ORIGINAL ARTICLE

Diagnostic Accuracy of Nugent Scoring in Comparison to Amsel's Criteria for Diagnosis of Bacterial Vaginosis in Women of Reproductive age Group at a Tertiary Care Hospital

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Abstract

Introduction: Bacterial vaginosis (BV) is the most common cause of vaginal discharge in women of reproductive age and is associated with adverse obstetric and gynecologic outcomes. **Aim:** To find out the diagnostic accuracy of Nugent's scoring when amsel's criteris considered as gold standard from the patients of reproductive age group suspected for bacterial vaginosis. **Material and Methods:** A total of 100 random samples were collected from the patients of reproductive age group having complaint of vaginal discharge suspected for vaginosis attending Obstetrics and Gynecology Out Patient Department (OPD). Bacterial vaginosis was diagnosed using Amsel's criteria and Nugent's method and Gardnerella vaginalis was isolated by inoculating on Columbia agar and identified by Hippurate hydrolysis test. **Result:** The sensitivity, specificity, positive predictive value, negative predictive value of Nugent's score when compared to Amsel's criteria was found to be 69.23%, 100%, 100%, 95.6% and 96% respectively. The rate of isolation Gardnerella vaginalis was found to be 15.3%. **Conclusion:** The Amsel's criterion has high sensitivity and was found to be highly efficient require lower cost, lesser time and have advantage of being done in outpatient department thus enabling precise and fast treatment for patient. Nugent scoring system can be as good as Amsel's criteria in terms of specificity and positive predictive value, however has lower sensitivity and negative predictive value at diagnosing bacterial vaginosis. Diagnosis of bacterial vaginosis by culture was least sensitive method.

Key Words

Gardnerella Vaginalis, Hippurate Hydrolysis Test,Columbia Agar,Vaginal Discharge,Vaginal Normal Flora

Introduction

Bacterial Vaginosis, Vaginal Candidiasis, Trichomoniasis, and Syphilis are the most prevalent infections of the reproductive system. Bacteria, fungus, and parasites cause these infections, which account for 90% of all causes of abnormal vaginal discharge.[1] The most prevalent of these is Bacterial Vaginosis (BV). In women of reproductive age, bacterial vaginosis is regarded as one of the most common causes of abnormal vaginal discharge.[2] Frequent complaints among women attending obstetrics and gynaecology OPD are abnormal colour, odour and appearance of vaginal discharge, itching, and burning sensation.[1]

Gardnerella vaginalis (previously Haemophilus vaginalis or Corynebacterium vaginale) is found in small quantities

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in the typical female genital system. Bacterial Vaginosis is a condition that occurs when bacteria are outnumbered. It is characterised by a change in the usual vaginal flora, with a decrease in lactobacilli counts and an increase in Gardnerella vaginalis concentration.[3,4]

Gardnerella vaginalis, Mycoplasma hominis, Mobiluncus species, and other anaerobic bacteria such as Peptostreptococcus spp. and Prevotella spp. are among the bacteria that cause bacterial vaginosis. [5] Gardnerella vaginalis (GV)-associated bacterial vaginosis is known for having a negative impact on pregnancy, leading to poor obstetric and neonatal outcomes.[6]

The Amsel criteria require the detection of clue cells. Gram staining of the vaginal discharge and examination under oil immersion can detect Clue cells (epithelial cells covered with tiny Gram-negative/Gram-variable rods). By Amsel's criterion, the presence of clue cells in at least 20% of the oil immersion fields should be considered as positive.[7]

The Nugent criteria form a standardized method of Gram-stain interpretation which is designed to evaluate the vaginal microflora in detecting BV. Vaginal swab smears should be graded on a 10- point scale based on the presence or absence of Lactobacillus morphotypes, gram variable and gram-negative rods, and curved gram-negative rods.[8]

Therefore this study was conducted to know the diagnostic accuracy of Nugent scoring when amsel's criteria considered as gold standard along with various risk factors, isolation rate of Gardnerella vaginalis for diagnosis of bacterial vaginosis in the reproductive age group patients attending tertiary care hospital of developing country with rural area around.

