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Diagnostic Accuracy of Griess Test for Asymptomatic Bacteriuria in Pregnancy

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RM, HK and SVK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SS, ASD and DHL managed the literature searches, analyses of the study performed the spectroscopy analysis and authors RM, HK and SVK managed the experimental process. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: Asymptomatic bacteriuria (ASB) is a microbiological diagnosis based on the isolation of a specified quantitative count of bacteria in a properly collected specimen of urine from pregnant women without signs and symptoms, which are referable to urinary tract infection. Global prevalence of ASB in pregnancy is 1.9-9.5%.
Objectives: 1. To evaluate the accuracy of Griess test as a tool for screening of ASB in pregnancy. 2. To measure the validity (sensitivity and specificity) of Griess test in comparison with urine culture (Gold standard) and its diagnostic ability by ROC curve.
Methodology: A Cross sectional study was done during June and July 2012. Predesigned, pretested questionnaire was used for collection of data regarding demographic profile. Midstream

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urine sample was collected in sterile container, Griess test was done and urine sample sent for culture.

Results: In the present study, the prevalence of asymptomatic bacteriuria was 18% among the pregnant women. The sensitivity of Griess test was 92.3% and specificity was 99%. Area under the curve is 0.96. Hence Griess test has good diagnostic value when compared to urine culture for detecting bacteriuria, it is statistically highly significant with p=0.000001.

Conclusion: ASB is not uncommon among antenatal mothers in the population studied. Hence routine urine examination and Griess test for detecting ASB should be included in the ANC on routine basis.

Keywords: Asymptomatic bacteriuria; Griess test.

1. INTRODUCTION

Asymptomatic bacteriuria (ASB) is a microbiological diagnosis based on the isolation of a specified quantitative count of bacteria in a properly collected specimen of urine from persons without signs and symptoms, which are referable to urinary tract infection. The term ASB is used when a bacterial count of the same species over 10^5/ml in mid-stream clean catch urine on two occasions is detected without symptoms of urinary tract infection (UTI) [1].

Normally, the urine is sterile until it reaches the distal urethra. Various defence mechanisms of the body prevent the infection of urinary tract. One of the most important defence mechanisms is the outward flow of urine that can clear 99% of the organisms experimentally inoculated in the bladder. The acidic pH (5.5) and low osmolarity of urine also discourage the bacterial growth. Similarly there are a number of factors that increase the risk of developing urinary tract infection, like sex, pregnancy, catheterization, congenital abnormalities in urinary tract, diabetes mellitus etc [2].

In pregnancy, the tendency of urinary tract infection increases partly due to the pressure of gravid uterus on the ureters causing stasis of urine flow and is also attributed to the humoral and immunological changes during pregnancy [3]. The hormonal changes in pregnancy leads to decreased bladder tone, diminished peristalsis and dilatation of renal pelvis and ureter. It has been claimed that pregnancy produces physical obstruction in the female urinary tract and obstruction is one of the important risk factor for the development of infection [3,4]. Pregnant women have recurrent urinary tract infection with commensal and non-commensal microorganisms [5]. A urinary tract infection itself is no threat to a pregnant women or the foetus, but a UTI may spread to the bladder. From the bladder, the infection can spread to the kidneys, where it can cause complications. Once a UTI infects the bladder and kidneys, a pregnant woman is at the risk of hypertension, preeclampsia, anaemia and amnionitis: An infection of the amniotic membrane. Bladder and kidney infections increase the chance of premature labour and low birth weight [6]. Twenty five percentage of pregnant women with asymptomatic bacteriuria go on to develop acute pyelonephritis [2]. According to Edward H. Kass et al. [5] 6-7% of pregnant women have bacteriuria; in almost half of these, pyelonephritis will develop in pregnancy and these women are also more liable to produce premature or still birth. Fortunately, prompt treatment of bladder and urinary tract infections with antibacterial drugs in most pregnant women usually prevents kidney infections.

