Original Article

DOI: 10.7860/JCDR/2012/4565.2547

Community Section

Can the Griess Nitrite Test and a Urinary Pus Cell Count of ≥5 Cells Per Micro Litre of Urine in Pregnant Women be Used for the Screening or the Early Detection of Urinary Tract Infections in Rural India?

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ABSTRACT

Objectives: Urinary Tract Infection (UTI) is a common problem in pregnancy due to the morphological and the physiological changes that take place in the genitourinary tract during pregnancy. Screening methods may be useful, because a full bacteriological analysis could be reserved for those patients who are symptomatic or those who have positive screening test results. The exact prevalence of UTI in rural, pregnant women is unknown. The present study was undertaken to estimate the prevalence of UTI in pregnant women and for ascertaining the utility of the Griess Nitrite test and the Urinary Pus Cell Count of ≥5 cells per micro litre test for the screening or the early detection of UTI in them at primary health care clinics. Occurrence of urinary complaints was compared in UTI and non UTI women.

Method: We conducted a study on 300 randomly selected, pregnant women from rural areas. Urine cultures, pus-cell counts and the Griess nitrite test were used for diagnosis of UTI. The screening tests for UTI were evaluated in terms of their sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and the percentage of correctly classified.

Results: In the present study, the prevalence of UTI was found to be 29/300 (9.6%, 95% confidence interval 9.57-9.63). The specificities of the two screening tests were comparable (97.05% and 94.47%). Also, the negative predictive values of the two tests were almost similar (97.77% and 96.96%). The percentage of correctly classified by the Griess nitrite test and the urine pus cell count were found to be 95.33% and 92.33% respectively. The proportion of the women with various urinary complaints was significantly higher (P<0.00) in the UTI subjects as compared to that in the non-UTI subjects.

Conclusion: Urine culture remains the gold standard for the detection of asymptomatic bacteriuria. The Nitrite test of uncentrifuged urine was observed to be the best among the screening tests which were evaluated in terms of their efficiency and validity.

Key Words: UTI, Pregnancy, Rural, Urine Culture, Griess Nitrite test, Screening tests, Urinary Pus cell Test

INTRODUCTION

Urinary Tract Infection (UTI) is a common problem in pregnancy due to the morphological and the physiological changes that take place in the genitourinary tract during pregnancy [1]. It can be symptomatic or asymptomatic [2]. The prevalence of UTI in India was reported to be 3.14-19.87% [3-5]. A high prevalence of symptomatic and asymptomatic bacteriuria was found, which was 19.87% and 4.34% respectively [5]. Most of the centres perform a routine analysis of the midstream urine specimen during one or more antenatal visits. However, a full bacteriological analysis is both time-consuming and expensive and a vast majority of the antenatal urine specimens will be negative to the culture [1]. Screening methods may be useful because a full bacteriological analysis could be reserved for those patients who are symptomatic or those who have a positive screening test results. The upper urinary tract infections in particular, may lead to significant morbidity for both the mother and foetus [6]. Bacteriuria is a significant risk factor for developing pyelonephritis in pregnancy, leading to adverse obstetric outcomes such as prematurity, low birth weight [7], higher foetal mortality rates [8], and maternal mortality [9]. Therefore, a proper screening and adequate treatment of UTI during pregnancy is necessary to prevent the UTI complications [10]. At least 25% of the women in the rural areas suffer at least one bout of UTI. In spite of this, UTI has not received adequate attention from the preventive perspective, especially in the developing countries. The positivity of the Griess nitrite test depends on three main factors- the nitrates content, the nitrate reducing bacteria, and sufficient time for allowing the reduction of nitrates to nitrites.

With this background, the present study was undertaken to estimate the prevalence of UTI in pregnancy in a rural setting and for ascertaining methods for screening them in primary care clinics. We also wanted to compare occurrence of urinary complaints in UTI and Non-UTI.

