

Evaluation of Three Screening Methods for Detection of Urinary Tract Infection in Antenatal Women.

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OBJECTIVES – To evaluate three easily available and rapid tests, namely, wet film, gram stain and acridine orange stain for their reliability as screening tests for urinary tract infection. **METHOD** – Wet film, gram staining and acridine orange staining were performed on the urine samples of 131 pregnant women attending the antenatal clinic. **RESULTS** – Acridine orange stain had the best sensitivity of 90% and negative predictive value (NPV) of 98.8% with moderately good specificity. Gram stain, with a sensitivity of 70% also had a good NPV (96.6%). Both these tests detected all the cases of asymptomatic bacteriuria. Wet film had the least sensitivity (40%), but high specificity (90.9%). A combination of wet film and gram stain increased sensitivity to 80% and NPV to 97.7%. **CONCLUSION** – Acridine orange staining is a rapid and useful negative screening test to rule out urinary tract infection in antenatal women. It can also reliably detect cases of asymptomatic bacteriuria. As an alternative, a combination of gram stain and wet film examination may be useful.

Key words : gram stain, acridine orange stain, urinary tract infections

Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections affecting humans throughout their lifetime. Rapid confirmation, early diagnosis and prompt treatment are essential for proper management of these cases. The gold standard for the diagnosis of UTI is a quantitative culture of the urine sample. But this is a costly and labor intensive test and needs 24-48 hours for final results.

Many urine screening methods such as the catalase, leucocyte esterase, nitrite detection, reagent combination dipsticks etc and automated methods like bioluminescence, turbidometry and electrical impedance are available. However, most commercial screening methods are neither easily available nor inexpensive to allow for their use in routine practice in India. Screening tests are advantageous as they are rapid and hence, useful in situations where a large number of negative cultures are being processed e.g. routine screening of the antenatal mothers. They may also be helpful in rapid identification of women having asymptomatic bacteriuria, which occurs in 5-10% of pregnant women¹.

The present study was undertaken to evaluate the

reliability of three simple, cheap and easily available screening tests to detect UTI in pregnant women as part of their routine antenatal checkup.

Materials and Methods

One hundred and thirty one pregnant women attending our antenatal clinic from 21st February, 2002 to 13th March, 2002 were subjects of this study. They were advised to collect mid-stream urine sample in sterile containers. Samples were processed within one hour of collection.

- Direct microscopy (Wet film preparation) – 50µl of well-mixed uncentrifuged urine was taken on a slide and a cover slip placed on it. It was viewed under high power objective. The presence of one pus cell / 7 hpf (high power field) was considered significant pyuria².
- Gram staining – Jensen's modification of Gram staining was employed³. At least 20 fields were examined and detection of one bacteria per oil immersion field was treated as significant⁴.
- Acridine orange staining⁵ – (100 µl of well mixed uncentrifuged urine put on a glass slide was air dried, fixed in 96% methanol for 2 minutes, flooded with acridine orange for 2 minutes, then washed in tap water, dried and examined under incident light fluorescent microscope (Nikon, Germany) using B1-A (Ex-470-490, DM 510, BA 520) filter assembly. A mixture of *Escherichia coli* and *Staphylococcus*

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Table - 1: Results of screening tests with respect to culture

Culture	Wet film		Gram Stain		Acridine Orange	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive (n=10) ($>10^5$ cfu/ml)	4	6	7	3	9	1
Negative (n=111)	10	101	25	86	26	85

Table II : Comparison of screening tests at significant bacteriuria ($>10^5$ cfu/ml)

Tests	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Wet film	40	90.9	28.5	94.3
Gram stain	70	77.5	21.9	96.6
Acridine orange Stain	90	76.6	25.7	98.8
Wet film + Gram Stain	80	78.4	25	97.7
All three	90	51.4	14.3	98.3

NPV – Negative predictive value, PPV – Positive predictive value

aureus was used as positive control and uninoculated broth as negative control. The presence of one or more morphologically similar orange colored organisms per 12 hpf was considered significant. Presence of more than two morphologically different organisms indicated presence of mixed flora.

- d) Culture – 1 μ l of urine was inoculated on Cysteine Lactose Electrolyte Deficient medium (CLED, Hi-Media Laboratories, Mumbai, India) using a standard loop of internal diameter of 1.34 mm (semi quantitative method). The plates were read after 24 hours of aerobic incubation at 37°C. They were incubated for another 24 hours before a negative report was issued. A single organism obtained in counts of $>10^5$ colony forming units (cfu)/ml was further identified by standard biochemical techniques. Anaerobic culture for urine samples is not routinely recommended or performed in our laboratory.