Material and Methods

The study was conducted over a period of twelve months in the Obstetrics and Gynecology & Microbiology department at Maharishi Markandeshwar Institute of Medical Science and Research, Mullana, Ambala. All the laboratory investigations were performed in the Department of Microbiology. 100 women of reproductive age group attending the gynecology O.P.D. were included in the study with complaints of vaginal discharge with one or more symptoms signifying lower genital tract infection (abnormal vaginal discharge, vulvar pruritus, malodour, burning micturition and dysuria). A detailed history, consent and information regarding age, symptoms, character and quantity of discharge, odour, appearance and pruritus were taken from the patients. The institutional ethical committee approval was also obtained.

Women that were menstruating or age < 15 years and > 45 years were excluded from the study. Bacterial vaginosis was diagnosed using Amsel's criteria and Nugent's score method. Gardnerella vaginalis was isolated by Columbia agar and identified by Hippurate Hydrolysis test.

A patient was suspected to be suffering from bacterial vaginosis if at least three or more of the four criteria of Amsel's were present. The clinical criteria used were:

a)increased homogenous greyish-white vaginal discharge,

b)increased vaginal pH >4.5

c)Release of fishy odour on addition of 10% KOH to vaginal fluid (Whiff test)

d)presence of clue cells on a wet mount preparation. Clue cells are vaginal epithelial cells with an overlay of micro-organisms.

The patient was considered not to have bacterial vaginosis or to be 'normal', if less than three criteria were detected. Each sample was examined by grading based on Nugent's scoring (NS) criteria after gram staining. The Nugent's score was calculated by:

a)assessing for the presence of large gram-positive rods (Lactobacilli morphotypes, decrease in number scored as 0 to 4),

b)small gram variable rods (Gardnerella vaginalis morphotypes, increase in number scored as 0 to 4)

c)curved gram variable rods (Mobiluncus, scored as 0 to 2).

A score of 7-10 was consistent with BV.

Results

A total of 100 samples of clinically suspected cases of bacterial vaginosis were collected and were subjected to macroscopic and microscopic examination (gram Staining, wet Mount examination). The diagnosis of patients with vaginal discharge with altogether examination showed 13(13%) cases of Bacterial Vaginosis, 16(16%) cases of Candidiasis, 1(1%) Trichomoniasis, 3(3%) Bacterial Vaginosis with Candidiasis, 1(1%) Bacterial vaginosis with Trichomoniasis and 66 (66%) cases were due to other

Table 1. Distribution Based on Pregnancy and Various Risk Factors

Parameters		BV positive@ (n=13)	BV negative# (n=87)	p-value
Pregnancy:				
Pregnant		3(23.07)	18(20.6)	---
Non-Pregnant		10(76.9)	69(79.3)	---
Risk factors:				
Douching	Present	6	16	
	Absent	7	71	0.024**
IUCD	Present	7	16	
	Absent	6	71	0.005*
OCP	Present	4	32	
	Absent	9	55	0.67 (NS)
Use of antibiotics/ Steroids	Present	8	28	
	Absent	5	59	0.039**
Cloth pad during menstruation	Present	5	13	
	Absent	8	74	0.04**

*@= Exclusive bacterial vaginosis positive cases; #= Bacterial vaginosis cases of mixed infection and other infection;*Highly Significant at 1% level of significance.** Significant at 5% level of significance.*

Table 2. Association of Symptoms in Patients with and Without Bacterial Vaginosis

Symptoms		No. of patients with BV (n=13)	No. of patients without BV (n-87)	P Value
Discharge	Colour:			
	Grey	8	11	<0.001*
	White	5	76	
	Foul odour:			
	Present	6	15	0.017**
	Absent	7	72	
	Appearance-			
	Thin	11	17	<0.001*
	Thick	2	70	
Pruritus	Present	5	13	0.040**
	Absent	8	74	

**Highly Significant at 1% level of significance. ** Significant at 5% level of significance.*

vaginal infections. Only 13 cases of Bacterial vaginosis were included and other clinical cases were excluded. The diagnostic accuracy of Nugent's Score when Amsel's criteria considered as Gold standard was found to be 69.23% Sensitive and 100% Specific while Positive predictive value & Negative predictive value was 100% & 95.6% respectively.