MATERIALS AND METHODS

The present cross sectional study was conducted among the pregnant women who attended the Antenatal clinic of the Rural Health Training Centre of the Medical College, Nagpur, India. On the basis of the pilot study findings, the overall prevalence of UTI was found to be 7%. In order to estimate the prevalence with a 95% confidence interval with an allowance of 5% error; the required sample size was 278. Three hundred pregnant women

who attended an antenatal clinic in the given setting were enrolled from June 2007 to December 2009. A systemic random sampling technique was used to select the study participants.

METHOD

Prior approval was sought from the institutional ethics committee. An informed consent was obtained from the study participants after explaining the relevance, importance and the objectives of the study to them. Pregnant women who were registered at any gestational age, who were with or without urinary complaints and who volunteered to provided urinary samples for simple urine tests, were included in the study. Pregnant women who were not willing to participate or who did not consent, and whose urine culture results indicated a contamination of the sample during its collection were excluded. The data which was related to the study variables like the socio-demographic information and an obstetric and a clinical history which were suggestive of the UTI symptoms were sought through a personal interview technique. The midstream clean-catch technique was used to collect the urine for a sample. The urine was divided into two parts. The first part was labeled, sealed and stored in a cold environment. These were then transported within half an hour to the institutional microbiology laboratory for urine culture and antibiotic sensitivity testing and were processed without delay. The second part was labeled, and immediately used for 2 screening tests viz. the urine pus cell count and the Griess nitrite test.

Urine culture: A semi-quantitative, calibrated loop technique was adopted for the primary isolation of the organism. A loopful of well-mixed uncentrifuged urine was streaked on to the surface of Blood agar and CLED agar. After incubating the plates aerobically for 24 hrs at 37°C, the Colony Forming Units (CFUs) per millilitre of the urine sample was described. The significant bacterial isolates were identified by standard procedures and they were subjected to antibiotic susceptibility testing by Kirby Bauer's disc diffusion method. The diagnostic criteria for ASB were considered when at least two consecutive urine specimens showed $\geq 10^5$ CFU/mL of urine of the same single species in the absence of symptoms.

The pus cell count: The uncentrifuged urine specimens were tested to estimate pyuria. A Neubauer's counting chamber was used. A pus cell count of ≥5 cells per micro litre of urine corresponded to an excrement rate of 4×10^5 leukocytes per hour, which was considered as significant.

The Griess Nitrite test: The uncentrifuged urine specimens were tested by a colorimetric Combur-10 multireagent test (Boehringer Mannheim and Co.), for the presence of nitrite, by following the manufacturer's instructions.

DATA ANALYSIS

The data were entered into an Excel 2007 Microsoft spreadsheet and were analyzed by using the Epi_Info (version 6.04) and the STATA – 10 (2009) software packages. The continuous and categorical variables were presented as mean ± SD and percentages respectively. The significance was assessed by using the Chi-square test. For all the inference purposes, p value of <0.05 was considered as statistically significant. The performance characteristics of each one of the screening tests for the diagnosis of UTI were evaluated against the gold standard (urine culture) in terms of their sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and the percentage which was correctly classified.

RESULTS

The present study was carried out at the Antenatal Clinic of the Rural Health Training Centre of the medical college. A total of 300 pregnant women were included in the study. The mean age (±SD) of the pregnant women was 23.17(±2.91) years. It was observed that a majority [281 (93.67%)] of the women were educated and that 19 (6.33%) were illiterate. Almost all the 291(97%) women were housewives. Around two third of the women belonged to the socioeconomic status, class III and class IV on Prasad's classification [11].

In the present study, 29 urine culture samples were found to be positive for UTI, thus yielding an overall prevalence of 9.6% (95% CI 9.53-9.63%). It was further revealed that the prevalence of symptomatic UTI was 6.67% and that the prevalence of asymptomatic UTI was 3%. It was also found that among the 271 non-UTI cases, 99 (36.53%) women had one or more symptoms of UTI. E.coli, Coagulase negative staphylococci, Klebsiella, Enterococci, Proteus and Candia albicans were the isolates which were reported in this study. Out of the 29 UTI subjects, 18 (62.06%) had E.coli and Coagulase negative staphylococci 3(10.34%) , and the remaining had infections which were caused by Klebsiella 2 (6.90%), Enterococci 2 (6.90%), Proteus 1(3.45%) and Candia albicans 1(3.45%).

