity of catalase in urine.

Overall positivity for catalase found out in the present study was 44.3%. While Sensitivity was found to be 88.8% and specificity of 97.1%. Millar *et al.* [14], in their study which evaluated URISCREEN, an advanced technique for detecting catalase activity in urine, found excellent sensitivity that is 100% and specificity to be 81%. Pezzelo *et al.* [21] stated that URISCREEN had a sensitivity of 95% and specificity of 58%. Waisman *et al.* [22] noted URISCREEN had high sensitivity of 100% but poor specificity with 68.6% value.

Wet mount is one of the most easily available laboratory test for patients of suspected urinary infection. It is one of the most sensitive test advocated by most workers, most ideal for peripheral or primary, health care levels. Out of 300 pregnant women in the present study 34 women had pyuria, of which 31 were culture positive. 3 cases which were negative for culture but showed pyuria, might be due to underlying inflammatory disorder. Millar *et al.* [14] stated

that the screening test with best sensitivity that is 93%, and in his study he detected pus cells and bacteria on microscopic examination of urine. Purpose of wet mount was for detection of pus cells and bacteria if any. Pyuria need not always indicate bacteriuria. It is almost 99% the sign of inflammation which demands exclusion of other conditions mimicking infections. Williams *et al.* [15] have quoted bacteriuria in almost 49% cases that showed pyuria. Lavanya *et al.* [19] stated that the pus cell counting method had sensitivity of 52.3% and specificity of 96.5%. Kacmaz *et al.* [16] also showed that urine wet mount examination was 50% sensitive and 96.7% specific. Bachman *et al.* [23] demonstrated that the wet mount study showed sensitivity of 25% and specificity of 99%, predicted it as a better parameter above others. Bandhopadhyay *et al.* [13] has stated that routine microscopic examination of urine for leucocytes has little relevance because of lack of specificity. Present study observed 91.17% sensitivity and 98.8% specificity of wet mount examination. Hence it is felt that; wet mount was a better predictive marker for identifying acute urinary infections.

Gram staining [11] is the easiest, least expensive, probably the most sensitive and reliable screening method for detecting significant bacteriuria. Presence of at least one organism per oil immersion field, after examining 20 fields, correlates with significant bacteriuria that is greater than 10^5 CFU/ml. It is a very sensitive, simple, inexpensive and practical test done to detect significant bacteriuria. Kass *et al.* [24] has stated that gram stain of freshly collected and un sedimented urine will differentiate contamination from true infection since organisms are readily found in urine when the concentration of bacteria is more than 10^5 / ml of urine. Lavanya *et al.* [19] found sensitivity of 95.2% and specificity of 98.6%. Millar *et al.* [14] *et al.* also suggested gram staining for diagnosing bacteriuria. Bachman *et al.* [23] also favour gram staining as a better criterion in microbiology laboratory. The present study revealed a higher sensitivity and specificity of gram staining that is 93.1% and 98.5% respectively. Thus gram staining and wet mount could be almost warranted as sensitive parameter for detecting bacteriuria. Both gram staining and wet mount correlated very well with culture positivity. Jayalakshmi *et al.* [25] also found higher sensitivity for Gram staining while nitrate test found low sensitivity [2].

Some of the culture methods for detecting bacteriuria are Quantitative [11], Semi-quantitative [11], Standard loop method [11], Filter paper method [11], Dip slide method [11] and semiquantitative method for urine culture by Kanai Mukherjee [11].

5. Conclusion

There are several ways to diagnose UTI, but urine culture still remains most reliable tool for its diagnosis [26]. An ideal screening test should be simple, rapid and accurate and must identify all positive cases, thus a sensitive test with a high NPV and specificity is desirable a screening for ASB in pregnant women must be done to discover infected cases to avoid complications of the five screening tests used, Gram staining and Wet mount were found to be having both, good sensitivity and specificity. Nevertheless, Nitrate reduction, catalase and TTC tests showed good specificity but lower sensitivity. Wet mount and Gram Staining can be applied to screen the pregnant women for ASB on a routine basis.

6. Recommendations

Gram's stain and wet mount of uncentrifuged urine and was found to be the most useful tests with good sensitivity, specificity and NPV, when only those urine containing a pure culture of a potential pathogen of more than 10^5 CFU/ml of urine were considered as positive. It, however, requires trained personnel to screen and the results are subjected to observer variation [27].

Wet mount and Gram Staining of urine do help in suggesting ASB and hence, being simple and rapid, these tests can be widely applied to screen the pregnant women on a routine basis.

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