The gold standard test for bacteriuria is urine culture but laboratory charges make this test expensive for routine screening in population that have a low prevalence of ASB and also this test takes 72 hours to give results [7,8]. Appropriate screening and treatment of asymptomatic bacteriuria will reduce the infection rate to 3% [9]. Wadland and Plante [10] performed analysis in a family practice obstetrics population and found screening for asymptomatic bacteriuria to be cost effective. The decision about how to screen asymptomatic women for bacteriuria is a balance between the cost of screening versus the sensitivity and specificity of each test. ASB detected at the earliest by using simple procedure like Griess test, urine microscopic examination and urine culture. Griess test can be used as a screening test for ASB. The Griess test is a chemical test used for screening of asymptomatic bacteriuria. Griess, a German scientist, developed in 1879 a reagent for the detection of nitrites in solutions. The reagent, of sulfanilic acid and α-naphthylamine, undergoes a diazotization reaction with nitrites to form a red

azodye [11,12]. The association between urinary nitrite and urinary tract infection was first reported by Cruickshank and Moyes in 1914 [13]. The Griess test utilizes the principle that nearly all of the bacterial species, which cause ASB, reduce the nitrate present in urine to nitrite if given sufficient time. The test does not require special skill and seems very accurate in detecting significant bacteriuria [11,12]. Approximate cost was less than 10 paise/sample [14].

Hence this study was taken up to evaluate the accuracy of the Griess test as a tool for screening of asymptomatic bacteriuria in pregnancy and to measure the validity (sensitivity and specificity) of Griess test in comparison with urine culture (Gold standard) and its diagnostic ability by ROC curve.

2. MATERIALS AND METHODS

The present cross sectional study was conducted in field practice area of urban health care attached to Department of Community Medicine. The urban health centre serves a total population of 8530 which includes 1483 eligible couple (Jan 2012) and 60 women registered in ANC register, were available as study participants, hence in the current study universal sampling method was adopted. But during the study, 71 pregnant women participated; study was done during the month of June and July 2012. Basic information of the pregnant women was collected by using predesigned proforma after obtaining informed consent. To classify socioeconomic status, modified B.G. Prasad classification was used. All pregnant women who were willing to participate and gave consent were included in the study. Pregnant women suggestive of urinary tract infection, diabetes mellitus, hypertension, having known congenital anomalies of urinary tract and who had taken antibiotics for any reason in the past 2 weeks were excluded from the study. The study subjects were asked to collect a mid-stream urine sample by aseptic method in a sterile container. Griess test was done within one hour of collection of urine sample. The same urine sample was immediately cultured by semi quantitative method on Mac Conkey agar by standard procedure.

2.1 Preparation of Griess Reagent [11,12]

1.5 g of Sulphanilic acid (chemically pure) was dissolved in 450 ml of 10% acetic acid. This solution was added to a solution of 0.6 g of α-naphthylamine (chemically pure) in 60 ml of boiling distilled water and filtered through Whatman number 1 filter paper. This colourless reagent was stored in an air tight amber coloured bottle to prevent oxidation. This reagent was stable for 2-4 weeks; decomposition was detected by the appearance of pink colour.

2.2 Procedure [11,12]

1 ml of the urine sample was taken in a clean test tube. To it 1 ml of the Griess reagent was added. The immediate development of pink to dark red colour shows the presence of nitrites and hence the presence of appreciable coliform infection.

2.3 Statistical Analysis

Statistical analysis was done by using SPSS version 11.0 software. Sensitivity, specificity, positive predictive value and negative predictive value were calculated. ROC curve was constructed based on the above findings.

3. RESULTS

In the present study, out of 71 pregnant women 13 were having ASB (18%). The maximum study group were in the 19-23 years age group constituting 53.5%. 83.1% literate were and 16.9% were illiterates. Maximum study subjects were in the SES of III and IV constituting 71.9% (Table 1). 50.7% of the husbands (counterparts) were in the age group of 28-32 years. About 89% of the study subjects were housewives. Maximum study subjects were Hindus (93%). About 56% of the study subjects belong to nuclear family.

Nulliparous pregnant women were 40.8% and primigravida were 40.8%. The maximum pregnant women were in 2nd trimester (56.3%) (Table 2). About 50% of the study subjects were having 1 or 2 living children, 13% of the study subjects had previous abortion.

Sensitivity of the Griess test with 95% confidence levels was 92.3% (66.69-98.63). Specificity of the Griess test was 99% (90.86-99.7). The positive predictive value was 92.31% (66.69-98.63). The diagnostic accuracy of the test was found to be 97.18% (90.3-99.22) (Table 3). Area under the curve was 0.96.Hence the Griess test has best diagnostic value when compared to urine culture for detecting bacteriuria, it is statistically highly significant with p=0.000001 (Fig. 1).