[Table/Fig-1] shows validity of the screening tests findings in comparison to the gold standard i.e. the urine culture. The Griess nitrite test could diagnose 23 (79.31%) of the culture positive UTI cases (true positives). It could not diagnose 6 (20.67%) UTI cases, (false negatives). Among the negative urine cultures, the Griess nitrite test was positive in 8 cases (2.95%), (false positives) and it was negative in 263 cases (97.05%), (true negatives). The test could detect almost E.coli (17/18 isolates), Coagulase negative staphylococci (2/3 isolates), Klebsiella (2/2 isolates), Proteus (1/1 isolate) and a mixed growth of E.coli and Klebsiella (1/1).

The urine pus cell count of ≥5 per high power field (hpf) could diagnose 21/29 (72.41%) culture positive UTI cases; (true positives). It could not diagnose 8 (27.59%) culture positive UTI cases; (false negatives). Among the cases with negative urine cultures, the urine pus cell count was positive (≥5 / hpf) in 15 cases (5.53%); (false positives) and it was negative in 256 cases (94.47%); (true negatives). Based on this data, the sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and the percentage which was correctly classified of both the screening tests were calculated by using urine culture as the gold standard for the diagnosis of UTI.

[Table/Fig-2] shows the performance characteristics of the 2 screening tests. The sensitivity of the Griess nitrite test (79.31%) was higher than that of the urine pus cell count (72.41%). Also, the positive predictive value of the Griess nitrite test (74.19%) was higher than that of the urine pus cell count (58.33%).

The specificities of the 2 screening tests were comparable (97.05% and 94.47%). Also, the negative predictive values of the two tests were almost similar (97.77% and 96.96%). The efficiency of the Griess nitrite test and the urine pus cell count was found to be 95.33% and 92.33% respectively.

[Table/Fig-3] shows that a burning micturition was reported by 55.17% of the UTI subjects and by 12.92% of non-UTI subjects (X^2= 33.15, df=1, p=0.00001 and the difference was highly significant). Among the UTI subjects, 51.72% complained of an increased frequency of micturition as compared to 21.03% of the

non-UTI subjects (x^2= 13.53, df=1, p=0.00 which was statistically significant). A significantly higher 17.24% of the UTI subjects had dysuria as compared to 4.79% of the non-UTI subjects (x^2=7.19, df=1, p=0.00001, Almost one fourth of 24.14% of the UTI subjects had fever with chills as compared to 2.58% of the non-UTI subjects (x^2=27.36, df=1, p=0.00 which was statistically significant) loin pain was significantly higher 10.34% of the UTI subjects as compared to 1.85% of the non-UTI subjects (x^2=7.29, df=1, p=0.00001).

Screening tests	Urine culture	
	Positive n=29 (%)	Negative n=271 (%)
Griess Nitrite Test		
Positive	23(79.31)	8 (2.95)
Negative	6(20.67)	263(97.05)
Urine pus cells count		
Positive (≥5 / hpf)	21(72.41)	15(5.53)
Negative (<5 / hpf)	8(27.59)	256(94.47)

[Table/Fig-1]: Validation of the screening tests.

