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Original Article

Pathology Section

Reagent Strip Testing (RST) for Asymptomatic Bacteriuria (ASB) in Pregnant Women: A Cost-effective Screening Tool in Under-resourced Settings

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ABSTRACT

Context: Bacteriuria in asymptomatic pregnant women, when it is not detected and treated, may lead to a number of maternal and foetal complications. Culture, though it is a gold standard test for the diagnosis of bacteriuria, it is time consuming and expensive and it is not available at all the healthcare settings. A pre-screen with a simple, rapid and less expensive near patient testing by using reagent strips (RST) can minimise the requirement of the culture, especially in antenatal women without urinary symptoms.

Aims: This study evaluated the performance characteristics of Reagent Strip Testing (RST) as compared to bacteriologic culture in detecting bacteriuria.

Settings and Design: A prospective, blinded study

Methods and Material: Urine samples from 100 consecutive women without urinary symptoms, who attended routine antenatal clinics were subjected to Reagent Strip Testing (RST) (by using four infection associated markers - leukocyte esterase, nitrites, blood and protein) and Bacteriologic Culture.

Statistical Analysis Used: The performance characteristics of Reagent Strip Testing were evaluated against the gold standard urine culture. Different markers singly, or in combination, were assessed to find out the best test strategy for the Reagent Strip Positivity.

Results: The prevalence of ASB in pregnant women, as was determined by culture, was 13%. Reagent Strip Testing (RST) for bacteriuria by using a single marker leucocyte esterase or nitrites yielded a sensitivity of 85% and 62%, which increased to 100%, with a negative predictive value of 1, if the criterion was broadened to positivity with one or more of four infection associated markers. This seemed to be a good rule out test strategy that could reduce the number of urine samples which were sent for culture. Thus, an algorithm of pre-screening the urine samples for infection with reagent strips by using four infection associated markers and performing the culture only on dipstick positive cases could prove to be cost effective in averting the complications which were associated with ASB.

Conclusions: Urinary dipstick testing in a near patient setting is a valuable resource to screen out the negative urine specimens at the point of care. If properly implemented, this programme can result in an improved use of the laboratory resources and it can aid the clinicians in instant clinical decision making.

Key Words: Reagent Strip Testing (RST), Asymptomatic Bacteriuria, Antenatal, India

INTRODUCTION

Infections of the urinary tract are common problems in pregnancy that are associated with serious maternal and perinatal morbidity. They may be symptomatic or asymptomatic. Untreated asymptomatic bacteriuria (ASB) leads to the development of pyelonephritis, intrauterine growth retardation and pre term birth and low-birth-weight infants. The prevalence of asymptomatic bacteriuria among pregnant women, as has been quoted in the western literature, varies from 2 to 10%. Fewer studies on this topic are available on the Indian scenario and the reported prevalence rate is as high as 8% [1,2]. The relatively high prevalence of asymptomatic bacteriuria during pregnancy and its significant consequences on women and on their pregnancies, plus the ability to avoid the sequelae with treatment, justify the screening of pregnant women for bacteriuria [1].

The debate lies on the manner of the screening. The gold standard for the detection of bacteriuria is urine culture. However, the full bacteriological analysis is both time-consuming and expensive and a vast majority of the antenatal urine specimens will be negative to the culture. Thus, a number of other screening methods have been proposed. These procedures are predicated on the concept that the pre-screening of the urine specimens for significant bacteriuria may reduce the need for culture of the non significant samples, increase the cost effectiveness of the culture and assist in immediate patient management. Dipstick urine analysis or Reagent Strip Testing (RST) is a simple and rapid test that can be done at the bedside of the patients and if it is effective, it can minimize the number of samples which are sent for urine culture and make the patient care better [3, 4].

We screened pregnant women who attended the antenatal clinic at our hospital for asymptomatic bacteriuria with dipstick and compared the results with urine culture. Leukocyte esterase and nitrites have been extensively studied for the screening of urinary infections. We included four infection associated markers (leukocyte esterase, nitrites, blood and protein) and assessed whether these could improve the diagnostic performance of the reagent strip testing. In India, there is insufficient local data on the prevalence of ASB and very few studies have been done on RST for bacteriuria

among pregnant women. This prompted us to do this study so that a cost-effective screening of ASB could be done.

