Comparative Analysis of Amsel Criteria and Nugent Score in the Diagnosis of Bacterial Vaginosis in Pregnancy

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Abstract: The Amsel criteria and Nugent score are common diagnostic methods for BV and efforts continue to establish which method is superior. The study was a prospective cross-sectional study which compared the diagnosis of BV using the Amsel criteria and Nugent score. Participants were 316 consenting antenatal clinic attendees in the second trimester. All participants had two high vaginal swab samples collected which were analyzed using both Amsel criteria and Nugent score. Data analysis was done using SPSS version 20.0 and P<0.05 was considered statistically significant. Among the 316 study participants, the prevalence of BV was 24.1% with Nugent score and 15.5% with Amsel criteria. Also, post-treatment BV persistence rate was 25.0% with Nugent score and 11.8% with Amsel criteria. Nugent score was superior to Amsel criteria for the diagnosis of BV (χ^2=74.764, P0.001). Among 76 women diagnosed BV positive by Nugent score, 36(47.4%) were diagnosed by Amsel criteria while among 240 women diagnosed BV negative with Nugent score, 14(5.8%) were diagnosed as positive by Amsel criteria. Nugent score had a higher sensitivity (93.3% vs. 80.4%), lower specificity (92.1% vs. 94.2%), higher positive (94.0% vs. 72.0%) and negative (90.0% vs. 85.0%) predictive values, lower false positive (2.0% vs. 5.8%) and false negative rate (15.0% vs. 52.6%) and higher accuracy (94.0% vs. 82.9%) compared to Amsel criteria. In conclusion, Nugent score offers an advantage over Amsel criteria in the diagnosis of BV in pregnancy, thus it should be the preferred diagnostic method.
Keywords: Bacterial vaginosis, Nugent score, Amsel criteria, vaginal discharge.

INTRODUCTION

Bacterial vaginosis (BV) is the commonest cause of vaginitis among both pregnant and non-pregnant women (Nelson & Macones, 2002; Lenox *et al*., 2013). It is a polymicrobial vaginal infection characterised by a reduction in the concentration of the dominant hydrogen peroxide producing lactobacilli and an increase in the concentration of anaerobic organisms in the vagina (Nelson & Macones, 2002; Lenox *et al.,* 2013; Abdelaziz *et al*., 2014). The prevalence of BV in pregnant women varies from 17.3% in Maiduguri, North East (Ibrahim *et al*., 2014) to 25% in Osogbo, South West (Adesiji *et al*., 2007) Nigeria; 19.4% in Ethiopia (Mengistie *et al*., 2014), 20.5% in India (Lata *et al*., 2010), 19.4% in Denmark (Svare *et al.,* 2006) and 8% to 23% in South America (Krauss-Silva *et al.,* 2014).Among non-pregnant women, the prevalence of BV ranges from 40.1% in Ilorin, Nigeria (Abdulateef *et al.,* 2017) to 29.2%in the USA (Koumans *et al.*, 2007).A major challenge however is that about 25 to 50% of women with BV in pregnancy are asymptomatic (Awoniyi *et al*., 2015; Krauss-Silva *et al.,* 2014); these women are unreported, undiagnosed and untreated with greater risk for adverse pregnancy outcomes (Amsel *et al.,* 1983). Aetiological organisms in BV include *Gardnerella vaginalis, Mycoplasma hominis, Prevotella* and various species of *Mobiluncus, Bacteroides, Fusobacterium, Veilonella, Propionibacterium, Bifidobacterium*, and *Eubacterium* (Lennox *et al*., 2013; Awoniyi *et al.,* 2015; Ibrahim *et al*., 2014; Krauss-Silva *et al*., 2014, Abdulateef *et al.,* 2017).

