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Comparison of Different Diagnostic Methods of Bacterial Vaginosis – Amsel’s vs Neugent

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ABSTRACT

Bacterial Vaginosis (BV) is the most common cause of vaginitis and is of special public health concern in India because of high burden of reproductive and pregnancy related morbidity. Early diagnosis and treatment might be useful in prevention of complications and can only be achieved by accurate, reproducible and inexpensive method. Although Nugent's criterion is considered as the gold standard in diagnosis of BV, routinely a combination of various methods is used for the diagnosis of bacterial vaginosis (BV). In the present study we compared Amsel's composite clinical criteria with Nugent's method for the diagnosis of BV. The study was undertaken from March 2016 - December, 2016 at a Tertiary Care Hospital in Punjab. The study consisted of women with complaints of vaginal discharge. BV was diagnosed based on Amsel's™ criteria and Neugent™ scoring. Nugent scoring system was considered the gold standard. Sensitivity, specificity, positive predictive value and negative predictive value of Amsel's™ criteria were compared with those of Nugent scoring system. The present study included 200 cases of abnormal vaginal discharge. Prevalence of BV was 34%. Age group 24-29 years was most affected. Amsel's™ criteria detected 60/200 whereas Nugent score identified 68/200 subjects as having bacterial vaginosis. In comparison with Nugent's™ criteria the sensitivity, specificity, positive predictive value and negative predictive value of Amsel's™ criteria were 88%, 100%, 100% and 94.2%. With limited resources in developing countries like ours, there is a great need for inexpensive diagnostic methods for bacterial vaginosis. Amsel's™ criteria is as good as Nugent's™ scoring in diagnosis of BV and it is simple, easy, cost effective, fast and reliable, and can be done in OPD which can be used for precise and fast treatment.

Keywords

Bacterial vaginosis,
Amsel's criteria,
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Introduction

Bacterial vaginosis (BV) is a polymicrobial syndrome characterized by replacement of vaginal *Lactobacilli* with predominantly anaerobic micro-organisms such as *Gardnerella vaginalis*, *Prevotella*, *Peptostreptococcus* and *Bacteroides spp.* with concurrent decrease in *Lactobacilli*, the dominant constituents in normal vaginal flora (Ling *et al.*, 2009). It's an extremely common health problem for women, occurring in 35% of women attending sexually transmitted

infection (STI) clinics, 15% to 20% of pregnant women, and 5% to 15% of women attending gynaecology clinics (Livengood, 2009). In addition to the troublesome symptoms often associated with a disruption in the balance of vaginal flora, BV is associated with adverse gynecological and pregnancy outcomes. Although BV is often asymptomatic, it still is the most common cause of vaginitis, and hence among the commonest reasons for women to seek

medical help (Laxmi *et al.*, 2009). Although not technically a sexually transmitted infection, Bacterial vaginosis is a sexually associated condition.

Most often, multiple criteria are used for the diagnosis of bacterial vaginosis. Clinical features were first described by Gardner and Dukes (Livengood, 2009), and range from asymptomatic to an increased thin vaginal discharge with or without a fishy odour. One of the methods of diagnosis is the Amsel's composite criteria which includes clinical diagnosis and a few simple laboratory tests. The presence of any three of the following four criteria is considered to be consistent with the presence of bacterial vaginosis: characteristic thin, homogenous vaginal discharge, vaginal pH greater than 4.5, release of a fishy amine odour on addition of 10% KOH (whiff test), and demonstration of clue cells in more than 20% of the total cell population (Amsel *et al.*, 1983).

Bacterial vaginosis can also be diagnosed by Spiegel's and Nugent's criteria. Both these criteria are based on the evaluation of the normal flora in the Gram stained smears of the vaginal discharge (Laxmi *et al.*, 2009) Nugent *et al.*, suggested a modification of Spiegel's method of scoring Gram-stained vaginal smears for the diagnosis of bacterial vaginosis (Nugent *et al.*, 1991). The score, calculated by assessing the presence of large Gram-positive rods (*Lactobacillus* morphotypes), small Gram-negative/Gram-variable rods (*G. vaginalis* morphotypes), and curved Gram-variable rods (*Mobiluncus* spp. morphotypes) can range from 0 to 10 with a score of 7 to 10 being consistent with bacterial vaginosis. Compared to the Amsel criteria, the Nugent's score allows for assessment of alteration in vaginal flora as a continuum rather than a dichotomy

In a developing country with limited resources such as India, diagnosis of bacterial

vaginosis by Nugent's score would place a great strain on available resources. The Amsel criteria method requires less infrastructural and manual resources; thus clinicians would be better placed if they knew the sensitivity and specificity of Amsel criteria in relation to Nugent's score before diagnosis. Hence this current study was undertaken not only to diagnosis and knows the prevalence of bacterial vaginosis but also to compare Amsel's criteria with the Nugent scoring.