Results

Out of 131 samples 96 (73.3%) were sterile. Organisms

in pure culture in significant numbers ($>10^5$ cfu/ml) were obtained in 10 cases (7.6%). Fifteen samples (11.5%) yielded mixed growth of urethral flora, $<10^4$ cfu/ml (growth of no significance). In four samples, pure growth of organism between 10^4 and 10^5 cfu/ml was obtained and six samples showed gross contamination. These 10 samples were excluded from the analysis.

E. Coli was the commonest isolate (70%) followed by *Klebsiella pneumoniae*, *Enterococcus fecalis*, *Acinetobacter calcoaceticus* and *S. aureus*. Four out of ten women with counts $>10^5$ cfu/ml had asymptomatic bacteriuria.

Out of 10 urine samples with positive cultures, pus cells were present in four samples, gram stain was positive in seven and acridine orange stain was positive in nine (Table I). Comparing the various screening tests (Table II), it was seen that acridine orange had the maximum sensitivity (90%) and highest negative predictive value (NPV) (98.8%). Wet film had the highest specificity (90.9%), but also had the least sensitivity (40%). Gram stain had moderately good specificity (77.5%) and high NPV (96.6%) but the lowest positive predictive value.

PPV) of 21.9%. The combination of all three tests had good sensitivity (90%) and NPV (98.3%) but a high number of false positives (48.7%). A combination of wet film and gram stain had a sensitivity of 80%, NPV of 97.7% and specificity of 78.4%.

Discussion

The mainstay of diagnosis of urinary tract infection is the semi-quantitative culture of a clean catch midstream urine sample. But this is expensive, laborious and results are available only after 24-48 hours. Since only 5-10% of pregnant women are likely to have asymptomatic bacteriuria¹, the number of expected negative urine cultures in antenatal women would be high (>90%) resulting in unnecessary expenditure. Screening tests have the advantage of being rapid, cheap and labor-saving. However, the ideal screening test would be one which can correctly identify negative samples i.e. one with high sensitivity and negative predictive value (>95%) with reasonably good specificity⁶.

In the present study, three easily available and rapid tests were evaluated for their efficacy as screening tests on antenatal urine samples. Wet film of uncentrifuged urine was used to detect pyuria. It has been shown that examination of well-mixed uncentrifuged urine is more reliable than that of centrifuged urine⁷. Significant pyuria, in the absence of significant bacteriuria (counts of $<10^5$ cfu/ml) in a symptomatic patient (e.g. acute urethral syndrome) is an indication for treatment and hence the importance of wet film examination. In this study, however, this test had poor sensitivity (40%) and PPV (28.5%) but good specificity (90.9%). Previous studies have shown this test to have sensitivity ranging between 25-95% and specificity of 41-97%⁸. But the PPV can be as low as 33%⁹. This test hence cannot be relied upon as a screening test.

The gram stain on urine samples has been shown to have a sensitivity and specificity of 52-100% in various studies⁸. In the present study, this test showed a sensitivity of 70% and NPV of 96.6% but very poor PPV. Gram stain has been found to be a reasonably good test for detecting UTI in infants because of its good sensitivity and specificity¹⁰. McNair et al¹¹, however, have shown that gram stain on centrifuged urine samples is unacceptable as a screening test in cases of asymptomatic bacteriuria. Similar results were obtained in another study, where it was shown that the sensitivity and specificity fell when lower levels of sensitivities were taken into consideration¹².

Acridine orange as a urinary screening test has not been evaluated as much as gram stain. Sensitivity ranges from

92 to 98% and specificity from 59 to 87%⁷. In the present study, this test had the highest sensitivity (90%) and NPV (98.8%) but also had the lowest specificity. Similar results were obtained in a study conducted in Delhi, where the sensitivity was found to be 91.2% and specificity 86.2%⁵. Hoff, et al¹³, have demonstrated that this test had a greater NPV of 99% when counts of $>10^4$ cfu/ml were taken as significant on culture and thus would eliminate the need for cultures in approximately 50% of the specimens. Hence, this technique can be recommended as a routine negative screening test especially in large laboratories. This would obviate the need for performing cultures on samples, which are tested negative by preliminary screening. However, a positive test will need confirmation by culture, as the PPV of this test is low.