Reports have associated BV in pregnancy with adverse pregnancy outcomes including premature rupture of membranes (PROM), preterm labour, preterm delivery, low birth weight, increased susceptibility to HIV infection and postpartum endometritis (Nelson & Macones, 2002; Ibrahim *et al.*, 2014; Mengistie *et al*., 2014; Krauss-Silva *et al.,* 2014; Abdulateef *et al.,* 2017, Ogunniran *et al*., 2021). Healthy microbiota of the lower genital tract predominantly consists of *Lactobacillus* spp. with *Lactobacillus crispatus, Lactobacillus jensenii* and *Lactobacillus iners* being the most prevalent species (Ravel *et al*., 2011). They form a critical line of defence against potential pathogens while the symbiotic relationship between vaginal lactobacilli and their human host is modulated by various hormones which stimulate the vaginal epithelia to produce glycogen (Hay 2005). Vaginal lactobacilli also metabolize glycogen secreted by the vaginal epithelia to produce lactic acid that is responsible for the acidic vaginal pH (pH < 4.5). The acidic environment of a healthy vagina is not permissive to growth of many potential pathogens while vaginal lactobacilli fend off pathogens through competitive exclusion via the formation of biofilms, production of hydrogen peroxide and bacteriocin-like substances (Aroutcheva *et al*, 2001).

The diagnosis of bacterial vaginosis can be made through various means although the Amsel criteria and Nugent scoring system are commonly used. The Amsel criteria require the fulfilment of three out of four criteria including elevated vaginal pH > 4.5, positive Whiff test (characteristic fishy odour on addition of 10% KOH to the discharge), presence of homogenous thin vaginal discharge and numerous exfoliated cells coated with bacteria called clue cells (Amsel *et al*., 1983). Nugent scoring systemis however preferred in the scientific community because about 50% of women with BV are asymptomatic so the absence of symptoms does not rule it out. It is regarded as the gold standard and it is based on Gram staining technique to detect and score the main bacterial morphocytes involved in BV (Nugent *et al*, 1991). Other less common diagnostic methods include Spiegel criteria which involve direct Gram staining of vaginal fluid based on the observation of an inverse relationship between the quantity of the *Lactobacillus* and the *Gardnerella* morphotypes. The Schmidt criteria involve microscopy of an unstained wet smear and the numbers of Lactobacilli and small bacterial morphocytes are counted and scored. The Hay and Ison criteria employ Gram staining followed by a grading system based on the quantity of Lactobacilli present in the smear. Others are Affirm VP III, polymerase chain reaction and Gas liquid chromatography (Ison & Hay, 2002). Generally, Nugent score is regarded as the gold standard because it is objective, highly sensitive and reproducible (Sha *et al*., 2005). Nugent score is however costly, requires laboratory equipment and expert slide readers (Sha *et al*., 2005; Mohammedzadeh *et al*., 2015) which are not readily available in most low-resource countries. Alternatively, Amsel criteria is superior to the syndromic management; it is highly specific and requires less equipment and manpower but rather utilize the clinical acumen of the health worker (Sha *et al*., 2005). This makes Amsel criteria common in low-resource countries. This study aimed at comparing the performance of Amsel criteria to Nugent score in the diagnosis of BV in pregnancy among pregnant women at a tertiary facility in Ilorin, Nigeria.

MATERIALS AND METHODS

Study site

The study was conducted at the Obstetrics & Gynaecology as well as the Medical Microbiology and Parasitology departments of the University of Ilorin Teaching Hospital (UITH), Ilorin, Kwara State, Nigeria.

Study design

A cross-sectional study.

Study participants

Pregnant women in the second trimester who were attending the antenatal clinic of the study site.

Inclusion criteria

Women in the second trimester of pregnancy who were receiving antenatal care at the antenatal clinic of the study site who consented to participate in the study were recruited into the study.

Exclusion criteria

Women who used antibiotics within two weeks prior to recruitment into the study and those with chronic medical disorders (e.g. diabetes mellitus, chronic hypertension, HIV infection, Hepatitis, etc.) were excluded from the study.

