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Original Article

Microbiology Section

Comparison of Clinical and Gram Stain Diagnosis Methods of Bacterial Vaginosis Among Pregnant Women in Ethiopia

ZEMENU MENGISTIE[1], YIMTUBEZINASH WOLDEAMANUEL[2], DANIEL ASRAT[3], MAHLET YIGEREMU[4]

ABSTRACT

Background: Bacterial Vaginosis (BV) is characterized by an increased thin homogenous white vaginal discharge accompanied by fishy odour and increased vaginal pH. It is associated with different gynecologic and poor obstetric outcome. Bacterial vaginosis can be easily diagnosed by combination of two Amsel's criteria.

Objective: The aim of this study was to evaluate the accuracy of Amsel's criteria individually or in combination of two for the clinical diagnosis of bacterial vaginosis among pregnant women in Ethiopia.

Material and Methods: In this crosssectional study 252 pregnant women were screened for bacterial vaginosis. Vaginal swabs were collected for pH determination, saline wet mount microscopic examination to detect clue cells, KOH preparation for whiff test and Gram-stain evaluation of vaginal flora for diagnosis of bacterial vaginosis by Nugent scoring system. Accuracy of clinical diagnosis using individual and two of Amsel's criteria was evaluated.

Results: The prevalence of BV was 18.3% by Amsel's two of three criteria and 19.4% Gram by Nugent's methods. Comparing with Nugent scoring methods, the clinical diagnosis by Amsel's criteria had sensitivity of 85.7%, specificity of 98%. The most sensitive and specific individual Amsel's criterion was clue cells. Amsel's criteria with the lowest sensitivity and specificity were whiff test and vaginal pH respectively. Combination of clue cells with vaginal pH test were the highest in sensitive while whiff test with clue cells were the highest in specificity than the other combined two Amsel's criteria.

Conclusion: Amsel's criteria diagnosis of bacterial vaginosis can be simplified by using a combination of the two criteria, vaginal pH and clue cells, in settings where time or Gram staining is not available.

Key words: Amsel's criteria, Bacterial vaginosis, Ethiopia, Nugent Scoring System, Pregnancy

INTRODUCTION

Bacterial Vaginosis (BV) is found worldwide among women of reproductive age. It is the most common infectious cause of vaginitis, being twice as common as candidiasis [1,2]. The prevalence of BV varies widely among the different populations studies and it has been most widely studied among women attending publically supported sexually transmitted infection clinics, family planning clinics, and obstetrical clinics [3,4]. Higher prevalence of BV is commonly reported from developing country than developed country (35% vs. 24.8%) [5].

The importance of diagnosis and treatment of BV in various clinical settings is increasingly recognized. Treatment with antibiotics might be helpful in some cases of idiopathic preterm labor but at present, knowledge and diagnostic methods are not sufficient in recommending antibiotic therapy in routine clinical practice. Rapid screening with available resource is essential for a favorable health care outcome. Classical initial method of BV diagnosis was done by isolation of *G. vaginalis* from clinical specimen [6,7]. Later on with the advent of the anaerobic culture technique other organisms are also detected from those women with disturbed flora [8].

The diagnostic approach of bacterial vaginosis varies from time to time and at different clinical setting and purpose. Despite this, Amsel's clinical diagnosis and gram stain evaluation by Nugent methods are mostly used worldwide particularly in developing countries. The Nugent scoring test requires health care experts, laboratory support, and access to high-power microscopy to obtain timely results for the diagnosis of BV [9]. 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. Therefore, knowledge of best diagnostic approach in a given area using the available resource helps to inform the preference method.

The objective of this study was to evaluate the accuracy of Amsel's criteria individually or in combination of two for the clinical diagnosis of BV.

MATERIAL AND METHODS

A hospital based cross sectional, observational study was conducted from November 2011 to April 2012 at Black Lion University Hospital, Addis Ababa, Ethiopia. During this period, 252 pregnant women in any gestational week were screened for bacterial vaginosis. After physical and gynaecological examination by the attending Physician all eligible pregnant women were referred (requested) with their card to participate in the study. The Nurse interviewers explained the purpose and practice of the study, and obtained informed consent. Standard Questionnaire was used to get relevant information and the existing Clinical data was recorded for each participant. Vaginal bleeding, antibiotic treatment in the previous 2 weeks and not volunteers to give consent were exclusion criteria.

Two vaginal swabs/discharges were collected from each pregnant women posterior vaginal fornix using sterile cotton tipped applicator by trained Nurse, one used for vaginal pH measurement and for whiff test after addition of 10% KOH. The second swab was used for preparation of saline wet mount and smear for Gram staining. After labeling, all materials were transported immediately to Microbiology Teaching Laboratory for Microscopic wet mount examination and Gram staining.