Materials and Methods

Study setting and duration

A prospective, Cross sectional study was conducted from March 2016 till December 2016 in the Department of Microbiology and Department of OBG, at Tertiary care hospital in Punjab. Approval of institutional ethical committee was taken for this study.

Study design

The present study was conducted, to detect cases of BV among Sexual Active Women of Reproductive Age Group. The Women in reproductive age (15-45 years) were included in the study. Descriptive variables obtained during evaluation included age, pregnancy status, parity, ethnicity, mode of contraception, number of sexual partners, presence or absence of symptoms, and a sexually transmitted diseases history.

Exclusion criteria

Women were excluded from the study if they had history of receiving systemic antibiotic therapy or local vaginal antimicrobial therapy within the preceding 15 days, were menstruating at the time of the examination, vaginal bleeding, placenta previa, spermicide use, recent douching, or sexual intercourse within 24 hours. Subjects had prenatal

assessments including thorough histories and physical examinations (Sarada Tiyyagura *et al.*, 2012).

Two high vaginal swabs were collected in a well-lit room from posterior fornix under aseptic precautions and transported immediately to Microbiology laboratory. While taking the swab character of vaginal discharge was observed. The pH of vaginal discharge was recorded using standard pH indicator paper with range 1 to 14. Diagnosis of bacterial vaginosis was done by Nugent's scoring and Amsel's criteria.

Diagnosis by Amsel's criteria

Amsel's composite criteria includes the presence of a homogeneous vaginal discharge, pH of the vagina being > 4.5, the presence of clue cells in wet mount of the vaginal discharge and a positive whiff test. According to Amsel, if 3 of the 4 criteria are positive, the patient has bacterial vaginosis (Laxmi *et al.*, 2011).

Vaginal pH determination

pH of the vagina was tested using a pH paper by dipping it in the secretions pooled in the posterior fornix. This was compared with a standardized colorimetric reference chart to estimate the actual pH.

Whiff test

A drop of the vaginal fluid was taken on a grease free glass slide. To this one drop of 10% KOH was added. An intense, putrid, fishy odour indicates positive reaction.

Presence of clue cells

A drop of the vaginal fluid was mixed with a drop of normal saline on a clean grease free glass slide; a cover slip was placed on it. Slide was observed under 10 x & 40 x

magnifications within 10 mins. The vaginal epithelial cells which were coated with cocobacillary organisms so that their edges which normally have a sharply defined cell border became indistinct or stippled were considered as the clue cells. Clue cells are characteristic feature of BV. If the clue cells constitute 20% or more of the epithelial cells in the high power field it is considered positive

Diagnosis by Nugent's criteria

Vaginal swab was rolled on a microscopic slide, air dried then Gram stained with gram staining protocol. Slides were read according to Nugent score as follows: Morphotypes were counted as the average number of bacteria in 10-20 oil immersion fields. The Nugent score was calculated by assessing for the presence of large gram-positive rods (*Lactobacillus* morphotypes; decrease in *Lactobacillus* scored as 0 to 4), small gram-variable and gram-negative rods (*G. vaginalis* and *Bacteroides* morphotypes; scored as 0 to 4), and curved gram-variable rods (*Mobiluncus* spp. morphotypes; scored as 0 to 2). After the amount of each morphotype detected on the smear was graded it was then allocated a score as shown in table 1. Then total score calculated from 0 to 10.

- A score of 1-3, considered normal
- A score of 4-6 considered intermediate (means an intermediate state between normal and BV)
- A score of 7 to 10 was consistent with BV.

This method is considered the gold standard for diagnosis of BV.

Results and Discussion

A total of 200 patients in reproductive age group with complaints of vaginal discharge were examined for diagnosis of Bacterial

vaginosis. Among these, 68 patients were diagnosed to be affected with BV by Nugent scoring providing a prevalence rate of 34 % for bacterial vaginosis and 60(30%) by Amsel's criteria. Thus the sensitivity of Amsel criteria was 88%, specificity was 100%, positive predictive value was 100% and negative predictive value was 94.2%.

Maximum patients belonged to the age group of 24-29 years. The mean age was 28 years. Highest prevalence of BV was noticed in the age group of 24-29 years followed by 30-35 years indicating that there is a high incidence of BV in young individuals in the reproductive age group. Vaginal discharge and malodour were very common, seen in 100% of cases followed by itching and dysuria.

Bacterial vaginosis (BV) is the most common cause of vaginitis in women of reproductive age group (Morris *et al.*, 2001). It is the most common infection encountered in the Gynaecological outpatient setting. Proper diagnosis of bacterial vaginosis is challenging. Most often, multiple criteria are used for the diagnosis of bacterial vaginosis. In addition to scientific considerations, choosing a method for laboratory diagnosis requires consideration of complexity including cost, and the frequency of uninterpretable specimens.

Amsel and Nugent's methods remain the most practical, viable and economical options for diagnosing bacterial vaginosis, especially in developing countries. Bacterial vaginosis is often misdiagnosed using clinical criteria alone because the components are subjective and depend on the acuity of the clinician and the availability of equipment (Nawani *et al.*, 2011).

