ORIGINAL ARTICLE

Bacterial Vaginosis in Pregnant Women and its diagnosis using Amsel's Clinical Criteria and Nugent's Method

NERGIS TAJ[*], M. SHAMS UL ALAM[‡], SARAH[†], *ZUNAIRA BAJWA[ɣ], ABDUL WAHEED[¶], EHSAN ULLAH[‡]*

ABSTRACT

Aim: To determine the frequency of bacterial vaginosis in pregnant women and to evaluate the accuracy of Amsel's clinical criteria taking Nugent criteria as gold standard.
Methods: It was a descriptive, observational study in which 380 asymptmatic pregnant women attending antenatal facility at two teaching hospitals were screened for bacterial vaginosis by Gram staining. Sensitivity and specificity of Amsel's criteria were also assessed.
Results: The frequency of bacterial vaginosis was 41(10.8%) by Gram's staining. The sensitivity and specificity of Amsel's clinical criteria was 54% and 98%. Positive and negative predictive value was 71% and 96% respectively. Clue cells had highest specificity. Positive and negative predictive values for combined (both) criteria were 63 and 98%, respectively.
Conclusions: Bacterial vaginosis was found in a higher proportion of asymptomtic pregnant women. Amsel's clinical criteria were useful but inferior to Gram's staining for diagnosis of bacterial vaginosis.
Key words: Bacterial vaginosis, pregnancy, Amsel's criteria, clue cells, whiff test, vaginal discharge

INTRODUCTION

Bacterial vaginosis (BV) is the most frequent vaginal disorder in reproductive age females which commonly manifest as abnormal vaginal discharge and pruritis but it may be asymptomatic as well[1,2]. Whereas vaginal discharge is only second to menstrual problem among the most common complaints reported by RAFs. Prevalence of BV among pregnant women ranges from 9-23%[3].

Bacterial Vaginosis (BV) is an infectious disease, the etiology of which is polymicrobial where normally present lactobacilli are replaced by some other bacteria such as Gardenella vaginalis, Mycoplasmas, and anaerobic gram negative rods[4]. Imbalance of vaginal flora with a reduction of lactobacilli morphotypes and increased numbers of anaerobes is an important phenomenon in the pathogenesis of BV[5].

The gold standard method of diagnosis for BV is based on Gram staining devised by Nugent et al[6]. Amsel clinical criteria provides a quick method to diagnose BV where laboratory facilities are scarce or the work load is too much[7].

**Department of Obstetrics and Gynaecology, Bahawal Victoria Hospital Bahawalpur*
†Department of Medicine, Bahawal Victoria Hospital Bahawalpur
‡Department of Pathology, Quaid-e-Azam Medical College Bahawalpur
ɣDepartment of Obstetrics and Gynaecology, Jinnah Hospital Lahore
¶Department of Microbiology, University of Health Sciences Lahore
Correspondence to Dr. Ehsan Ullah 218-A, Revenue Society, Johar Town Lahore Email: e_ullah@yahoo.com

MATERIALS AND METHODS

It was a descriptive observational study. A total of 380 asymptomatic pregnant women who attended the antenatal clinic at Bahawal Victoria Hospital between August 2012 and July 2013 were enrolled in the study after their informed consent. Purposive, non-probability sampling technique was used to select the subjects. Exclusion criteria included women who were menstruating or having vaginal bleeding, using any vaginal suppository drugs, previously diagnosed with human immunodeficiency virus (HIV) infection, having visible vaginal or cervical mass or a suspected cancer and those who had aborted or gave birth within last six weeks.

Samples of the vaginal discharge were obtained with two dry cotton-wool tipped swabs from vaginal fornices. One of the swabs was pressed briefly against an indicator paper to measure the pH, then this swab was mixed into two drops of normal saline solution in the glass tube. One drop of the mixture was dropped onto the glass slide then a cover slip was placed over the mixture. It was taken care that presence of clue cells was declared only after assuring that >20% of epithelial cells were 'clue cells'. Moreover, it was necessary for a clue cell to be covered by bacteria on >75% of the cell boundary. The other swab was smeared onto the glass slide, then this swab was mixed into two drops of 10% potassium hydroxide solution for amine testing. The composite clinical diagnosis was defined as presence of at least three of the following: homogeneous vaginal discharge vaginal pH >4.5, positive amine test and presence of 'clue cells' (greater than 20% of all visible epithelial cells)[8]. The smear on the glass

slide was air-dried. Gram staining was performed and the stained smears were independently examined under the light microscope under oil immersion at 1000x magnification Nugent's score was assigned as shown in Table 1. A score of >7 indicated BV infection[6].

RESULTS

A total of 380 women were enrolled in the study. The mean age was 28.3±4.9 years. The majority of the women were in the first trimester of gestation. Homogeneous vaginal discharge was found in 55 (14.5%) study participants. However, true and false positive cases were 24 and 31 respectively as shown in Table 2. Thus the sensitivity and specificity was 58.5 and 90.9% respectively. Positive and negative predictive values were 43.6 and 94.8% respectively.