SUBJECTS AND METHODS

In this prospective blinded study, clean catch midstream urine samples from 100 consecutive pregnant women who attended the routine antenatal check up were collected after taking their informed consent. Institutional Ethics Clearance was also obtained prior to the start of the study. The mean age of the patients who were studied was 24 years (range: 19- 33 years). Of the 100, 15 presented during the Ist trimester, 45 during the IInd trimester and 40 during the IIIrd trimester. The patients were excluded if they had urinary symptoms or if they had used antibiotics during the preceding two weeks.

All the urine samples were subjected to RST and bacteriologic culture. RST was done at the point of care with a Bayer's 10 parameters urine reagent strip (which included the four infection related markers- leucocyte esterase, nitrites, protein and blood). The samples were collected in a sterile container and they were taken to the Microbiology Laboratory within 1 hour. If there was a delay of more than 1 hour, the specimens were stored in a refrigerator for not more than 4 hours. The specimens were cultured in blood agar and MacConkey's agar by using the standard loop technique. The criterion for clinically significant bacteriuria was either a pure or predominant culture of > 10^5 colony forming units (CFU)/ml, two organisms in similar proportions at > 10^5 CFU/ml, or 10^4–10^5 CFU/ml [1] of a gram negative organism or two organisms where the gram negative organism clearly predominated. All the specimens were also examined microscopically for pyuria and bacteriuria [1]. Microscopy was considered as positive if the results had > 5 pus cells/hpf or >20 bacteria/hpf in the spun samples. Culture was used as a reference method for determining the performance of the urine microscopy and the dipstick data. The performance characteristics of the reagent strips were calculated for the two specific markers, leucocyte esterase and nitrites individually and also for different combinations of the four infection associated markers, in order to find out whether the marker combinations improved the diagnostic efficiency. The sensitivity, specificity, positive predictive value, negative predictive value, the positive likelihood ratio and the negative likelihood ratio were calculated by using the Med Calc statistical software [Tables/Fig-1 & 2].

RESULTS

The prevalence of ASB in the present study was 13% (13/100) as was determined by the quantitative culture by using the criteria which was stipulated by Patel et al. The most common organism which was isolated was E.coli (4/13), followed by Klebsiella (2/13) and coagulase negative Staphylococcus (CONS) (2/13). The performance characteristics of RST for leucocyte esterase and nitrites and the 6 different marker combinations are given in Tables 1 and 2. The sensitivity and the specificity for leucocyte esterase were 85% and 71% and for nitrites, they were 62% and 71%. By using different marker combinations, any one of the four positivities (i.e. positive for one or more of leucocyte esterase or nitrite or blood or protein) yielded the maximum sensitivity (100%) and any one of two positivities (positive for either leucocyte esterase or nitrite) yielded the maximum specificity (83%).

DISCUSSION

Asymptomatic bacteriuria is the presence of actively multiplying bacteria at a time when the patient has no urinary symptoms and this poses a risk for many maternal and foetal complications. In the present study on 100 asymptomatic pregnant women who attended the routine antenatal check-up, 13 were found to be urine culture positive (13%). This was comparable to the figures which were quoted in the international literature (4% to 23.5%). There are not many studies on the incidence of ASB in India. In a study which was by Lavanya SV *et al.,* the incidence of ASB was 8.4% in a south Indian population. E.coli, Klebsiella and CONS were the most grown organisms on culture, whose uropathogenic spectra were similar to that of others [2].

The early detection of ASB is essential for an early treatment and for the avoidance of complications. Bacteriologic culture which is needed to confirm urinary infection, is time consuming and it requires laboratory facilities and competent personnel, which may not be available at all levels of healthcare. So, it may be prudent to have a screening test that is inexpensive, simple and rapid, that

Markers	Total Number of Cases n = 100					
	Positive	Negative	True Positive	True Negative	False Positive	False Negative
L	36	64	11	62	25	2
N	33	67	8	62	25	5
L/N	27	73	12	72	15	1
L/B/P	33	67	12	66	21	1
L/N/P	28	72	12	71	16	1
L/N/B	34	66	11	64	23	2
N/B/P	29	71	8	66	21	5
L/B/P	33	67	12	66	21	1
L/N/P	28	72	12	71	16	1
L/N/B/P	36	64	13	64	23	0

[Table/Fig-1]: Reagent Strip Testing as compared to gold standard Urine Culture.