Clinical diagnosis of bacterial vaginosis was considered positive if two of the following three criteria were met: vaginal pH exceeded 4.5, whiff test was positive, and clue cells were present on saline wet smear preparation [10]. The character of vaginal secretion was not used in the Amsel criteria for this study. Gram stain diagnosis

was based on a criterion score described by Nugent and considered positive if the score was 7–10. The Nugent criteria score vaginal flora as normal (0–3), intermediate (4–6), and bacterial vaginosis (7–10) [9].

Data was entered by using EPI data then exported to SPSS version 16.0 for analysis. Univariate analysis was done to calculate the frequencies and proportions. In addition, sensitivity, specificity, positive and negative predictive value of Amsel's criteria was calculated by using OpenEpi soft ware.

The research proposal was ethically cleared and approved by Research and Ethical Review Committee (REC), School of Medicine, Addis Ababa University. Symptomatic pregnant women positive for Bacterial vaginosis were treated by 500mg oral metronidazole twice daily for seven days while yeast infected pregnant were treated by Miconazole 2% cream 5 g intravaginally for 7 days.

RESULTS

The age range of the study participants was 18-40 with mean age was 27.6 years. At the time of data collection 36(14.3%) of the study participants were at their first trimester gestational age while 121(48%) were at their third trimester gestational age. Of 252 participants 91 (36.1%) pregnant women had Primigravidia.

The prevalence of bacterial vaginosis was 18.3% by Amsel's 2 of 3 criteria and 19.4% by Nugent scoring [Table/Fig-1]. Amsel's criteria had sensitivity of 85.7% and a specificity of 98% when compared using Gram stain evaluated by Nugent scoring method as standard.

When we compare individual Amsel's criteria with Nugent scoring, in the present study we found that clue cells was the criteria with the highest sensitive and specificity [Table/Fig-2]. The sensitivity of the remaining individual criteria ranged from 69% to 82%. All criteria had high negative predictive value (93-97.5%). Specificity of the combination of any two Amsel's criteria as shown in [Table/Fig-2] ranged from 99–100%. The Combination of Amine test with clue cells had hundred percent specificity and positive predictive value but had less specificity [Table/Fig-3]. Even though combinations of clue cells with other criteria increase its specificity and predictive value of positive, it markedly decreases its sensitivity. The combination of pH with clue cells had the highest sensitivity and negative predictive value.

During wet mount preparation *T. vaginalis* from saline wet mount and Yeast cells from KOH wet mount were assessed. Yeast cells were diagnosed from 28 (11.1%) pregnant women while none of the participants had *T. vaginalis*.

DISCUSSION

The prevalence of bacterial vaginosis by Amsel's criteria and Gram stain is 18.3% and 19.4% respectively. Consistent with our study, almost equal prevalence of bacterial vaginosis (6.7% vs. 8.6%) by the two methods was reported from the study that was conducted among 502 New Delhi pregnant women [11]. The researcher was considered three of four Amsel's criteria for clinical diagnosis of bacterial vaginosis. In addition, the finding of this research also similar with the report from general population in Southern India (18% vs. 19%) [12]. In contrast, the study wsa conducted or 200 symptomatic women in the rural setting had documented higher prevalence of bacterial vaginosis by three of four Amsel's criteria than Nugent gram stain (49% vs. 35%) [13]. These differences may be due to difference in number of Amsel's criteria used or in study subject.

When we correlate any two of three Amsel's criteria (excluding type of discharge) with Gram stain Nugent criteria for diagnosis of bacterial vaginosis, we found that the sensitivity, specificity, PPV and NPV Amsel's was 85.7%, 91.3%, 98 and 96.6% respectively which is almost equal with the clue cells performance. Comparison result

Nugent scoring				p-value	SN	SP	PPV	NPV
		Positive	Negative					
Amsel criteria	Positive	42	4	<0.05	85.7	98	91.3	96.6
	Negative	7	199					
	Total	49	203					

[Table/Fig-1]: Comparison of Amsel's criteria and Nugent scoring for the diagnosis of bacterial vaginosis among pregnant women attending ANC in Tikur Anbessa Hospital (November 2011 – April 2012)
N - Number; SN - Sensitivity; SP - Specificity; PPV - Positive predictive value NPV - Negative predictive value

Amsel's Criteria	n (%)	SN (95% CI)	SP(%)(95% CI)	PPV	NPV
Vaginal pH	65 (25.8)	81.6 (68.6, 90)	87.7 (82.5, 91.5)	61.5	95.2
Amine test	39 (15.5)	69.4 (55.5, 80.5)	97.5 (94.4, 98.9)	87.2	93
Clue cells	48 (19.0)	89.8 (78.2, 95.6)	98 (95, 99.2)	91.7	97.5

