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Short report

A comparative study of griess nitrate test and urinary culture in detection of asymptomatic bacteriuria in children.

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ABSTRACT

Background: Screening of asymptomatic bacteriuria (ASB) in children is strongly recommended to detect and treat renal diseases like urinary tract infections, obstructive uropathy, reflux nephropathy, hereditary nephritis and End stage renal disease . Culture is the gold standard method to detect ASB but cannot be adopted everywhere as it is available in few major cities is time consuming and expensive. 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 asymptomatic school children of both sexes aged between 5 to 14 yrs selected from schools of Mysore city. 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 1000 cases were examined for bacteriuria with Griess test and also inoculated on media. There were 8 cases positive for bacteriuria with Griess test. Culture was positive in 25 cases and in the culture positive , 14 cases were found mixed growth (contaminants) and 11 cases were culture positive. sensitivity and specificity of griess test was 76.6% and 100% respectively. **Conclusion:** Although Griess test cannot be compensatory for culture it can be applied as a screening test due to good sensitivity and specificity in resource constrain settings. It is easy to perform with instant results and to interpret without going special training for it.

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1. Introduction

In 1879, Griess, a German chemist developed a reagent for the detection of nitrite in solution. The reagent, an acid solution of sulfanilic acid and alpha naphthylamine, undergoes a diazotization reaction with nitrites to form a red azo dye. The test was originally intended for assessing bacterial contamination in municipal water supplies based on the principle that nitrate present in sewage will be reduced to nitrite by the action of bacteria. However, in 1914, Cruickshank and Moyes demonstrated a direct correlation between

the presence of nitrite in urine and the presence of coliform bacteriuria [1-5].

The Griess nitrite test for the detection of significant bacteriuria is based on the finding that most of the bacterial species which cause urinary tract infections reduce nitrate to nitrite. It has been shown that sodium nitrite in concentrations of as little as 0.1 ug/ml, gives a positive test. Human diets ordinarily contain a certain amount of nitrate. Under normal circumstances the urine should contain no trace of nitrite. Its presence therefore, can be considered as positive evidence for the presence of bacteria that reduce nitrate to nitrite as one phase of their metabolic activity [7].

Objectives:

- (1) To evaluate the reliability of the Griess test in the diagnosis of urinary tract infections comparing it with the culture method and the conventional routine urinalysis and

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- (2) To determine the applicability of the Griess test in our clinical setting particularly in areas where urine culture is not available
- (3) To know the sensitivity and specificity of Griess Nitrate test.

2. Materials and Methods

2.1. Source of Data: Asymptomatic school children of both sexes aged between 5 and 14 yrs selected from schools of Mysore city.

2.2. Method of Collection of Data: A cross sectional study was conducted on 1000 school children of both sexes aged 5 to 14 yrs. Informed consent is taken. The following procedure was followed:
A. First morning voided urine specimens were obtained using the midstream clean-catch technique.

B. Urine culture was done. Specimens were cultured in eosin-methylene blue agar, Bacto agar and trypticase soy agar. A culture is said to be positive if the bacterial colony count is 100,000 and above.

C. Modified Griess test was done as follows: We obtained about 8 ml. of urine in a test tube and centrifuged this for 15 minutes. The supernatant was decanted. To the precipitate, we added 0.5 ml. of a 10% solution of potassium nitrate. This was incubated for one half hour at room temperature. Then, we added 1 ml of the Griess reagent. The development of a pink or a red color in a matter of seconds was considered to be a positive test. Asepsis was strictly observed.

Preparation of the Griess Reagent. One and a half gms of sulfanilic acid (chemically pure) was dissolved in 450 ml. of 10% acetic acid. This solution was added to a solution of 0.6 gm alpha naphthylamine (chemically pure) in 60 ml of boiling distilled water and filtered through Whatman No. 1 filter paper. Preparation required approximately 15 minutes. This combined reagent, now colorless, was stored in a tightly stoppered bottle to prevent oxidation. The reagent in this form remained stable for 2 to 4 weeks and decomposition was detected by the appearance of a pinkish colored solution. The activity of the reagent was then tested by adding a few drops to few milliliters of 10% sodium nitrite solution and we noted the development of a red color.

D. Routine urinalysis was done with emphasis on the presence and number of pus cells and bacteria in the sediment after centrifugation at 1,500 rpm for 15 minutes. Pyuria was considered to be present if there were 5 or more leucocytes per high power field. Presence of bacteria was considered significant regardless of their number.

E. The results of the Griess test were analyzed and correlated with the results of routine urinalysis and culture.

3. Results

There were 1000 specimens of urine processed for asymptomatic bacteriuria. The results are shown in table. Out of 1000, Griess test reported 992 cases as negative for infection and 8 were indicated positive. On culture, 975 cases found true negative. The cases positive for infection indicated by Griess test were also

culture positive for significant growth ($>1 \times 10^5$ CFU/ml). Culture was positive in all 8 cases where Griess nitrate test was positive. Griess nitrate test was negative in 3 culture positive cases. The commonest organism grown in all study groups was E.coli followed by Klebsiella. Thus there were 992 true negative (TN) cases also indicated negative by Griess test but false positivity was not seen in any of cases in our study. False negativity of Griess test may be due to frequent bladder emptying in children or due to low nitrates in diet. Sensitivity of Griess nitrate test was 76.4%. Its specificity was 100%.

Griess Nitrate Test Vs Culture

RESULT	GRIESS NT+VE	CULTURE+VE	BOTH+VE
NO.OF CASES	8	11	8

4. Discussion

Our study highlights some salient features in the urine examination of apparently healthy schoolchildren. The finding of urinary abnormalities in nearly 10% of the study group is significant and should settle the age-old debate as to whether routine urinalysis in apparently healthy children is warranted, in the affirmative. Results show that although bacteriuria amongst school children, especially girls, rarely leads to end stage renal failure [5-8]].

It may be the first clue to underlying anatomical abnormalities in some patients. Children with bacteriuria have more recurrent infection and urological abnormalities and are at high risk of developing bacteriuria during pregnancy. The incidence of asymptomatic bacteriuria in our study was 1.1% with similar incidence to other studies like Joseph and Zainal with 0.12 % respectively except with that of Kumar where incidence was 10.37 %. In the latter case, this may be attributable due to variation in defining Asymptomatic bacteriuria. The organism most frequently isolated in asymptomatic bacteriuria and urinary tract infection includes species of Enterobacteriaceae especially E.coli and other gram negative bacteria. Bacterial count of $>10^5$ organisms of mid stream urine on successive cultures in children should be significant bacteriuria [9,10]. Detection of urine nitrites has been used as a screening test for urinary tract infection. The major problem with this test is the high number of the False negative results. Its specificity is known to be good. Griess nitrate test was positive in 8% of the culture positive cases. False positivity was not seen in any of the cases of the study. The results of this study are similar to the study done by Seema Sood. There was only one comparative study used for Nitrate test because our test procedure was based on the procedure used by Seema Sood.

5. Conclusion

Our study has shown that asymptomatic children had bacteriuria in 1.1% of the cases. Rapid diagnostic tests as Griess nitrate test can be used as an alternative to culture for screening purpose in resource constrained settings. Hence screening for urinary findings in asymptomatic children is of great importance as early diagnosis and intervention can reduce the mortality and morbidity.

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