Marker	Sensitivity (CI)	Specificity (CI)	PLR	NLR	PPV	NPV
L	84.62% (53.7-97.3%)	71.3% (60.4%-0.2%)	2.94	0.21.	0.31	0.96
N	61.6% (32.3-84.7%)	71.3% (60.4-80.2%)	2.14	0.54	0.24	0.93
L/N	92.30% (63.97-99.81%)	82.75% (73.16-90.02%)	5.35	0.09	0.45	0.99
L/B/P	92.31% (63.97-99.81%)	75.86% (65.5-84.4%)	3.82	0.1	0.36	0.99
L/N/P	92.31% (63.97-99.81%)	81.61% (71.86-89.11%)	5.02	0.09	0.43	0.99
L/N/B	84.62% (54.55-98.08%)	73.56% (63.02-82.45%)	3.2	0.21	0.32	0.97
N/B/P	61.55% (31.58-86.14%)	75.90% (65.5-85.40%)	2.55	0.5	0.27	0.93
L/B/P	92.31% (63.97-99.81%)	75.86% (65.5-84.4%)	3.82	0.1	0.36	0.99
L/N/P	92.31% (63.97-99.81%)	81.61% (71.86-89.11%)	5.02	0.09	0.43	0.99
L/N/ B/P	100% (75.29-100%)	73.60% (63.56-82.45%)	3.8	0	0.4	1

[Table/Fig-2]: Performance characteristics of the different infection associated markers in RST

Sensitivity= True Positive/True Positive + False Negative; Specificity = True Negative/True Negative +False Positive; Positive Likelihood Ratio (PLR) = Sensitivity/1-Specificity; Negative Likelihood Ratio= 1- Sensitivity/ Specificity; Positive Predictive Value (PPV) = True Positives/ True Positives + False Positives; Negative Predictive Value (NPV)= True Negatives/ True negatives + False Negatives

which has high sensitivity and reasonable specificity and that which subjects only the positive samples to the culture. This can ensure the optimal use of the lab resources in the improvement of patient care. Reagent Strip Testing (RST) is a potential screening tool for urinary infections, as it is simpler and rapid and as it can be done at the point of care. We evaluated the performance characteristics of these reagent strips against bacteriologic culture by using the four infection associated markers- leucocyte esterase, nitrites, protein and blood- which were available in the multi parameter reagent strips. Most of the workers have studied leucocyte esterase and nitrites and they have reported a variable sensitivity of 16.7 to 92% and a specificity of 83.4 to 100% in detecting urinary infection [4,5] and our results were comparable with theirs- a sensitivity and a specificity of 85% and 71% for leucocyte esterase and a sensitivity and a specificity of 62% and 71% and nitrites respectively. Leucocyte esterase is an enzyme which is produced by neutrophils and its positivity suggests pyuria and not necessarily bacteriuria. So, its negative results do not exclude infection. The test for nitrites relies on the break down of urinary nitrates to nitrites by many gram positive and negative organisms, especially if they are found in significant numbers. Its negative results do not rule out infection. In order to improve the diagnostic efficiency of RST, we decided to use four infection associated biochemical markers (leucocyte esterase, nitrites, blood and protein) in different combinations. The criterion for positivity with any one the four infection associated markers yielded the highest sensitivity (100%), a specificity of 73.6%, a positive likelihood ratio of 3.8, a negative likelihood ratio of 0, a positive predictive value of 0.4 and a negative predictive value of 1 [Table/Fig-2].

A high NPV implied that when the test yielded a negative result, it was most likely that it was correct in its assessment. This made RST a good rule-out test. Our results were comparable to those of Patel et al., (98.3% sensitivity and 0.98% negative predictive value). The ultimate goal of a diagnostic testing is to refine the pre test probability to the point where the physician can confidently make a treat or no-treat decision. The low false negative rate and the difference in the pre test (0.13) and the post test probability (positive result – 0.36, and negative result – 0.0) makes this combination an effective rule out strategy, considerably reducing the number of samples (from 100 to 64) that needed to be sent for culture.

To conclude, the high prevalence of ASB (13% in our study) and the associated complications warrant the screening of pregnant women for asymptomatic bacteriuria. The strategy of the pre screening of urine samples by Reagent Strip Testing (RST) by using positivity for any one of the four infection associated markers, followed by urine culture, ensures a high diagnostic performance andpotential cost savings and it reduces the laboratory workload considerably. It's time that we have a look at this strategy for improving the healthcare and for reducing the maternal and foetal morbidity and mortality.

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