Table 1. Demographic profile of the study subjects

Age years	Total number	Total percentage	ASB present	ASB present percentage
<= 18	01	1.4	01	7.7
19 - 23	38	53.5	07	53.8
24 - 28	26	36.6	03	23.1
29 - 33	03	4.2	01	7.7
34+	03	4.2	01	7.7
Education status	**Total number**	**Total percentage**	**ASB present**	**ASB present percentage**
illiterate	12	16.9	04	30.8
Primary school	16	22.5	02	15.4
Secondary school	14	19.7	04	30.8
PUC/Diploma	22	31.0	01	7.7
Degree	06	8.5	02	15.4
Post-graduation	01	1.4	0	0.0
SES	**Total number**	**Total percentage**	**ASB present**	**ASB present percentage**
I	09	12.7	02	15.4
II	11	15.5	00	0.0
III	33	46.5	06	46.2
IV	18	25.4	05	38.5

ASB: Asymptomatic Bacteriuria; PUC: Pre-university College; SES: Socioeconomic status (Modified B. G. Prasad classification)

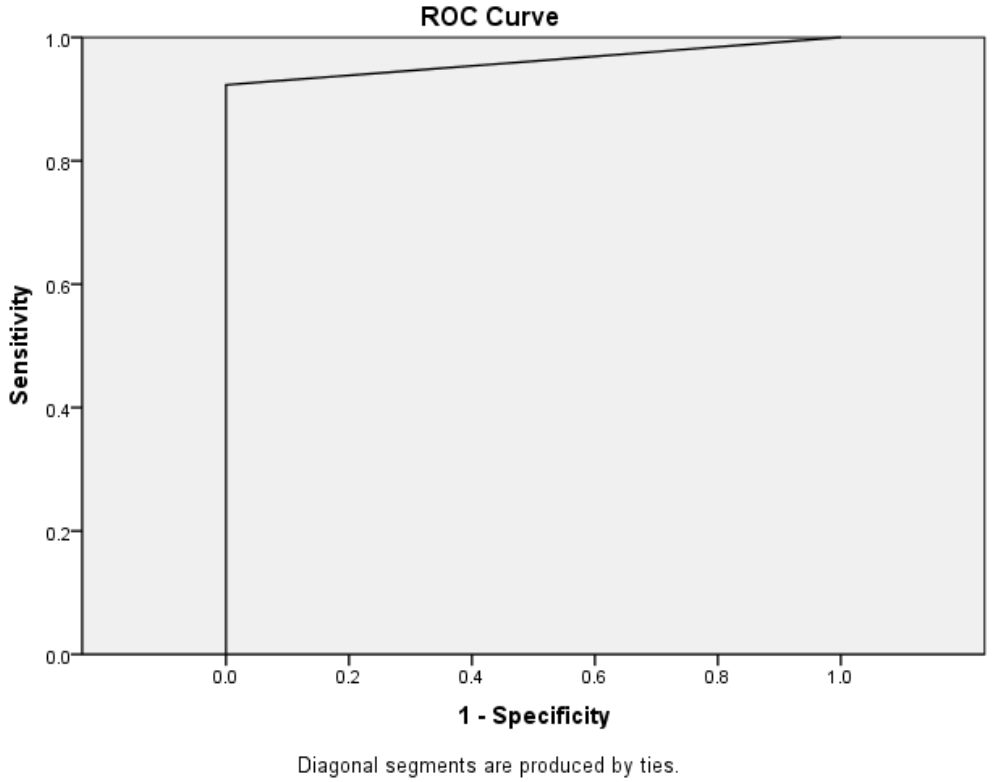

Fig. 1. Diagnostic ability of Griess test
Area under the curve is 0.96

Table 2. Distribution of study subjects according to obstetric history

Gravida	Total number	Total percentage	ASB present	ASB present percentage
1	29	40.8	09	69.2
2	17	23.9	01	7.7
3	21	29.6	03	23.1
4	03	4.2	00	0.0
5	01	1.4	00	0.0
Para	**Total number**	**Total percentage**	**ASB present**	**ASB present percentage**
0	29	40.8	08	61.5
1	22	31.0	03	23.1
2	16	22.5	02	15.4
3	04	5.6	00	0.0
Trimester	**Total number**	**Total percentage**	**ASB present**	**ASB present percentage**
1st	03	4.2	02	15.4
2nd	40	56.3	07	53.8
3rd	28	39.5	04	30.8

ASB: Asymptomatic Bacteriuria

4. DISCUSSION

In the present study, the prevalence of asymptomatic bacteriuria is 18% among pregnant women. Global prevalence of ASB in pregnancy varies from 1.9 to 9.5% [5]. In Monireh Rahimkhani et al study, the bacteriuria in pregnant women was found to be 29% [15]. Carla Baleiras et al. [16] in their study, reported the prevalence of asymptomatic bacteriuria from 5-10%. Kutlay S et al. [17] found the prevalence of ASB was 10.6%. It was 6.2% in a study conducted by Gayathree S et al. [18] in South Karnataka, India. The differences in the prevalence of ASB could be due to various cultural practices with regard to hygienic practices.