McNair et al¹¹ found that the combination of gram stain and wet film was useful because of the high number of false negatives in cases of asymptomatic bacteriuria¹¹. However, we found that, using this combination, the sensitivity, specificity and NPV could be increased to 80%, 78.4% and 97.7% respectively. In the absence of acridine orange stain, this combination can be used for screening. The combination of three tests had a high sensitivity (90%) and high NPV (98.3%) but did not offer any advantage over acridine orange as a single screening test.

In the present study, in all the four women who had asymptomatic bacteriuria, both gram stain and acridine orange stain were positive while the wet film failed to detect any pus cells. In view of the consequences of asymptomatic bacteriuria, these findings assume significance because application of appropriate screening test as a routine, will rapidly detect such cases.

The cost of chemical constituents, consumables and other overhead charges were calculated for each of the screening tests and for culture. We found a 10-fold reduction in expenditure when screening tests were used to rule out presence of urinary tract infection. This cost factor makes the screening tests to be a viable and attractive option in peripheral centers where facilities for culture are not available.

Acridine orange staining as a single screening test is recommended as a rapid and inexpensive method to rule out UTI in antenatal women. Since this test requires a fluorescent microscope, which may not be available in all laboratories, a combination of wet mount and gram stain can be used as an alternative. Since no single screening test has been found to have 100% sensitivity, it is recommended that, in spite of a negative screening test, if the symptoms persist for 24-48 hours, another

sample should be submitted for urine analysis and culture.

We suggest that larger studies are needed to validate our findings.

References

1. Savage WE, Hajj SN, Kass EH. Demographic and prognostic characteristics of bacteriuria in pregnancy. *Medicine (Baltimore)*, 1967; 46: 385-407.
2. Collee JG, Duguid JP, Fraser AG et al. Laboratory diagnosis of infective syndromes. In Collee JG, Fraser AG, Marmion BP, eds. Mackie and McCartney Practical Medical Microbiology. 14th ed. *Edinburgh. Churchill-Livingstone* 1996: 53-64.
3. Duguid JP. Staining methods. In Collee JG, Fraser AG, Marmion BP eds. Mackie and McCartney Practical Medical Microbiology. 14th ed. *Edinburgh. Churchill-Livingstone*, 1996:793-812.
4. Garcia LS, Procop GW, Roberts GD et al. Infections of the urinary tract. In Forbes BA, Sahm DF, Weissfeld AD eds. Bailey and Scott's Diagnostic Microbiology 10th ed. *Missourie. Mosby Inc.* 1998:350-62.
5. Sood S, Singh BU, Das BK. et al. Acridine orange staining for bacteriuria screening. *Ind J Med Microbiol* 1995, 13: 200-3.
6. Kellogg JA, Manzella JP, Shaffer SN et al. Clinical relevance of culture versus screening for detection of microbial pathogens in urine specimens. *Am J Med* 1987;83:739-45.
7. Lewis JF, Alexander J. Microscopy of stained urine smears to determine the need for quantitative culture. *J Clin Microbiol* 1976;4:372-4.
8. Daly JA. Non-automated rapid methods for bacteriuria detection. *J Med Technol* 1985;2:759-61.
9. Lohr JA, Portilla MG, Gender TG et al. Making a presumptive diagnosis of urinary tract infection by using a urinalysis performed in an onsite laboratory *J Pediatr* 1993;122:22-5.
10. Lockhart GR, Lewander WJ, Cimini DM et al. Use of urinary gram stain for the detection of urinary tract infection in infants. *Ann Emerg Med* 1995;25:31-5.
11. McNair RD, MacDonald SR, Dooley SL et al. Evaluation of the centrifuged and Gram-stained smear, urinalysis, and the reagent strip testing to detect asymptomatic bacteriuria in obstetric patients. *Am J Obstet Gynecol* 2000, 182:1076-9.
12. Crout FV, Tilton RC. Rapid screening of urine for significant bacteriuria by gram stain, acridine orange stain and AutoBac MTS system *Diagn Microbiol Infect Dis* 1984;2:179-86.
13. Hoff RG, Newman E, Staneck JI. Bacteriuria screening by use of acridine orange stained smears. *J Clin Microbiol* 1985; 21:513-6.