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Effectiveness of Griess Nitrite Test on Screening Asymptomatic Bacteriuria in Pregnancy: A Cross Sectional Study in Harare, Zimbabwe

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Authors' contributions

This work was carried out in collaboration between all authors. Author JMR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PN, MFG, COH and BSP managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to evaluate effectiveness of the Griess test on screening asymptomatic bacteriuria when compared to culture.
Study Design: This study was conducted using the cross sectional study design.
Place and Duration of Study: The study was conducted at four Harare Primary Care Clinics. The University of Zimbabwe, Nursing Science Department and Medical Microbiology Laboratory were also used.

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Methodology: Pregnant women reporting at four purposively selected Primary Care clinics in Harare for registration for antenatal care at gestation between 6 and 22 weeks were randomly selected. Women who were unwilling to participate and declined to provide a signed consent were excluded. Mid-stream clean catch urine samples were collected and tested first by Griess nitrite test followed by culture. Presence of more than 10^3 colony forming units per milliliter of urine of a similar bacteria was considered positive for asymptomatic bacteriuria. Sensitivity, specificity, positive predictive value and negative predictive value was calculated for the Griess test. Data was analyzed using the Statistical Package for Social Sciences Statistics version 20.

Results: Seventeen out of 80 participants we recruited had asymptomatic bacteriuria. The prevalence of asymptomatic bacteriuria in this study was 21.3% (95% CI, 13.5 to 31.8). The sensitivity (100%), specificity (93.7%), positive predictive value (81%) and negative predictive value (100%) of the Griess test were high. *Coagulase negative staphylococcus* was the popularly (47%) isolated uropathogen, followed by *Escherichia coli* (29%).

Conclusion: The Griess nitrite test was very effective to detect asymptomatic bacteriuria. The Griess test should be considered for screening asymptomatic bacteriuria in pregnancy especially in low resource settings. The culture test should be reserved for positive sample for quantifying bacteria. This is expected to reduce the burden of culture test which is unaffordable especially in low resource settings.

Keywords: Screening; screening test; asymptomatic bacteriuria; Griess nitrite test; pregnancy; urine culture; specificity; sensitivity.

1. INTRODUCTION

Asymptomatic bacteriuria (ASB) refers to significant growth (> 10^5 colony forming units per milliliter of urine) of a similar bacteria in a culture-tested midstream urine sample, obtained from a patient who did not present with urinary tract infection symptoms [1,2]. This is one of the most common diseases occurring during pregnancy, with prevalence ranging between 2% and 10% globally [3]. Higher prevalence is reported in developing countries like India (13.2%), Nigeria (45%) and Brazil (12.2%) [4].

When ASB is undetected and untreated early in pregnancy it is associated with complications including pyelonephritis, preterm labor, preterm amniotic sac rupture, oligohydramnios or polyhydramnios and recurrent loss of pregnancy [1,4]. Pyelonephritis is the most severe complication of ASB with resultant life threatening possible challenges including urosepsis and pulmonary insufficiency [4,5]. Up to 40% will develop pyelonephritis later in pregnancy [6,7]. Pyelonephritis is also associated with high risk of preterm delivery, currently the leading cause of neonatal mortality worldwide [3]. A direct association of ASB with preterm birth has also been reported [8]. The complications and adverse pregnancy outcomes associated with the disease have raised a huge public health concern. A large number of pregnant women could be affected by ASB if no screening and treatment is done during antenatal care. Several studies have confirmed that if ASB is detected and treated early in pregnancy its complications will be significantly (10 fold) reduced [9]. This background justifies the need and recommendation for screening pregnant women for ASB during antenatal care for early detection and treatment of those found positive [10]. Several international and national organizations including the World Health Organization, US Preventive Services Task force, Canadian Task Force for Preventive Health Care, March of Dimes and Centre for Disease Control recommend that the screening be done by urine culture at initial antenatal visit, especially by 16 weeks or later if registration is delayed [11]. Screening and treating ASB reduces risk of pyelonephritis by almost 20% [12].

Screening involves identification of an unrecognized disease. It enables early initiation of treatment which prevents development of complications which often result in high morbidity and mortality [13]. Screening tests are important and commonly used in health practice for the purposes of early detection of disease in asymptomatic individuals in a population at risk. A screening test is effective when it identifies those individuals with the disease but are asymptomatic [14]. However some screening tests falsely identify positives and negatives. A screening test is often essential when the disease being screened has adverse outcomes if undetected and untreated early. Screening is

also necessary when treatment before symptoms occur is more effective than when delayed. The screening test is also required when prevalence of a disease is more than 2% [4]. A screening test must be worthwhile, easy to administer, provide rapid results, of minimal discomfort, reliable, and valid [13]. A screening test is not a diagnostic test.

