

Original Article

Development of modified diagnostic criteria for bacterial vaginosis at peripheral health centres in developing countries

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Abstract

Introduction: Bacterial vaginosis (BV) is the commonest cause of abnormal vaginal discharge in women of child-bearing age and is associated with increased susceptibility to HIV-AIDS and sexually transmitted diseases. In obstetrics, BV has been implicated in causing various complications. In clinical practice, BV is diagnosed using Amsel criteria and Nugent scoring. Using modified Amsel criteria, we determined that the fulfilling of any two instead of three criteria can be diagnostic of BV.

Methodology: This prospective cross-sectional study involved pregnant females complaining of excessive vaginal discharge admitted in a tertiary health centre in central India. Four vaginal swabs were collected for the diagnosis of BV by Amsel criteria, modified Amsel criteria, and Nugent scoring on Gram stain. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of each individual criterion and combinations of criteria were calculated by using Nugent scoring as the gold standard and by chi square test.

Results: In the present study overall prevalence of BV was 12%. The sensitivity of individual or a combination of two criteria was almost same or higher than that of Amsel criteria. Specificity of each combination of criteria was comparable to that of Amsel criteria. However, a combination of high pH and amine odor test had the highest sensitivity (88%) and highest PPV (62%). Diagnostic accuracy of all combinations in our study was equal to or slightly higher than that of Amsel criteria.

Conclusion: Diagnostic accuracy of new modified Amsel criteria is as reliable as that of Amsel criteria.

Key words: Amsel criteria; bacterial vaginosis; BV; Nugent scoring

J Infect Dev Ctries 2012; 6(5):373-377.

(Received 01 October 2010 – Accepted 08 July 2011)

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Introduction

Bacterial vaginosis (BV) is the commonest cause of abnormal vaginal discharge in women of child-bearing age according to the Centers for Disease Control and Prevention (CDC), and it is furthermore associated with increased susceptibility to HIV-AIDS and sexually transmitted diseases. For these reasons, the Centers for Disease Control have included bacterial vaginosis on its list of sexually transmitted diseases [1]. The World Health Organization (WHO) has developed a set of syndromic management guidelines for the treatment of men and women with symptoms suggestive of sexually transmitted diseases, in which diagnostic criteria for BV was included although BV is not a sexually transmitted disease [2]. Health experts are not sure what role sexual activity plays in developing BV. A change in sexual partners or having multiple sexual partners

may increase a woman's chances of getting the infection [3]. Cigarette smoking, using an intrauterine device (IUD) and douching also may increase the risk of getting BV [4]. In obstetrics, BV has been implicated in causing higher rates of late miscarriage, preterm premature rupture of membrane, chorioamnionitis, spontaneous preterm labour, preterm birth, and postpartum endometritis [4]. The manifestations vary from an asymptomatic state to increased homogenous grayish white vaginal discharge; other symptoms may include pain during urination and itching around the vagina. In clinical practice, BV is diagnosed using Amsel criteria and Nugent scoring. Amsel criteria [5] have four requirements, out of which at least three should be fulfilled for confirmation of diagnosis of BV: (1) thin white homogenous discharge; (2) clue cell on microscopy; (3) vaginal pH > 4.5; and (4) release of

fishy odor on adding alkalies (10%KOH). In the laboratory, the Nugent scoring system [6] is a reliable, convenient, and cost-effective method for evaluation of cases of bacterial vaginosis [7,8]]and is considered the gold standard test for diagnosing BV [7,8,9,10,11]. It has a scoring system of 0-10 and allows for intermediates between normal and abnormal. The criteria for BV is a total score of 7-10, while a score of 0-3 is normal and a score of 4-6 is intermediate BV [12]. Treatment of BV is simple; antibiotics such as metronidazole or clindamycin are used to treat women with BV.

The majority of women who are at great risk of the sequelae of BV are in settings where conventional diagnostic methods are not possible due to lack of expert technical hands and laboratory support. Our main aim, therefore, was to develop a new, cost effective diagnostic tool for BV in a rural setting where a health worker can diagnose BV using modified Amsel criteria which suggest that the fulfilling of any two instead of three criteria can be diagnostic of BV [13]. While this method can be very useful in rural settings in developing countries where microscopy may not be feasible, there is hardly any published literature on diagnostic accuracy of modified Amsel criteria. Here we report the diagnostic accuracy of modified Amsel criteria for diagnosis of BV.

Methodology

Study design

We performed a prospective cross-sectional study involving 205 pregnant females with abnormal excessive vaginal discharge admitted in tertiary health centre in central India.