Sample size determination

The sample size was calculated using the formula for comparative study (Araoye, 2004)

$$n = \frac{z^2 pq}{d^2}$$

n =sample size

z = standard normal deviation (a constant which is 1.96 at 95%confidence interval

p = known prevalence of bacterial vaginosis in pregnancy i.e. 0.25 (25%) by Adesiji *et al*., 2007.

d = observed difference at 0.05(5%) level of significance.

q = 1-p = 1- 0.25 = 0.75

$n = \frac{1.96^2 \times 0.25 \times 0.75}{(0.05)^2} = 288$

Making provision for attrition rate of 10% (28 participants), the minimum sample size for the study was 316.

Sampling method

Purposive (non-probability) sampling in which all consecutive consenting and eligible women were recruited into the study until the sample size was completed was employed.

Study procedure/ laboratory analysis

For this study, the Nugent score is considered the standard for comparison because it has been documented from previous studies to be the gold standard due to its high level of objectivity, reproducibility, sensitivity and reliability for the diagnosis of BV (Moussavi & Behrouzi 2004; Sha *et al*., 2005; Bansa *et al.,* 2019).

Recruitment was done at the antenatal clinics using the study protocol; participants were screened for eligibility followed by an informed consent. Relevant information was thereafter obtained using the study questionnaire.

Thereafter, a sterile speculum examination was performed and two swab samples were obtained from vaginal fluid or secretion in the posterior or lateral fornix in each participant using sterile cotton-tipped swab sticks. A smear was made on a glass slide by rolling the swab on it and allowed to air dry. The slides were then transported to the microbiology laboratory for Gram staining and microscopy. The speculum was removed and a pH indicator paper was applied to the specimen on the speculum and pH recorded. Thereafter, assessment for Whiff test was done by adding a drop of 10% potassium hydroxide to the specimen on the speculum and the odour was noted.

Further processing at the laboratory involved wet sample preparation which involved addition of a drop of 0.9% Normal saline to the smear made and then examining under the microscope at a magnification of 40X for clue cells (epithelial cells coated by bacteria). Thereafter the air dried slides were fixed with 2 drops of ethanol followed byGram staining. The fixed smear wasfirst covered with Crystal violet stain for 30-60 seconds and then washed off with water. Lugol iodine was then applied for 30to 60 seconds to serve as mordant and washed off with water. Decolourization followed rapidly with acetone-alcohol and was also washed off with water. Thereafter the smear was covered with Safranin stain and left for a period of two minutes then washed off with water and allowed to air dry. The smear was then viewed under the microscope for the various morphotypes initially at 40X objective to check the staining and distribution of material followed by 100X objective to report the bacteria.

Microscopy was done in the laboratory by identification; quantification and scoring of the three characteristic morphotypes i.e. *Lactobacillus* (large gram positive rods), *Mobiluncus* (curved gram variable rods) and *Gardnerella* (Gram variable coccobacilli) were identified, quantified and then scored. Diagnosis of BV was made using both Nugent score (Nugent *et al.,* 1991) and Amsel criteria (Amsel *et al.,* 1983) on each sample collected. Amsel criteria were positive if three of the four criteria were present (clue cells, pH > 4.5, fishy odour on addition of 10% potassium hydroxide and characteristic homogenous vaginal discharge). Nugent score was performed as indicated in Table 1:

Table1: Diagnosis of Bacterial Vaginosis using Nugent score (Nugent *et al.,* 1991)

Organism	**Number on oil Immersion field**	Score
Lactobacilli (Gram Positive Rods)	>30	0
	5– 30	1
	1– 4	2
	<1	3
	0	4
Gardnerella/ Bacteroides (Tiny Gram variable *Coccobacilli* and rounded pleomorphic Gram Negative Rods)	>30	4
	5- 30	3
	1-4	2
	<1	1
	0	0
Mobiluncus(Gram Negative Curved Rods)	>5	2
	1- 4	1
	0	0

BV was diagnosed with a total score of ≥7, 4 to 6 was intermediate while 0 to 3 was normal. Participants who were diagnosed with BV using Nugent score were treated with oral Metronidazole 400mg thrice daily for 7 days. Four weeks after treatment, all participants with BV were reassessed with a post-treatment testing using both Amsel criteria and Nugent score.