Urine culture is the recommended and gold standard test for diagnosis of ASB, as it is for the other urinary tract infections [15,16]. The test is expensive as it needs a proper laboratory set up, a laboratory scientist and expensive equipment [17]. In addition to the high cost, results delay in coming as it requires a minimum of 72 hours of waiting. One popularly used screening test for ASB in most primary health care centers is simple urinalysis [18]. The test needs no expert, produces results immediately and does not require expensive laboratory equipment. However it is unreliable due to its low sensitivity and specificity [19]. There has been no final agreement on the screening test to use for ASB in pregnancy. Griess nitrite test is another screening test for ASB. It was discovered in 1879 by a Germany chemist, Peter Griess. This test is a nitrite detection test as it measures nitrite in a sample, based on chemical diazotization reaction. The system uses two reagents namely sulfanilamide and N- 1- naphthylethylenediamine dehydroxide under acidic conditions [20]. When these reagents are added in urine with nitrite, a diazotization reaction occurs which results in change of urine color to purple [21]. Presence of nitrite (NO_2^-) in urine as with other substances like blood and leucocytes is a potential clinical sign that a urinary tract bacterial infection is present. It is required that urine stays in the bladder for up to 4 hours to allow accumulation of detectable nitrite levels. The Griess test therefore operates with the principle that almost all bacterial species causing urinary tract infections reduce nitrate which is normally present in urine, to nitrite [20,22]. The test is usually more than a tenth cheaper than culture [20]. It is simple to perform and results are available immediately. It does not need special testing field and even an improvised environment will do [23].

Screening for ASB is unavailable in antenatal care guidelines for majority of developing countries including Zimbabwe and is not being practiced at Primary Care Clinics. The main challenge could be high cost of the recommended gold standard culture test as a screening test. Culture test is not feasible especially in low resource settings. Meanwhile Zimbabwe ranks 4th (16.6%) among countries with highest preterm birth rate in the world [24]. Identification of a cost effective and reliable screening method for ASB may be useful so that only those with positive results will have a urine culture to reduce costs. This study was conducted to evaluate effectiveness of the Griess test in detecting ASB in pregnancy based on study results. The study was done to ascertain a cost effective screening method for asymptomatic bacteriuria especially for use in low resource settings. The null hypothesis was that specificity and sensitivity of the Griess nitrite test was equal to 99% and 92% respectively. The alternative hypothesis was that specificity and sensitivity of the Griess test was not equal to 99% and 92% respectively. The study was conducted as one of primary objectives for the main thesis for the main author.

2. MATERIALS AND METHODS

A cross sectional study was conducted at 4 purposively selected Harare Primary Care Clinics. High volume centers for antenatal care bookings and socioeconomic status of residents were considered. The period of recruiting and testing extended for 6 weeks from 15 March to 27 April 2017. Sample size was calculated using the formula: $n= [Z_{\alpha/2} \sqrt{P_0 (1-P0)} + Z_{\beta} \sqrt{P_1 (1-P_1)}]^2 / (P_1 - P_0)^2$, $Z_{\alpha/2}$ =1.96, Z_{β} = 0.84, P_0 = 92% (predetermined values of sensitivity), P_1 = 99% (predetermined value of specificity). The probability used for type I and type II errors were 0.05% and 0.20% respectively. A minimum sample size of 77 participants was required for this study. Simple random sampling method was used to select participants. Potential participants were identified by obtaining first date of last menstrual period (LMP) from which gestation was calculated for eligibility assessment. Included were all pregnant women who reported at the sites for initial antenatal care visit at gestation between 6 and 22 weeks and were asymptomatic for urinary tract infection. Only those who voluntarily gave a signed consent for their participation were included. Participants also voluntarily provided a signed consent for urine transportation from the clinics to the University of Zimbabwe Medical microbiology laboratory for culture test. Women who could not remember their LMP, declined to participate and unwilling to give a signed consent were excluded in this study. Excluded also were chronic renal patients and those with a known urinary tract structural problem. Ethical approval for

conduction of this study was granted for the main study by the responsible local and national ethical review boards. Questionnaires were available in English and local Shona language for easy understanding.