Setting

The study was conducted in the Department of Microbiology and the Department of Obstetrics and Gynaecology, Chhatrapati Shahaji Maharaj Medical University (CSMMU), (Erstwhile KGMU) Lucknow, India.

Pregnant women complaining of vaginal discharge were enrolled in the study. Patients with vaginal bleeding and sexually transmitted diseases were excluded.

Ethics

The study was conducted in accordance with the ethical rules of CSMM University, Lucknow, India. Informed written consent to participate in the study was obtained from all participants.

Methodology

A non-lubricated speculum was passed into the vagina and the nature of the discharge, (colour and consistency) was noted. Four samples of vaginal discharge were taken with the help of sterile cotton-tipped swabs from the posterior vaginal fornix. The samples were processed using the following methods: the Nugent scoring method and the Amsel criteria method.

The four swabs from each patient were used for four different tests as follows:

First swab: The clinician rolled the first swab along a glass slide and made a smear which allowed air-drying. This was sent to laboratory. Gram's staining was done for Nugent scoring. Women with intermediate flora (NS4-6) were excluded from analysis, to avoid false positive results. The results from the analyses of the first swabs put the total numbers of cases at 200, which was the sample size of our study (Table 1).

Second swab: At the same time that the first swab was collected, the clinician placed the secretions from the second swab on pH indicator strips with a pH range of 3.5 to 6 to determine vaginal pH.

Third swab: Two drops of 10% KOH solution were added to the third swab. Release of fishy amine odor signified a positive whiff test.

Fourth swab: The sample from the fourth swab was combined with two drops of normal saline on a slide and covered with a cover slip and examined under high-power microscopy to identify the clue cells.

Comparison of different combinations of criteria to the Amsel criteria

Results were grouped in different categories. Basic criteria such as thin homogenous vaginal discharge (VD), pH > 4.5, KOH Test and clue cell were examined individually and then compared with the combinations of two methods such as pH > 4.5 and KOH; pH > 4.5 and vaginal discharge; and KOH and vaginal discharge in addition to standard Amsel criteria (combination of any three methods) (Table 2)

Statistical analysis

Our investigation was a triple-blinded study because the patients (participants) were not aware of which method was used to make their diagnoses and neither the clinician nor the laboratory worker knew the other's results. Furthermore, analysis of the

Table 1. Results of Nugent scoring

Nugent scoring	Total cases (n = 205)
< 4	176
4-6	5
7-10	24

samples was performed by a third person so the analyser was also unbiased.

Using Nugent scoring as the gold standard, we calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each individual criterion and combinations of the criteria by chi square test. Diagnostic accuracy for each individual criterion and combination of the criteria were evaluated on the basis of two dimensional likelihood ratios. Computer software for statistics was not used.

Results

A total of 200 samples, excluding intermediate flora, were included in the study. Table 3 shows the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy for each individual criterion and combinations of the criteria. The pH (> 4.5) was the criterion with highest sensitivity. The sensitivity of the remaining individual criteria ranged from 75% to 88% and any combination of two criteria ranged from again 75% to 88%. Amsel criteria had sensitivity of 83%. Vaginal discharge had highest specificity of 86% out of all the individual criteria. Amine odour test (KOH test) had the lowest specificity (62%). Specificity of any combination of two criteria ranged from 90% to 93%. Amsel criteria had specificity 90%. Diagnostic accuracy of all the individual criteria was 65-85%. Amine odour test had the lowest accuracy while vaginal discharge had the highest accuracy. Diagnostic accuracy of the combination of two criteria ranged from 88% to 92%. A combination of pH and KOH had the highest accuracy at 92%. Diagnostic accuracy of Amsel criteria was 89%.

Discussion

The prevalence of BV in a developing country such as India is 20% to 47% in non pregnant women and up to 31% in pregnant women [14, 15]. Bhalla *et al.* reported a prevalence of BV in 32% of sexually active non pregnant women, in Delhi, India [16]. In our study, prevalence of BV in pregnant women was lower at ~ 12% is (Table 1).

We chose Nugent scoring as the gold standard test for diagnosis of BV because the Nugent scoring system is an excellent, method for laboratory evaluation of cases of bacterial vaginosis and it is more reliable than Amsel criteria [11]. We excluded intermediate flora from our analysis because they might give rise to false positive results. [7]

The Nugent scoring test requires health care experts, laboratory support, and access to high-power microscopy to obtain timely results for the clinical diagnosis of BV. Since these necessities are not always available in developing countries, it is important to have simple and reliable clinical criteria that clinicians can use in practice.