Ethical issues and data analysis

An institutional approval was obtained from the Ethical Review Committee of the University of Ilorin Teaching Hospital before the commencement of the study while informed written consent was obtained from each participant. The data was analysed using the Statistical Package for Social Sciences software (SPSS version 20.0), the results was presented in frequency tables with percentages while number of participants positive for BV were compared using chi-square; P<0.05 was termed statistically significant.

RESULTS

A total of 316 asymptomatic pregnant women participated in the study. Biosocial characteristics of participants showed that the mean age was 29.52±4.57 (range 20-39 years), 286(84.8%) had tertiary education, all were married, 289(91.5%) were in monogamous marriage and 103(32.6) were in high social class (classes I &II) as shown in table 1.

From table 2, the prevalence of BV was 24.1% (Nugent score) and 15.8% (Amsel criteria) at recruitment and 25.0% (Nugent score) and 11.8% (Amsel criteria) for post-treatment persistence infection.

From table 3, among 76 women diagnoses with BV using Nugent score, 37(47.4%) were BV positive using Amsel criteria while among 240 women diagnosed BV negative using Nugent score, 50(15.8%) were diagnosed as BV positive using Amsel criteria. Nugent score was superior to Amsel criteria in the detection of BV in pregnancy (χ^2 =74.764, P0.001)

Table 4 compares the diagnostic performances of Nugent score and Amsel criteria. The Nugent score had higher sensitivity (93.3% vs. 80.4%), positive (94% vs. 72%) and negative (90% vs. 85%) predictive values and accuracy of diagnosis (94% vs. 82.9%) compared to Amsel criteria. However, Amsel criteria had higher specificity (94.2% vs. 92.1%), false positive (5.8% vs. 2%) and false negative (52.6% vs. 15%) values compared to Nugent score.

DISCUSSION

This study showed the superiority of Nugent score over Amsel criteria as diagnostic tool for detecting BV in pregnancy. The report suggests that when the emphasis is to accurately confirm true positive cases of BV, limit both false positive and negative results with a high predictive power; Nugent score should be preferred. However, Amsel criteria had a comparative advantage in its ability to diagnose true negatives (specificity).

The higher detection of BV in pregnancy recorded with Nugent score compared to Amsel criteria (24.1% vs. 15.8%) in this study supports the comparative advantage of Nugent score. This compares to reports from Ethiopia (19.4% vs. 18.3%) by Mengistie *et al*. (2015), India (36.9% vs. 30.76%) by Bansa *et al* (2019) and Pakistan (78% vs. 62%) by Taj *et al*. (2012) which reported higher detection rates for Nugent score. This is due to the objectivity employed in the Nugent scoring system compared to the Amsel criteria. This is relevant because most women with BV in pregnancy are asymptomatic; thus, a reliable method of diagnosis is desired to prevent the adverse pregnancy outcomes associated with undetected and untreated BV infection in pregnancy. Nugent score also recorded a higher detection rate of persistent post-treatment BV infection among BV positive women (25.0% vs. 11.8%) compared to Amsel criteria in this study showing its relevance for monitoring treatment response. However, the use of Nugent score requires additional laboratory equipment and experienced staff to read the slides. These are not always available in health facilities in low-resource settings and have been identified as a source of additional strain on the fragile health system and a hindrance to the universal utilization of Nugent score in the diagnosis of BV in these areas (Bansa *et al.,* 2019).