Discussion

Bacterial vaginosis, which was earlier designated as 'non-specific vaginosis' is the commonest cause of vaginal discharge occurring in women attending gynecology clinics in India.[5] Out of total 100 samples, cases of bacterial vaginosis was 13%, candidiasis was 16% while for trichomoniasis, mixed infection of Bacterial vaginosis and candidiasis it was 1% and 3% respectively. However, the rates were found to vary in other studies conducted by Dai Q *et al.*[9] (2010) at Sichuan province, China from 51.6%, 6.5%, and 2.5% to 6.4%, 21.6%, 9.7% in study

Table 3. Frequency Distribution of Different Parameters of Amsel's Criteria in Women with Bacterial Vaginosis

Amsel's Diagnostic Criteria	No. of Patients with Bacterial Vaginosis N=13 (%) Present	P value
Homogenous Discharge	13(100)	0.023**
pH	12(92.3)	
Whiff Test	9(69.2)	
Clue Cells	13(100)	
	Combination of Amsel's Criteria	
Discharge + pH + clue cells	4(30.7)	
Discharge + pH + whiff test	0(0)	
Discharge + whiff test + clue cells	1(7.6)	
Discharge + pH + whiff test + clue cells	8(61.5)	

** *Significant at 5% level of significance.*

Table 4. Diagnosis of Bacterial Vaginosis by Nugent's Gram Stain Score

Nugent's Score	No. of patients N=100(%)
0-3 (Normal flora)	85(85)
4-6 (Intermediate flora)	6(6)
7-10 (BV Positive)	9(9)

Table 5. Rate of Gardnerella Vaginalis on Culture Media and Biochemical Test

No of patients with bacterial vaginosis	Growth of *Gardnerella vaginalis on Columbia agar* (%)	*Gardnerella vaginalis* positive by Hippurate Hydrolysis Test (%)
13	2(15.3)	2(15.3)

of Shaikh S *et al.*[10] (2018) at Solapur, India which can be due to the varying degree of prevalence rate among people of different communities which might be due to certain factors such as hygiene behavior and socio-demographic characteristics.

The rate of BV in present study was observed high in non-pregnant women 76.9% as compared to pregnant women (23.1%). The similar results were seen in studies done by Sabour S *et al.*[11] (2018) and Mulu W *et al.*[12] (2015) at referral hospital, Ethiopia where the prevalence in non-pregnant was 28% and 17.3% and in pregnant women was 16.5% and 13.3% respectively. Among all the risk factors studied IUCD usage was more significantly associated with BV and less significant association was observed with douching, antibiotic intake and use of cloth pad during menstruation. This was in concordance with studies done before where Om HS *et al.*[13] (2015) at Allahabad, India reported significant increase in risk of BV (p=0.017) among IUCD users. The study conducted by Ranjit E *et al.*[14] (2018) at Lalitpur, Nepal showed statistically significant increase in risk of BV with douching (p=0.015). Another study conducted by Bahram A *et al.*[15] (2009) at Zanjan, Iran showed statistically significant increase in risk of BV with menstrual and individual vaginal hygiene ($p<0.01$ and $p<0.001$). In present study, among all the symptoms studied colour and consistency of discharge were found to be more significantly associated with BV. In study conducted by Ranjit E *et al.*[14] (2018) at Lalitpur, Nepal showed statistically significant association with consistency and odour of discharge with BV along with amount of discharge.