In our study, among the individual Amsel criteria, the clue cell was found to be the most reliable predictor of BV, but this test requires technically experienced hands and laboratory support which is not always possible in rural setting. A second criterion is vaginal discharge, which is nonspecific and non sensitive. Thin homogenous vaginal discharge is not accurately interpreted. In our study there were cases of BV in other types of vaginal discharge also. The third criteria is high pH (> 4.5). Many studies suggest that raised pH is recognized as the most sensitive but least specific criteria [8,17], which was also confirmed in our investigation (Table 3). In our study, high pH (> 4.5) had higher sensitivity than the Amsel criteria (90.3%) but it was less specific (78.4%). A fourth criterion is the Amine odor test (KOH test). Gutman *et al.* suggested in their study that the KOH test was a highly sensitive and specific method [8]; however, our findings did not support this hypothesis. In our study the sensitivity of the KOH test was as good as that of Amsel criteria, but its specificity was accurate. Our results indicate that any individual criterion on its own is not sufficient to diagnose BV, but if we modify Amsel criteria by using a combination of any two criteria, there is decreased sensitivity and increased specificity. Previous studies also suggest making modifications in Amsel criteria, such as the combination of high pH (> 4.5) and amine odor test [8], or clue cell and positive amine test [4]. In our study, the specificity of each pair of criteria is more or less similar to that observed for Amsel criteria; however, the combination of high pH (> 4.5) and amine odor tests has high sensitivity (88%) and high PPV (62%) as compared to Amsel criteria. Diagnostic accuracy of all combinations in our study is more or less equal to or higher than that of Amsel criteria.

Table 2. Results of diagnostic indices of tests used for diagnosis of BV

Diagnostic criteria	Total number of positive cases (n = 200)	Positive by Nugent scoring (n = 24)
Amsel	37 (18.5%)	20 (83%)
vaginal discharge (VD)-thin homogenous	42 (21%)	18 (75%)
pH>4.5	60 (30%)	22 (91.3%)
KOH Test	88 (44%)	21 (87.5%)
pH+KOH	34 (17%)	21 (87.5%)
pH+VD	34 (17%)	17 (70.8%)
KOH+VD	34 (17%)	18 (75%)

Table 3. Analysis of diagnostic indices of tests used for diagnosis of BV

Diagnostic criteria	Test + (TP)	Test - (FP)	Sensitivity (%) (CI %)	Specificity (%) (CI %)	PPV (%) (CI %)	NPV (%) (CI %)	Diagnostic Accuracy (%)
Amsel	20	17	83.3 (61.8 - 94.5)	90.3 (84.8 - 94.1)	54.1 (37.1 - 70.2)	97.5 (93.4 - 99.2)	89.5
pH > 4.5	22	38	91.7 (71.5 - 98.4)	78.4 (71.5-84.1)	36.7 (24.9 - 50.2)	98.6 (94.4 - 99.8)	80.0
KOH Test	21	67	87.5 (66.5 - 96.7)	61.9 (54.3-69.1)	23.9 (15.7 - 34.4)	97.3 (91.8 - 99.3)	65.0
Thin white homogenous vaginal discharge (VD)	18	24	75.0 (52.9 - 89.4)	86.4 (80.2-90.9)	47.4 (28.1 - 58.9)	96.2 (91.6 - 98.5)	85.0
pH+KOH	21	13	87.5 (66.5 - 96.7)	92.6 (87.4-95.9)	61.8 (43.6 - 77.3)	98.2 (94.4 - 99.5)	92.0
pH+VD	17	17	70.8 (48.8 - 86.6)	90.3 (84.7-94.1)	50.0 (32.8 - 67.3)	95.8 (91.2 - 98.1)	88.0
KOH+VD	18	16	75.0 (53.0 - 89.4)	90.9 (85.4-94.6)	52.9 (35.4 - 69.8)	96.4 (91.9 - 98.5)	89.0

Conclusion

Our study supports the idea of simplifying the clinical diagnosis of BV by using two clinical criteria, especially high pH (> 4.5) and amine odor tests, without significant loss of sensitivity and specificity, for screening of women for BV at outpatient clinics. The pH and amine odour tests can be performed easily by health workers in rural settings to diagnose BV, and they are cost effective. Early diagnosis of BV can help prevent further complications, especially in pregnant women, by commencing appropriate treatment.

Acknowledgement

We are very thankful to Mr Zeashan Haider Zaidi, lecturer in biostatistics in the Department of Community Medicine of Era's Lucknow Medical College, Lucknow, India, for calculating all the statistics.

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Conflict of interests: No conflict of interests is declared.