The reported high specificity (94.2%) reported for Amsel criteria in this study is similar to reports by other authors including 98% from Ethiopia (Mengistie *et al.,* 2013), 95% from India (Bansa *et al.,* 2019) and 88% from Iran (Moussavi & Behrouzi, 2014). However, this has been overshadowed by its low accuracy (82.9%), positive (72%) and negative (85%) predictive values, as well as high false positive (5.8%) and false negative (52.6%) values as reported in this study. This brings to the fore the concern about the reliability of Amsel criteria in the diagnosis of BV (Bensalet *et al.,* 2019). Furthermore, the process in Amsel criteria has been described as cumbersome (Mengistie *et al.,* 2015) which further reduces its position in the pecking order. An assessment of the relevance of individual parameters of Amsel criteria by Bansal *et al.* (2019) showed that pH>4.5 had high sensitivity (97.5%) but lowest specificity (77.78%) which may be attributed to the fact that pH is easily affected by semen, blood, lubricant gel as well as the cervical mucus pH. The presence of clue cells showed sensitivity of 72.5% and specificity of 96.67%; although this is limited by its dependence on the availability of skilled personnel in wet mount microscopy. The Whiff test was reported to have sensitivity of 92.5% and specificity of 90.0%; but it was noted to be subject to the observer's strength to detect odour. Therefore, suggestions have been put forward to simplify the Amsel criteria and improve its sensitivity thereby increasing the overall relevance of the method. The suggestions include greater emphasis on the pH and Whiff test components by Bansa *et al.,*(2019) while Mengistie *et al* (2013)

emphasized the importance of the pH and presence of clue cells.

Rangari Amit *et al.* (2013) noted that relying on Amsel criteria to diagnose BV could be misleading due to its high false positive result while Moussavi & Behrouzi (2004) recommended that results obtained based on Amsel criteria should be confirmed by Gram staining. On the other hand, Mohammadzadeh *et al* (2015) suggested that Amsel criteria can be an alternative to Nugent scoring system in resource-limited settings with high prevalence of BV to prevent the adverse pregnancy outcomes occasioned by complications from undiagnosed and untreated BV infection.

CONCLUSION

This study concludes that Nugent score is superior to Amsel criteria in the diagnosis of BV in pregnancy. In view of the potential implications of undiagnosed or missed diagnosis of BV in pregnancy, a test with high sensitivity and accuracy like Nugent score should be the preferred choice.

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Table 1: Demographic and obstetric variables of participants

Variable	Frequency	Percentage
Age (years)		
Mean ± SD	29.52 ± 4.57 (range 20-39)	
Age Group		
20 – 24	40	12.7
25 – 29	123	38.9
30 – 34	94	29.7
35 – 39	59	18.7
Occupation		
Artisan	14	4.4
Trader/Business	96	30.4
Civil servant	88	27.8
Professional	71	22.5
Unemployed	47	14.9
Level of education		
Primary	3	0.9
Secondary	45	14.2
Tertiary	268	84.8
Type of marriage		
Monogamy	289	91.5
Polygamy	27	8.5
Husband's Occupation		
Artisan	25	7.9
Civil servant	121	38.3
Trader/Businessperson	90	28.5
Professional	67	21.2
Unemployed	13	4.1
Social Class		
I	201	63.6
II	67	21.2
III	31	9.8
IV	14	4.4
V	3	0.9
Parity		
0	103	32.6
1 – 4	213	67.4

Table 2: Pre and post-treatment result of testing using Nugent scoring and Amsel criteria

Laboratory testing method	Pre-treatment		Post-treatment	
	n=316	%	n=76	%
Nugent score				
≥7	76	24.1	19	25.0
4-7	73	23.1	25	32.9
1-3	167	52.8	32	42.1
Amsel's criteria				
Bacteria Vaginosis positive	50	15.8	9	11.8
Bacteria Vaginosis negative	266	84.2	67	88.2

Table 3: Comparison of the performances of Amsel criteria and Nugent score in the diagnosis of BV

Variable	Nugent score ≥ 7 n (%)	< 7 n (%)	Total n (%)	χ^2	p value
Amsel criteria					
Positive	36 (47.4)	14 (5.8)	50 (15.8)	74.764	0.001
Negative	40 (52.6)	226 (94.2)	266 (84.2)		
Total	76 (100.0)	240 (100.0)	316 (100.0)		

χ^2: Chi square

Table 4: Diagnostic performance of Amsel criteria and Nugent score in the diagnosis of BV

Evaluation	Nugent score	Amsel criteria
Sensitivity	93.3%	80.4%
Specificity	92.1%	94.2%
Positive predictive value	94.0%	72.0%
Negative predictive value	90.0%	85.0%
False positive	2.0%	5.8%
False negative	15.0%	52.6%
Accuracy	94.0%	82.9%