Instructions on urine collection were given to participants repeatedly for clarity with emphasis on preventing contamination by cleaning hands first, avoiding touching inside specimen bottle and lid and adding midstream clean catch urine. Cleaning of the genitalia prior to urine collection was discouraged. A disinfected labelled specimen bottle was used for collection of urine sample. Participants were given hand sanitizer to rub on both hands before urine collection. Mid-stream clean catch urine amounting to 20 milliliters was obtained from each participant. All samples were first tested by Griess test within 30 minutes. A solution that changed color from clear to purple was recorded as positive for ASB, but was considered negative when no color change was observed. All negative and positive samples were stored in a cooler box with frozen ice packs. The samples were transported within an hour to Medical Microbiology laboratory of the University of Zimbabwe for culture test for confirmation of results and quantitation of isolated bacteria.

Each non-centrifuged urine sample was streaked on two culture media, blood agar and Cysteine Lactose Electrolyte Deficient (CLED) agar. The plates were incubated at 37°C and were observed after 24 hours for growth of bacteria. Plates which had no growth were labelled as negative but those with growth were further examined for quantification of bacterial colonies per milliliter of urine. A colony count of the same bacterial species of 1000 (10^3) and above was considered positive for ASB, to cater for early and middle stage of the disease in pregnancy. A sample with mixed growth had the dominant bacterial species quantified and if found at or above 10^3 cfu/ml, was considered positive. A sample with insignificant mixed growths was labelled as contaminated. Identification of the bacterial species was also done by first classifying the pathogens as gram negative and gram positive and later bacteria name identified. A microbiologist for the laboratory was blindly involved in culture test of all samples and printed results without knowledge of Griess nitrite test results for each of the samples to reduce bias.

Griess nitrite and culture test results were compared. Sensitivity, specificity, positive predictive value and negative predictive values for Griess test was calculated. The Statistical Package for Social Sciences Statistics version 20 was used to analyze results.

3. RESULTS AND DISCUSSION

Our study was conducted at 4 Primary Care Clinics in Harare in Zimbabwe. Eighty pregnant women were enrolled in this study. Fig. 1 is an illustration of the flow of participants in this study. Participants' age ranged between 17 and 41 years. The mean age was 26 years. All (100%) participants attended formal education, with majority (68.8%, n=55) ending at secondary level. Only 5% (n=) had tertiary education. Majority (97.5%, n=78) of the participants were married and only 2 (2.5%) were single. Majority (62.5%, n=50) of the participants were unemployed and had no monthly income at all. Thirty percent (n=24) were self- employed and 10 (41.7%) of them earned less than US100 per month. Only 4 (5%) worked for private companies and among them was the highest paid ($400- $499 per month). Majority (61.2%, n= 48) were multiparous and 38.8% (n=31) were nulliparous. The minimum gestation was 8 weeks and 6 days whilst the maximum was 22 weeks. Majority (76%, 13 out of 17) of positive samples were obtained from those registering at gestation between 17 and 22 weeks, as illustrated on Table 1. Twenty four percent (n= 4) of the positive samples were obtained from those registering at gestation less than 17. Most of the participants (68.8%, n=55) were reporting for their initial antenatal registration at gestation above 16 weeks.

With the Griess test 21 (26.3%, 95% CI, 17.6 to 37.2) samples were positive for ASB but 59 (73.8%, 95% CI, 62.8 to 82.4) tested negative compared to 17 (21.3%, 95% CI, 13.5 to 31.8) positive and 63 (78.8%, 95% CI, 68.2 to 86.5) negative by culture (see Table 2). Four (23.5%) urine samples were wrongly labelled positive whilst 17 (100%) of them were correctly labelled positive with the Griess test. There was no contaminated growth reported from culture tests. In this study the prevalence of ASB was 21.3% (95% CI, 13.5 to 31.8). The sensitivity and specificity of the Griess test in our study was 100% and 93.7%. The positive predictive value and negative predictive value was 81% and 100% respectively. There were no observed or reported adverse events from performance of this test.

Table 3 presents uropathogens identified in this study. The predominant isolated uropathogen in was *Coagulase Negative Staphylococcus (CNS)* (47%, n= 8), followed by *Escherichia coli* (29%, n= 5). The other bacteria isolated included *Bacillus* species (12%, n=2), *Salmonella* species (6%, n=1) and *Streptococcus viridians* (6%, n= 1).

