

ACCURACY OF GRIESS TEST TO PREDICT ASYMPTOMATIC BACTERIURIA DURING PREGNANCY

¹Aziz Marjan Khattak, ²Habibullah Khan, ²Wasim Akhtar,
¹Ihsanullah Mahsud and ³Bushra Ashiq

¹Department of Pathology & Medicine, Gomal Medical College and

²Department of Microbiology BMSI, JPMC Karachi

ABSTRACT

Background: Screening of asymptomatic bacteriuria (ASB) is strongly recommended to detect and treat as preventive health services for safe outcome of pregnancy. Untreated ASB is a potential risk factor for mother and baby. Culture is the gold standard method to detect ASB but cannot be adopted everywhere in our province as it is available in few major cities. The objective of this study was to see the accuracy of Griess test as a screening test to detect nitrates reducing bacteria in urine.

Method: In this study the pregnant women coming for antenatal check up and registration in the Gynae/Obs Department of JPMC were included according to prescribed criteria during the period of September 2001, to March 2002. Urine was collected as midstream voided specimen and immediately transported to the laboratory, where the specimens were processed for Griess test and culture. The results were recorded and calculated for sensitivity and specificity of the test.

Results: The specimen of 290 cases were examined for bacteriuria with Griess test and also inoculated on media. There were 18 cases positive for bacteriuria with Griess test. Culture positive 24 cases were found. The culture positive 6 cases were found mixed growth (contaminants) and rest of the growth were pure growth giving 75% sensitivity and 97.79% specificity of the test.

Conclusion: Although Griess test cannot be compensatory for culture it can be applied as a screening test due to good sensitivity and specificity. It is easy to perform with instant results and to interpret with out going special training for it.

Key words: Bacteriuria, Pregnancy, Urine nitrites, Urine culture, Griess Test.

INTRODUCTION

Urinalysis is the commonly used test for evaluation of patients with potential urinary tract infections^{1,2}. Urinary tract infections are one of the commonest infections to occur during pregnancy. Their frequency and seriousness have been well recognized for more than a century³.

Although they may be relatively asymptomatic, urinary tract infections must be diagnosed since they have morbid potential in the form of cardiovascular and obstetrical complications and in eventual renal failure⁴. The early detection and treatment of asymptomatic bacteriuria (ASB) is of great potential value for pregnant women⁵. ASB occurs in 2-7% of pregnant women; of those who are not bacteriuric at initial screening, 1-2% will develop bacteriuria later in pregnancy^{6,7}. Several indirect chemical tests have been proposed to screen urine samples for significant bacteriuria. The Griess test is one of chemical tests to use as screening procedure for asymptomatic bacteriuria. Griess, a German scientist, developed in 1879 a reagent for the detection of nitrites in solutions. The reagent, an acid solution of sulfanilic acid and α -naphthylamine, undergoes a diazotization reaction with nitrites to form a red azo

dye⁸. The association between urinary nitrite and urinary tract infection was 1st reported by Cruickshank and Moyes in 1914⁹. The Griess test utilizes the principle that nearly all of the bacterial species, which cause ASB reduce the nitrate that is normally present in urine to nitrite if given sufficient time³. The test is easy to perform, easy to read and seems very accurate in detecting significant bacteriuria¹⁰.

The most accurate test for bacteriuria is urine culture but laboratory charges make this test expensive for routine screening in population that have a low prevalence of ASB⁵. Wadland and Plante¹¹ 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. The gold standard for detection of bacteriuria is urine culture but this test is costly and takes 24 to 48 hours to obtain results¹². The accuracy of faster screening methods (leukocytes esterase dipstick, nitrite dipstick, urinalysis and urine gram staining) has been evaluated and were found not to be of use in the screening for asymptomatic bacteriuria^{6,13,14,15}.

A prospective study was performed to know the accuracy of Griess test as a screening tool for asymptomatic bacteriuria.

MATERIALS & METHODS

This study was conducted in Microbiology department of BMSI. Pregnant women coming for antenatal check up and registration in the Gynae/obs department of JPMC were included according to prescribed criteria during the period of September 2001, to March 2002. Urine was collected as mid-stream voided specimen in a sterilized container. Before taking the specimen the process of collecting it was told to the patients. It was assured that the stay of urine in bladder was more than 4 hours as early morning specimen of urine was difficult to collect. After collection urine specimen were immediately transported to the laboratory, processed for pH, Griess test and culture.

The Griess reagent was prepared by dissolving 1.5 gm of sulphanilic acid in 450 ml of 10% acetic acid. This was then added to a solution of 0.6 gm of α -naphthylamine dissolved in 60 ml of boiling distilled water. The reagent remains stable for several months when stored in a stopper amber bottle and refrigerated. Deterioration of the test reagent is detected by the development of a pink color. Urine is tested by adding 1.0 ml of urine to 1.0 ml of the reagent in a clean test tube. The immediate development of a pink color to dark red color indicates the presence of nitrites in urine and is called a positive reaction⁸. The α -naphthylamine was handled carefully for its possible hazards. Aerobic culture was done on Blood agar and MacConkey agar at 37 °C. The microorganisms of significant growth were identified by standard methods.

The data obtained was tabulated and statistically analyzed for sensitivity and specificity of Griess test.

RESULTS

There were 290 specimens of urine processed for asymptomatic bacteriuria. The results are shown in table.

Out of 290, Griess test reported 272 cases as negative for infection and 18 were indicated positive. On culture, 266 cases found true negative. The microorganisms of 06 false negative cases are given in the table. The cases positive for infection indicated by Griess test were also culture positive for significant growth ($>1 \times 10^5$ CFU/ml). All of them were coliforms but 06 cases were mixed growth (more than two organisms and were taken as contaminated. The rest of cases were pure significant growths.