Amsel's composite criteria include clinical diagnosis and a few simple laboratory tests.

Bacterial vaginosis can also be diagnosed by Nugent's criteria. This test is based on the evaluation of the normal flora in the Gram stained smears of the vaginal discharge (Laxmi *et al.*, 2009) (Table 2).

We conducted a study on 200 cases complaining of vaginal discharge. Of these, 68(34%) were diagnosed as BV. Similar prevalence rates were found in other studies, 41.5% by Nawani *et al.*, (2011) and 53% by Tiyyagura *et al.*, (2013).

Bacterial vaginosis was most common in the 24-29 years age group in our study. The disease occurs mainly in young women in the reproductive age group which also correlates with other studies done earlier. Changes in structure and composition of vaginal ecosystem maybe influenced by age, infections, methods of birth control by using contraceptives, frequency of sexual activities and number of sexual partners. These features are most likely seen in women of reproductive age group (Nawani *et al.*, 2011).

The most common symptoms of BV were vaginal discharge found in all the 60 cases followed by malodour, itching and dysuria. These were also the findings of studies done by Falagas *et al.*, (Falagas *et al.*, 2007). Among the 200 patients 60(30%) were diagnosed having bacterial vaginosis by Amsel Criteria, i.e.100% patients had vaginal discharge, 56(93%) had a positive whiff test, 42(70%) had clue cells and 40(67%) had pH>4.5.

68(34%) patients were diagnosed by Nugent's Scoring (Table 3). These similar results were reported by Gratco *et al.*, 1999. Amsel and Nugent's methods remain the most practical, viable and economical options for diagnosing bacterial vaginosis, especially in developing countries. Bacterial vaginosis is often misdiagnosed using clinical criteria alone

because the components are subjective and depend on the acuity of the clinician and the availability of equipment.

vaginosis can be seen to vary considerably from study to study (Bradshaw *et al.*, 2005; Chaijareenont *et al.*, 2004; Sha *et al.*, 2005).

In this study, the prevalence of bacterial vaginosis among patients with the primary complaint of abnormal vaginal discharge was 34%. Using Nugent’s method as the diagnostic criteria, the prevalence of bacterial

A study from southern India found the prevalence of bacterial vaginosis to be 20.5% (Rao *et al.*, 2004), which closely matches the findings in the current investigation.

Table.1 Nugent scoring of Gram stained smear for bacterial vaginosis

Morphology	0-3 (Normal)	4-6 (Intermediate)	7-10 (Bacterial vaginosis)
Lactobacillus – like (parallel sided, gram positive rods)	4+ to 3+	2+ to 1+	0
Mobiluncus- like (curved, gram negative rods)	0 to 1+	2+ to 3+	>4+
Gardnerella/bacteroides- like (tiny, gram variable coccobacilli and pleomorphic rods with vacuoles)	nil	nil	1+ to 4+
Clue cells	nil	nil	present

Table.2 Amsel’s criteria

S.No	Variables	No. of patients(%)
1	Vaginal discharge	60(100)
2	Clue cells	42(70)
3	Whiff test	56(93)
4	pH>4.5	40(67)

Based on Amsels Criteria, 60 patients were labelled to have BV

Table.3 Nugent’s scoring

S no	Score	No of cases	
1	0-3	72	NEGATIVE
2	4-6	60	INTERMEDIATE
3	7-10	68	POSITIVE
	Total	200	

Based on Nugents Criteria, 68 cases were labelled to have BV

It is difficult to determine the exact prevalence of bacterial vaginosis because only one third to three quarters of the patients are symptomatic (McCue, 1989). Reported

prevalence also varies in different population subtypes. Prevalence in ambulatory gynecology patients has been reported to be 15% to 19%; however, in special groups the

data varies (10% to 30% in pregnant patients, and 24% to 40% in patients carrying concurrent sexually transmitted diseases (Hill *et al.*, 1983; Bump and Buesching, 1988).

Data from most studies suggests that women of child bearing age are more prone to developing bacterial vaginosis. In our study this might be reflected in a lower age of presentation of symptoms. The average age of the bacterial vaginosis group in this study (mean age = 28.33 ± 7.90 years) was slightly lower than that of the non suffering group (mean age = 31.13 ± 11.19 years), but the difference was not found to be statistically significant. The lack of significance might be a result of the low number of patients in the study. We also tried to determine if marital status, education, and parity brought about significant differences in the prevalence of bacterial vaginosis: no statistically significant difference was found.

The present study found that mutual agreement between the two diagnostic tests was lacking ($\kappa = 0.58$), which necessitates development of a set of unified and universal diagnostic criteria to lessen the ambiguity in diagnosis.

There is a great need for an inexpensive diagnostic method that is both reliable and unifies clinical and microbiological parameters to make it more sensitive while retaining its specificity. It may be beneficial to further review Amsel criteria to assign differential weights to various parameters with evidence generated by a systematic review of related studies.

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