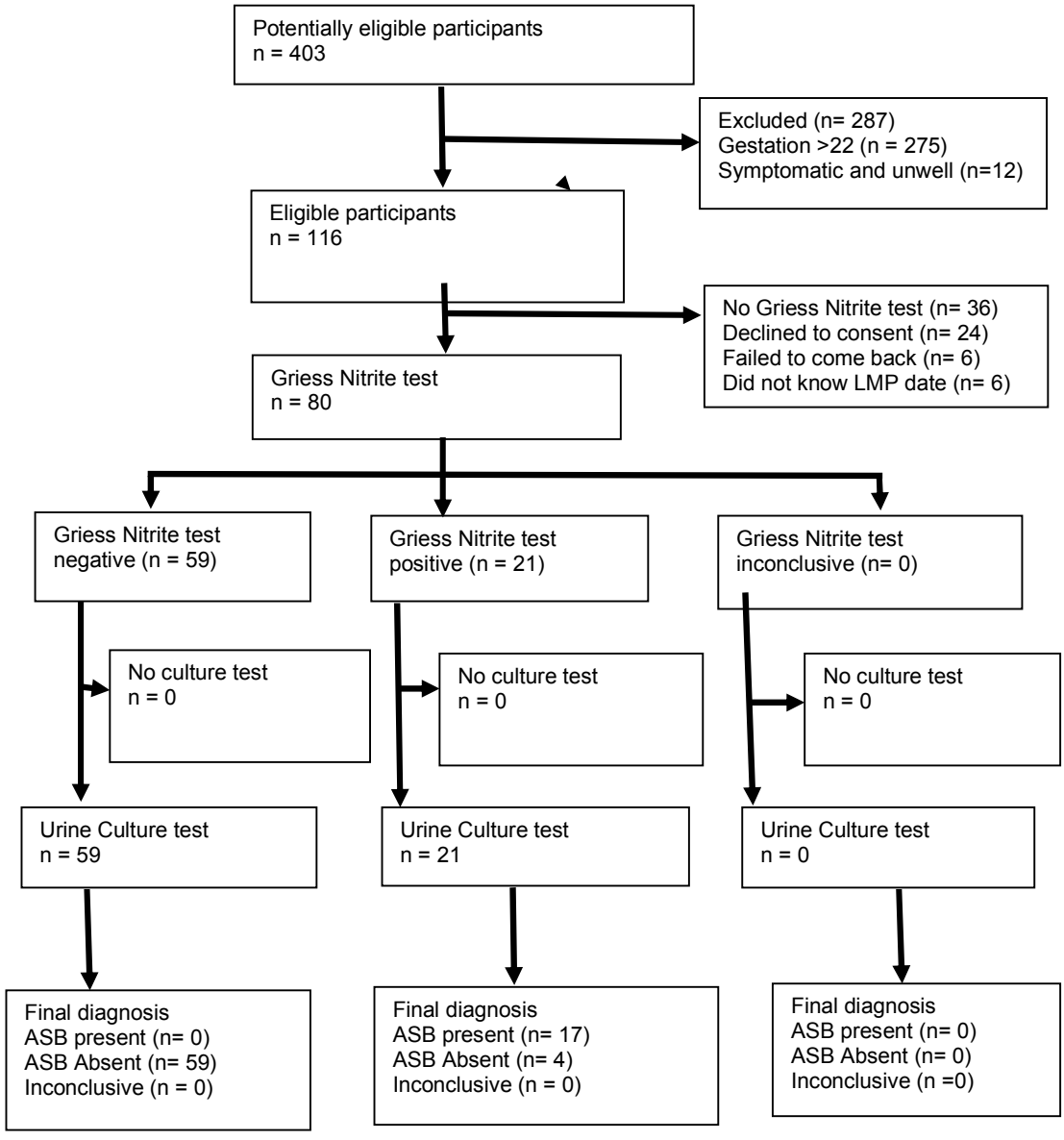

Fig. 1. Flow of participants through the study

Table 1. Gestation category and ASB culture results

Gestation category (weeks. days)	Culture test result		Total
	Positive	Negative	
8.0 –12.6	2 (12%)	9 (14%)	11 (14%)
13.0 –16.6	2 (12%)	18 (29%)	20 (25%)
17.0 –22.0	13 (76%)	36 (57%)	49 (61%)
Total	17 (100%)	63 (100%)	80 (100%)

Table 2. Griess nitrite test and culture results

		Culture test result		Total
		ASB	No ASB	
Griess test result	Positive	17 (81%)	4 (19%)	21 (100%)
	Negative	0 (0%)	59 (100%)	59 (100%)
Total		17 (21.3%)	63 (78.7%)	80 (100%)

Table 3. Isolated uropathogens in ASB

Isolated uropathogen	Frequency	Percent (%)
Coagulase negative staphylococcus	8	47
Escherichia coli	5	29
Salmonella species	1	6
Bacillus species	2	12
Streptococcus viridians	1	6
Total	17	100