The maximum number of affected participants (53.8%) were between the age group 19-23 years, followed by 24-28 years (23.1%). In Paul Erhunmwunse Imade study, the maxium affected group was between 21-35 years (71.4%), similarly R J Girishbabu et al. showed 80% of affected pregnant women were between 18-35 years of age [19,20]. ASB was more common among the literates (69.2%) than the illiterates (30.8%). As education helps to change the myths and beliefs and ultimately changing the attitudes towards health and hygiene, so it can play an important role to decrease the prevalence of ASB, but this is not true in the present study, it could be due to sampling variation. Mid socioeconomic (SES III) women were more affected (46.2%) by ASB; it is closely related to socioeconomic status. Turck et al. [21] reported, 2% in non-indigent pregnant women of middle socioeconomic status were affected by ASB compared to 6.5% of indigent patients [22]. Women belonged to lower socioeconomic status were affected more (60%) as compared to women belonging to middle class (40%) in Robina Ali et al. [23] study.

Table 3. Comparison of Griess test with urine culture

		Urine culture	
		No	**Yes**
Griess test	Negative	57	01
	Positive	01	12

Chisquare: 64.4, df=1 p<0.000001 HS

Sensitivity	92.3%	66.69-98.63
Specificity	99%	90.86-99.7
Positive predictive value	92.31%	66.69, 98.63
Negative predictive value	98.28%	90.86, 99.7
Diagnostic accuracy	97.18%	90.3, 99.22

In the present study the nulliparous (61.5%), primi gravida (69.2%) were more affected than others. Christian Obirikorang et al. [24] study observed that nulliparous women (8.6%) were affected less than the multiparous women (9.7%), but it was not statistically significant ($\chi 2 = 0.043$; $p = 0.8366$). Robina Ali et al. [23] observed that multigravida (80%) were affected more than primigravida (20%). Monireh

Rahimkhani et al. [15] found that ASB was significantly higher in pregnant women (29.1%) than non-pregnant women (5.4%). ASB in present study was common in second trimester (53.8%) of pregnancy, which is in accordance with Robini Ali, who found maximum ASB in second trimester with 55% [23]. Another study conducted in Nigeria also found maximum ASB during second trimester constituting 48% [19]. R J Girishbabu et al. [20] found equal prevalence (40%) in second and third trimester of pregnancy. But Paul Erhunmwunse Imade et al. [19] observed no significant difference in the prevalence of asymptomatic bacteriuria with respect to trimester (P=0.2006).

In the present study, Griess test was found to be 92.3% sensitive and 99% specific, whereas the study done by Aziz Marjan Khattak et al. [25] found the Griess test to have 75% sensitivity and 97.79% specificity. Similar study conducted by Gayathree L et al. [18] found very high sensitivity (70.96%) and specificity (99.28%). Birgul Kacmaz [26] found sensitivity 60% and specificity 99.2% for Griess test. Area under the ROC curve in the current study was 0.96. Hence Griess test has good diagnostic value when compared to urine culture for detecting bacteriuria, which was statistically highly significant with p=0.000001.

The limitations of present study were the small sample size and short study period. Follow-up of the ANC cases can yield the more information about the pregnancy outcome and complications. Hence for better understanding about ASB, the large sample size and long term follow-up studies can be considered.

5. CONCLUSION

The definitive study of asymptomatic bacteriuria (ASB) is based on the urine culture which takes 72 hrs for the results and is also considered expensive. Griess test is rapid, accurate and inexpensive. It would be of great advantage in the routine diagnosis of UTI. Test can be conducted in remote areas where microscopic facilities are not available. It can also be included in routine antenatal care in National programme as it is very simple to administer even by health workers. If the test shows positive, they can be referred to higher centre for further management and prevention of pregnancy wastages.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Institutional Ethics Committee of S. N. Medical College Bagalkot, Karnataka, India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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