Screening Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficiency (%)
Griess Nitrite Test	79.31	97.05	74.19	97.77	95.33
Urinary Pus Cell Count ≥ 5/ hpf	72.41	94.47	58.33	96.96	92.33

[Table/Fig-2]: Comparison of Griess Nitrite test and urinary Pus cells count >5/hpf test with urine culture in the detection of UTI

Urinary complaints	UTI (N=29) No. (%)	Non-UTI (N=271) No. (%)	Total (N=300) No. (%)
Burning micturition	16 (55.17)	35 (12.92)	51 (17.00)*
Increased frequency of micturition	15 (51.72)	57 (21.03)	72 (24.00)*
Fever with chills	7 (24.14)	7 (2.58)	14 (4.67)*
Dysuria	5 (17.24)	13 (4.79)	18 (6.00)*
Loin pain	3 (10.34)	5 (1.85)	8 (2.67)*

[Table/Fig-3]: Distributon of urinary complaints in UTI and Non-UTI women.
* p<0.05

DISCUSSION

The present study was carried out among 300 pregnant women in a rural area, to estimate the prevalence of UTI during pregnancy. We evaluated the performance of the Griess nitrite test and the urine pus cell count as the screening tests for UTI against the pus cell count- the gold standard.

The Prevalence of UTI

The prevalence of UTI among the pregnant women in the present study was 9.67%. The prevalence of symptomatic and asymptomatic UTI was 6.67% and 3% respectively. These findings corroborated well with those of other Indian studies [1,3-5]. The higher prevalence (19.87%) for symptomatic bacteriuria which was reported by Bandyopadhyay S et al., [7] could be because of the inclusion of non-specific symptoms like low backache and lower abdominal pain. In our study, we included only the urinary symptoms to define the symptomatic bacteriuria.

The Performance Characteristics of the Screening Tests for UTI

The Griess nitrite test: The sensitivity of the Griess nitrite test in our study was found to be 79.31%.and the specificity was 97.05%. This test had a Positive Predictive Value (PPV) of 74.19% and a Negative Predictive Value (NPV) of 97.77%. The lower PPV which was found in this study could be because of the low prevalence of UTI in the study setting. Another reason could be that the collection of the randomly voided urine samples was done due to feasibility issues, while the first void morning urine sample would have been ideal for this purpose. These findings were similar to that of other Indian studies [12-14]. However, J Jayalakshmi et al., [1] reported a higher PPV of. 94.5%.

The Urinary Pus Cell Count: Our study found a low sensitivity (72.41%), a high specificity (94.47%), and also a low PPV (58.33%). This could partly be explained by the fact that we could not use centrifuged urine due to the non-availability of a centrifuge machine at the rural study settings. Pus cells in urine are seen in all types of inflammations (genitourinary TB, gonococcal infection, etc) and they are not specific for UTI. Also, the patients with asymptomatic bacteriuria often do not excrete an increased number of leucocytes in the urine. So, they might have been under diagnosed by the urine pus cell count. These findings are similar to those of other Indian studies [13-15]. Although the sensitivity and the specificity are of interest from the public health point of view, other qualities of the test like the NPV and the PPV are also equally important for validating the screening test. In a setting like ours, the Griess nitrite test and the pus cell test with a high NPV of 97.77% and 96.96% respectively, provided the reassurance that the subjects were unlikely to have UTI. This high NPV value would be particularly of importance if the UTIs were relatively common. An early detection and treatment of ASB may be of considerable importance, not only to forestall acute pyelonephritis and chronic renal failure in the mother, but also to reduce prematurity and foetal mortality in the offspring.

The nitrite test is an indirect measure of the nitrate reducing bacteria, which includes all the enterobacteriaceae, most of the non-fermenters and the gram-negative cocci, provided the urine contains sufficient dietary nitrates and if it has been retained in the bladder for longer than 4 hours. The first voided urine specimen has been proven to be accurate, but the sample collection was not possible in all of the patient population. Similar difficulties were discussed by previous workers [1]. Although 6 positive cases were missed by this test, by and large, the nitrite test was acceptable by itself as a screening test in our study setting.