Table 1. Nugent's scoring on Gram stain[6]

Score	Lactobacillus	Gardnerella/ bacteroids	Mobiluncus
0	>30	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	-
4	0	>30	-

Table 2. Homogenous vaginal discharge

Test	Disease present	Disease absent	Total
+ve	24	31	55
-ve	17	308	325
Total	41	339	380

Table 3. Vaginal pH > 4.5

Test	Disease present	Disease absent	Total
+ve	18	13	31
-ve	23	226	325
Total	41	339	380

Vaginal pH >4.5 was found in 31(8.2%) study participants out of which 18 were true and 13 were false positives as shown in Table 3. Thus, the sensitivity and specificity for this criterion (alone) was 43.9 and 96.2% respectively. Positive and negative predictive values were 58.1 and 93.4% respectively. Amine odour when potassium hydroxide solution was added to vaginal secretions was found in 37(9.7%) out of which 21 were truly and 16 were falsely positive (Table 4). Thus, sensitivity, specificity, PPV and NPV for Whiff test were 51.2, 95.3, 56.8 and 94.2% respectively.

Table 4. Whiff test

Test	Disease present	Disease absent	Total
+ve	21	16	37
-ve	20	323	343
Total	41	339	380

Clue cells were found on microscopy of vaginal discharge in 31(8.2%) cases where it was subsequently proven to be true positive in 25 and false positive in only six cases (Table 5). Thus, the sensitivity, specificity, PPV and NPV for clue cells were 61, 98.2, 80.6 and 95.4%.

Table 5: Presence of clue cells

Test	Disease present	Disease absent	Total
+ve	25	06	31
-ve	16	333	349
Total	41	339	380

Thirty one (8.2%) study participants were found to meet at least three out four Amsel's criteria (Table 6). The sensitivity, specificity, PPV and NPV for Amsel's criteria were 68.3, 97.1, 73.7 and 96.2%.

Table 6. Total Amsel's criteria

Test	Disease present	Disease absent	Total
+ve	28	10	38
-ve	13	329	342
Total	41	339	380

DISCUSSION

Correct diagnosis of bacterial vaginosis is demanding. Moreover the choice of diagnostic test requires consideration of expertise, cost, and the frequency of un-interpretable specimens. Recently, some alternative diagnostic methods have been developed, such as the polymerase chain reaction (PCR), rapid nucleic acid hybridization test, proline amino peptidase activity and sensor arrays, have also been introduced for the diagnosis of bacterial vaginosis[9]. But, most of these tests are expensive and their diagnostic accuracy in terms of sensitivity and specificity do not offer a clear advantage over the classical methods[9]. Thus, the clinical criteria by Amsel and Nugent's method based on Gram staining remain the most practical, viable and economical options for diagnosing bacterial vaginosis, especially in developing countries[10]. Thus, the current study was designed to evaluate the accuracy of Amsel's clinical criteria taking Nugent criteria as gold standard. As a by-product, the frequency of BV was also determined in the study population of 380 pregnant women attending antenatal facility.

In this study, the frequency of bacterial vaginosis was found to be 10.8% using Nugent's criteria on Gram staining. Using Nugent's method as the diagnostic criteria, the prevalence of bacterial vaginosis can be seen to vary considerably from study to study[11-13]. A study from Rawapindi found the frequency of bacterial vaginosis to be 11.3% in a sample of 328 women which closely matches with our findings[14]. Aslam et al. found a frequency of

bacterial vaginosis as 18.7% in a small sample of pregnant women[15].

The sensitivity and specificity of Amsel's criteria were 68.3 and 97.1% whereas positive and negative predictive values were 73.7 and 96.2% respectively in current study. Modak et al. (2010) found that sensitivity and specificity of Amsel's criteria were 66.7 and 94.7% whereas the PPV and NPV were 80 and 90% in their study.[10] In same study, the sensitivity, specificity, PPV and NPV for characteristic vaginal discharge were 66.7, 71.1, 42 and 87% and those for pH of >4.5 were 83.3, 86.8, 67 and 94%. Modak et al. found that whiff test was 100% specific and 42% sensitive whereas clue cells were 100% sensitive but 76% specific for the diagnosis of BV. Our findings about the sensitivity of Amsel's criteria are similar to Modak et al. however the specificity and NPV are quite improved in current study which may be due to larger sample size as compared to Modak et al.

Beverly et al. (2005) found that total Amsel criteria had a sensitivity of 37% and specificity of 99% whereas vaginal pH of >4.5 had sensitivity and specificity of 83% and 69%. Similarly the presence of clue cells has sensitivity of 33% and specificity of 98%. Positive whiff test and characteristic vaginal discharge had sensitivities and specificities of 45%, 35%, 96% and 85% respectively[16].

Both clinical and the Gram stain criteria are acceptable methods of diagnosis of BV, however, subtle differences are apparent when these methods are compared to each other. A study showed that sensitivity and specificity of the Nugent's score compared to the Amsel's criteria were 97% and 98%, respectively[17]. Schwebke et al. showed that vaginal Gram stain (Nugent's score) was more sensitive than Amsel's criteria for diagnosis of BV[18].

CONCLUSION

Bacterial vaginosis was found in a quite higher proportion (10.8%) of asymptomtic pregnant women. Amsel's clinical criteria were found useful but inferior to Gram's staining for the correct diagnosis of bacterial vaginosis.

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