Thus there were 266 true negative (TN) cases also indicated negative by Griess test but 06 cases were indicated false negative (FN). The 18 cases indicated positive for bacteria were also found having infections but truly positive (TP) for the study were only 12. Likewise 06 cases were indicated false positive (FP) for this study. Taking the above figures for statistical analysis sensitivity (TP÷TP + FN) (18÷18+6) and specificity (TN÷TN + FP) (266÷266+6) of Griess test become 75% and 97.79% respectively. The pH of all specimen tested was found 6 or above.

DISCUSSION

ASB occurs in 5-10% of pregnant women and poses a significant health risk for them¹⁷. Clinically only one urine sample is obtained because the high cost of urine culture makes it an expensive screening technique¹⁷. The female urethra is relatively short (3-4 cm in length) and is located in close proximity to the vagina, which is colonized with organism from the GIT. E coli is the primary pathogen in 65% to 80% of cases¹⁷. The sensitivity of Griess test (75%) found in our study is also good but as a screening test cannot be taken significant as the prevalence rate of ASB is low^{6,7,17}. However the specificity of the test found in our study (97.79%) is significant to refer the Griess positive cases for culture. This will reduce culture load 98%. This test further takes importance for its application where it can be utilized for preventive gynecological/obs health services in our rural health centers without undergoing special training and arrangements. It will reduce 92% workload of culture. As long the pathogens of urine are concerned during pregnancy, these are same as in gen-

Table: Griess test reports with relation to culture results.

S. No.	Griess Test results	Growth results	Organisms isolated	Significance
1	272 (negative)	06 (positive)	Candida albican=1 Strep. agalactiae=1 Staph. aureus =1 Staph. saprophyticus=3	Sensitivity =75%
2	18 (positive)	12=pure growth 06= mix growth	Enterobacteriaceae=12	Specificity = 97.79%

eral population^{5,7,12}. The pathogens isolated in our study are majorly the Enterobacteriaceae, the Griess test for which is positive. There is great variation for other minor pathogens^{2,7,12} as false negative reported in our study. Increase frequency of Enterobacteriaceae in urine as a significant pathogen would increase the specificity of the test. The ability to reduce nitrates to nitrites is possessed by all the Gram-negative bacilli belonging to the genera *Eschericia*, *Klebsiella*, *Citrobacter*, *Proteus* and, *Shigella*, by *Pseudomonas aeruginosa*, by *Staphylococcus aureus*, *Staphylococcus albus* and by the *Providencia* group of paracolons, but this property is never found in *Streptococci*. This test might be less successful if it was applied to urine from patients infected with an organism which either does not reduce nitrate at all for example enterococcus or does so at a much slower rate for example *Pseudomonas aeruginosa*¹⁰. The ability of the test to detect *Staphylococcus* was not seen in our study as reported¹⁰. However the specificity (97.74%) of Griess test in our study is higher than other study (55.30% on clinic specimens, 92.20% on 1st morning specimens)⁸.

It cannot be assumed that this chemical test would be as accurate in screening symptomatic patients when the bladder incubation time would be shortened by a frequency of micturation, catheter drainage and the forcing of fluids¹⁰.

The Griess test was found positive with *Eschericia*, *Klebsiella*, *Staphylococcus*, *Proteus* significant infection but negative with enterococci⁸. The oral ingestion of potassium nitrate the night prior to urine collection did not increase either the sensitivity or the reliability of the Griess test⁸. Maximum reliable results are obtained when more prolong incubation of urine within the bladder prior to voiding is provided⁸. Limited nitrate concentration in urine was not a significant cause of false-negative nitrite results. However in vitro incubation at 37 °C in presence of $>1 \times 10^5$ CFU/ml of nitrite reducing organisms, nitrite will be lost⁸. Ascorbic acid, abnormal amounts of urobilinogen, and urinary pH below 6.0 are all possible causes of false-negative nitrite determinations⁸.

The nitrite reduction test, which detects nitrites produced by urinary bacteria (usually limited to gram-negative bacteria) has variable sensitivity (35-85%) but good specificity (92-100%)⁵. The nitrite test which having a high specificity, has a low sensitivity for bacteriuria when 1st voided morning urine is not used, will not detect the presence of *Staph saprophyticus*, a non nitrate reducing bacterium¹⁶.

Bachman and associates⁶ compared these screening methods with urine cultures and found that while it was more cost effective to screen for

bacteriuria with esterase dipstick for leukocytes only one half of the patients with bacteriuria were identified compared with screening by urine culture.

CONCLUSION

As a screening procedure for significant bacteriuria it is not possible for all pregnant patients to collect clean catch midstream urine specimens immediately after arising and to keep the specimen refrigerated until brought to the health center. The time of urine in bladder if assured to be 4 hours or more it can then be Griess tested, and the positive reactions should be followed by urine quantitative culture. If the urine is Griess negative, then in the asymptomatic patient further culture studies are not indicated. However since the Griess test is not perfect all symptomatic patients with Griess negative reaction should continue quantitative urine culture. It is hoped that this study will stimulate other family physicians and practicing obstetricians to incorporate a search for bacteriuria by Griess test into their 1st prenatal visit routine.

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Address for correspondence:

Dr Aziz Marjan Khattak,
 Assistant Professor Microbiology,
 Department of Pathology,
 Gomal Medical College,
 Dera Ismail Khan.
 Phone: 0966-9280338