The pus cell count test of unspun urine which is done by using a Neubauer's counting chamber, is an accurate method for diagnosing UTI. It is also useful for screening ASB. But it is a very cumbersome procedure. Moreover, it requires trained personnel to screen and the results are also subjected to an observer variation [16]. Our study yielded a low sensitivity but a high specificity and a high NPV. Another study which was done by Jailaxmi et al., also reported a low sensitivity and a low NPV. Whether the low sensitivity for pyuria is suggestive of the bladder colonization or an actual infection, is rather controversial [16]. In our study, the prevalence of the 4.1% sterile pyuria may be attributed to the infections which were caused by organisms like *Chlamydiae* , which failed to grow in the media which were used for their isolation [18]. Besides, the hypotonic urine or alkaline urine which is due to the presence of

Proteus, *Klebsiella* and *Pseudomonas,* can cause disintegration of the pus cells.

In our study, the most common organism which was isolated in the urine culture was E.coli (62.06%), followed by Coagulase negative Staphylococci (10.34%).The antibiotic susceptibility of the organisms was as follows: It was the maximum for nitrofurantoin (92.85%), followed by gentamycin (78.57%), cefotaxime (67.85%) and amikacin (57.14%). The maximum resistance was noted for cotrimoxazole (75%), followed by norfloxacin (64.28%) and ampicillin (53.57%). Similarly, J.Jayalakshmi et al., [1] isolated E.coli as the most common isolate (57.4%), followed by Klebsiella (19.21%). Lavanya SV et al., [5] also found that E.coli was the most common organism which was isolated (83.3 %), followed by E.coli + Klebsiella (4.7%).The organisms were sensitive to cephalexin, nitrofurantoin, amoxycillin and norfloxacin, in the decreasing order. Sutapa Bandyopadhyay et al., [7] found that among all the significant growths, 64.1% was of E.coli, 15.4% was of E.faecalis and that 7.7% was of Klebsiella. Among all the isolates, 44% each was resistant to ampicillin and cotrimoxazole. A resistance to nitrofurantoin and norfloxacin was noted in 5% of the isolates only. Other studies which were done by Lynnae Millar et al., [14] Robbye D McNair et al., [18] Taneja Neelam et al., [19] and Stuart K. Shelton et al., [20] also found E.coli as the commonest uropathogen among pregnant women. This could be due to the fact that urinary stasis is common during pregnancy and that E.coli prefer such an environment.

All the urinary complaints were significantly higher in UTI subjects as compared to those in non-UTI subject. The most common symptoms which were suggestive of UTI were burning micturition, an increased frequency of micturition of > 10/day and dysuria. These findings were consistent with those of Sharma et al., [21,22]. The symptoms are the best clinical predictors for the detection of UTI; however, these manifestations appear very late and in the mean time, the infection affects the maternal and the foetal health. Urinary Tract Infections (UTI) can lead to poor maternal and perinatal outcomes.

CONCLUSION

Urine culture remains the gold standard for the detection of asymptomatic bacteriuria. The nitrite test of uncentrifuged urine was observed to be the best among the screening tests which were evaluated. It is an easy to perform test, the results are immediately available and it does not require trained professionals or any equipment. The high specificity of the Griess nitrite test which was found in our study underlines its importance in referring only positive cases for the culture, thereby reducing the culture load. This test can also be utilized for the preventive gynecological/ obstetric health services in the rural health centres, as it does not require any special training, nor does it put an extra burden on the existing resources.

ACKNOWLEDGEMENT

The development of ideas and the mode of their presentation in this work owe much to Dr. Archana Patel, M.D. Paediatrics, Professor and Head, IGGMC, Nagpur, India from whom I have learned and continue to learn a great deal. Her kin interest, constructive criticism and constant encouragement were the prime factors that made my task easy and enabled me to complete this work successfully.

With great pleasure, I acknowledge my gratitude to respected Dr. Mohan Khamgaonkar, Professor and Head, Department of community medicine, Indira Gandhi Government Medical College, Nagpur, India for his encouragement.

My special thanks to all the staff members of rural health Training Centre, Saoner.

I take this opportunity to thank all study participants for their support.

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FINANCIAL OR OTHER COMPETING INTERESTS:

None.

Date of Submission: **May 19, 2012**
Date of Peer Review: **May 29, 2012**
Date of Acceptance: **Oct 15, 2012**
Date of Publishing: **Nov 15, 2012**