[Table/Fig-2]: Diagnostic accuracy of individual clinical criteria among pregnant women attending in Tikur Anbessa Hospital (November 2011-April 2012).
N - Number; SN - Sensitivity; SP - Specificity; PPV - Positive predictive value NPV - Negative predictive value

Amsel's Criteria	n (%)	SN (95% CI)	SP (95% CI)	PPV	NPV
Vaginal pH +Amine test	32 (12.7)	61.2 (47.3, 73.6)	99 (96.5, 99.7)	93.8	91.4
Vaginal PH + clue cells	40 (15.9)	77.6 (64, 87)	99 (96.5, 99.7)	95	94.8
Amine test + Clue cells	32 (12.7)	65.3 (51.3, 77)	100 (98, 100)	100	92.3

[Table/Fig-3]: Diagnostic accuracy of combination of two Amsel's criteria among pregnant women attending Tikur Anbessa Hospital (November 2011-April 2012).
N - Number; SN - Sensitivity; SP - Specificity; PPV - Positive predictive value NPV - Negative predictive value

of clinical criteria with Gram stain among asymptomatic pregnant women in Texas showed that clinical diagnosis had; a lower sensitivity of 56%, a comparable specificity of 96% and a lower positive and negative predictive value of 83% and 85% respectively, than the current study clinical diagnosis [14]. This difference in sensitivity and predictive value may be due to difference of study population clinical case and higher prevalence of bacterial vaginosis (27%). The sensitivity and specificity of Amsel's criteria comparing with Gram stain result was 35% and 99% respectively [15]. Amsel's method was found to be 78% sensitive and 95.6% specific as compared to Nugent's method [12]. Consistent with these two studies we found that almost equal specificity of Amsel's criteria.

From individual criteria for predicting the gram stain result, clue cells detection from wet mount microscopic examination is the single most reliable predictor of bacterial vaginosis. It had a higher sensitivity, specificity and positive and negative predictive value. This is consistent with the study done by other researcher's [16-19]. But this is not consistent with Dadhwal et al., [11]. This difference may be due to the subjective nature inherent in the evaluation of the test. Vaginal pH is the lowest in specificity and positive predictive value from the other two clinical diagnostic criteria. This result is similar with the finding by Mastrobattista and his colleagues [14]. Many studies suggested that raised vaginal pH is recognized as the least specific criteria [14,19] and it is confirmed in our investigation. The lower specificity and positive predictive value of vaginal pH compared to others clinical criteria in this study indicates the presence of other genital tract infection or factor which increases pH without the presence of disturbed vaginal flora. Whiff test, as a clinical diagnostic criterion is the lowest in sensitivity (69.4%) but high in specificity (97.5%). However, our findings did not support the suggestions in which whiff test was a highly sensitive and specific method [20,21]. The decrease in sensitivity of whiff test may attribute to subjective nature of the test due sensation ability of the person doing the test. The other factor may be absence or presence of low number of amine producing abnormal microorganism.

Our results indicate that clue cells from individual criterion by its own sufficient to diagnose BV, but if we modify Amsel's criteria by using

a combination of any two criteria, there is decreased sensitivity and increased specificity. The combination of two criteria had sensitivity of 61.2% to 77.6%, specificity of 99% to 100%. Amine test plus pH as diagnosis of clinical test is mostly recommended among the study done in different setting, population and countries [11,13,19]. In contrast to these three studies, we found that combination of clue cells and pH had the highest sensitivity and very good specificity than pH and amine test. In addition, in our study we found that raised vaginal pH lacks specificity and whiff test lacks sensitivity in comparison with clue cells. So in conditions where there is no enough time and gram stain procedure, combination of vaginal pH and clue cells detection can be used with only seven false negative diagnosis but comparative sensitivity and specificity.

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PARTICULARS OF CONTRIBUTORS:
1. Faculty, Department of Biomedical Science, College of Health Sciences, Mizan Tepi University, P.O. Box 260, Mizan, Ethiopia.
2. Faculty, Department of Medical Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University, P.O. Box 9086, Addis Ababa, Ethiopia.
3. Faculty, Department of Medical Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University, P.O. Box 9086, Addis Ababa, Ethiopia.
4. Faculty of Medicine, Department of Obstetrics and Gynecology, Addis Ababa University, P.O. Box 9086, Addis Ababa, Ethiopia.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Zemenu Mengistie,
Faculty, Department of Biomedical science, College of Health Sciences, Mizan Tepi University,
P.O. Box 260, Mizan, Ethiopia.
Phone: +251 9 13 51 37 66: Fax: +251 47 3 360019, E-mail: zemenumengistie@yahoo.com

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