3.1 Discussion

In this study 80 urine samples were obtained from pregnant women registering for antenatal care at selected primary care clinics. Their age ranged between 17 and 41 years and majority (93.5%) were under 35 years. Majority (62.5%) of pregnant women registering for antenatal care at primary care centers would not afford culture test for screening. Fifty of them were unemployed and had no monthly income. Majority (97.5%) of the participants were married. Participants in other studies which focused on screening pregnant women for ASB had a similar age range [25,26]. In another study 98.4% of pregnant were married and only 1.6% were single [25]. This is in congruence with marital status for our participants. Possibly the single pregnant women register late or they do not book until delivery. Their gestation ranged from 8.6 to 22 weeks. Most women are registering for antenatal care much later than 22 weeks and these were not included in this study. Participants in this study did not fully represents all subgroups and this could have introduced bias to study and also affected generalizability of results.

Although bacterial count equal or above 100 000 ($\geq 10^5$) colony forming units per milliliter (cfu/ml) is the acceptable definition for asymptomatic bacteriuria, a lower count of 10^3 cfu/ml of a similar bacteria could occur in true positive case at an early infection stage whilst a count of $>10^2$ cfu/ml may represent contamination [26]. Data on contaminated samples and mixed growth was missing and this could have introduced selective reporting bias. However majority of samples with a low bacterial count is also common in symptomatic individuals. A prevalence of 21.3% obtained in this study was almost similar (21%) to another obtained in a study conducted in Ibadan in Nigeria [27]. A different prevalence was reported from India (12.3%) and Sri Lanka (3.6%) [28,29]. ASB is a common problem in pregnancy although the prevalence differs from setting to setting. However the differences in sample sizes for different studies may be responsible for the differences in prevalence.

In our study sensitivity (100%) and specificity (93.7%) of the Griess test was significantly high as well as the positive predictive value (81%) and negative predictive value (100%). This means that the Griess test managed to identify all true positives. We therefore rejected the null hypothesis and accepted the alternative hypothesis. The sensitivity and specificity results cannot be used to predict probability of disease occurrence. The explanation for a few (5%, n=4) that were wrongly labelled positive, could be contamination of culture media which was reported. A study conducted in India reported a high sensitivity (92.3%) and specificity (99%) of Griess test [20]. In another study sensitivity (75%) and specificity (97.79%) were high too [22]. The Griess test proved to be effective as a screening test for ASB. The test was very effective in identifying ASB. All positive samples were identified and all negative were truly negative. Only a few that was screen positive but did not grow bacteria by culture. Positive samples will however need culture test for identifying and quantifying the bacteria. The culture test, however remains the gold standard test for the diagnosis of ASB.

The Griess test did not only identify ASB caused by gram negative bacteria but also by gram positives. This shows that some gram positive

bacteria also reduce nitrate to nitrite [30]. *CNS* was predominantly (47%) isolated pathogen followed by *Escherichia coli* (29%) and only 1 (6%) with *Shigella species*. However in a study conducted in India there was no *CNS* and the most common pathogen isolated was *Escherichia coli* (14 out of 30). A variety of Coagulase negative bacteria exist, some have the enzyme nitrate reductase whilst others do not have. The Griess test could therefore detect the CNS species responsible for asymptomatic bacteriuria in pregnancy. Coagulase negative staphylococcus species were also detected by Griess test in a separate study [23]. The most common uropathogens reported to be commonly isolated in ASB are the gram negative bacteria [20].

According to Cochrane review in 2015 there was low quality evidence to treatment of ASB in pregnancy for reducing pyelonephritis (RR 0.23, 95% CI, 0.13 to 0.41), preterm birth (RR 0.27, 95% CI, 0.11 to 0.62) and low birth weight (RR 0.64, 95% CI, 0.45 to 0.93) [31]. Study design limitation like lack of allocation concealment and blinding could have interfered with the results of the review [31]. However debate is still ongoing on the role of the disease in perinatal outcomes [32]. Screening for ASB in pregnancy should be considered a priority during antenatal care due to adverse effects associated with the disease. The Griess test is an inexpensive and effective screening test for ASB and can be done in any setting. Low resource settings could consider the Griess test for screening ASB in pregnancy. Use of the Griess test for screening ASB will lessen the burden of culture test.

4. CONCLUSION

Asymptomatic bacteriuria is common in pregnancy. If untreated ASB is associated with adverse pregnancy and birth outcomes. The Griess nitrite test is inexpensive, easy and effective screening test for ASB. This test should be considered for screening ASB especially in low resource settings where the culture is unaffordable and unavailable especially at primary care centers. Culture remains the gold standard test and should be reserved for quantifying identified bacteria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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