In the present study, among the exclusively BV diagnosed patients ,100 % were found to have homogenous discharge and clue cells in the wet mount, 92.3% were found to have pH >4.5 and 69.2% were found to have positive whiff test. All these results were found to be statistically significant (p=0.023). Similar results were seen in study by Jabuk S *et al.*[16] (2014) at Hilla city, Iraq where homogenous discharge was found in 83.4% cases, pH >4.5 was found in 89.6% cases, whiff test was found positive in 79.1% cases, and presence of clue cell in 93.8% in BV positive cases. However, studies done by Rao DS *et al.*[17] (2016) at Hyderabad, India showed 100% homogenous discharge in BV positive cases, 69.9% cases showed clue cells, whiff test was positive in 98.26% cases and pH>4.5 was seen in 67.6 % cases. The combination of three criteria was fulfilled by 38.4% of the positive samples and all the four criteria were fulfilled by 61.5% of the positive samples.

In the present study, out of the 100 women, Nugent score of 7-10 (BV cases) showed 9% cases, Nugent score of 4-6 (Intermediate flora) showed 6% cases while Nugent score of 0-3 (Normal flora) denoted to 85% cases. Gram-positive bacilli were dominantly present in 85% of the women. The results were in concordance with study done by Muthusamy S *et al*[18] (2016) at Chennai, India. However, variable results were seen in the study done by Rao R *et al.*[17] (2016) at Hyderabad, India, where 0-3 (normal flora) grading of nugent score had 26.51% cases, 4-6 (Intermediate flora) grading of nugent score had 25.69 % cases and 7-10 grading of nugent score (BV cases) had 47.7% cases. These differences in the incidence rates can be attributed to difference in the geographical distribution, hygienic measures and sexual habits between our research area and those studies' research populations.

In Present study positivity of BV cases by Amsel's Criteria was found to be 13% while by Nugent's score positivity was 9%. In study by Muthusamy S *et al.*[18] (2016) at Chennai, India, positivity of BV cases was 35.3% by Amsel's criteria as compared to Nugent's score where the positivity was found to be 14.6%. The high and low percentage of positivity by Amsel's criteria and Nugent score can be due to low and high specific nature of two test. [17]

The diagnostic accuracy of Nugent's Score when Amsel's criteria considered as Gold standard was found to be 69.23% Sensitive and 100% Specific while Positive predictive value & Negative predictive value was 100% & 95.6% respectively. In study by Chaijareenont K *et al*.[19] (2004) at Bangkok, Thailand the sensitivity of Nugent's criteria was 65.6%, specificity was 97.3%, positive predictive value (PPV) was 80.8% and negative predictive value (NPV) was 94.2% and accuracy of 92.6%. Nugent's score might not be suitable to use as a screening test for diagnosis of BV due to its low sensitivity in the present study which can be due to the subjective interpretation error or technical error. In the study done by Tanuja C *et al*.[20] (2008) at Surat, India had high sensitivity (95%) was observed with low specificity (88%) as compared to Amsel's criteria. Amsel's criterion is a convenient and inexpensive method of diagnosing bacterial vaginosis whereas Nugent's criteria require an experienced slide reader and considerable time and skill.

In Present study, only 2(15.3%) cases showed growth of Gardnerella vaginalis on Columbia agar and were tested positive for Hippurate Hydrolysis test.

This can be correlated with study by Nagaraja P *et al.*[21] (2008) in Kuwait where the rate for isolation of Gardnerella vaginalis was 15.5%. In other studies, done by Khan S *et al.*[22] (2009) at Islamabad, Pakistan and Baruah FK *et al.*[5] (2014) at Guwahati, India the rate of isolation was 28% and 8.7% respectively.

The Prevalence of Gardnerella vaginalis reported by various workers varies from 6-94 % probably because different authors have studied different types of population and have considered different criteria for selecting the cases of bacterial vaginosis.

Conclusion

The Amsel's criterion was shown to have great sensitivity in BV diagnosis and was found to be very efficient, requiring little expenses and time and being able to be performed in the outpatient setting, allowing for precise and quick treatment. Standardization of the technique and a solid training programme for microbiologists can, however, improve the sensitivity of Nugent's score.

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Conflicts of Interest
There are no conflicts of interest.

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