Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2016; 4(6C):2027-2031 **ISSN 2347-954X (Print)**
©Scholars Academic and Scientific Publisher
(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2016.v04i06.032

Original Research Article

Diagnosis of Bacterial Vaginosis: Amsel's Criteria vs Nugent's scoring

Dr. S. Rajeshwar Rao, Dr.K. GirishaPindi, Dr.Usha Rani, Dr. G. Sasikala, Dr. Vijendra Kawle
Department of MicrobiologyOsmania General Hospital& Osmania Medical College, Hyderabad, Telangana- 500095, India

***Corresponding author**
Dr. S. Rajeshwar Rao
Email: sunkara_dr2000@yahoo.com

Abstract: Bacterial Vaginosis(BV) is the most common cause of vaginitis. If untreated, it is associated withserious complications.BV is a poly-microbial, synergistic infection, involving over-growth of multiple bacterial pathogens. Vaginal flora becomes altered leading to increased pH due to decrease in lactobacilli (which produceH_2O_2).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 February - November, 2015 at a Tertiary Care Hospital in Hyderabad. The study consisted ofwomen with complaints of vaginal discharge. BV was diagnosed based on Amsel's criteria and Nugent's scoring. For each case, the following were interpreted: pH, Whiff test (a test in whichvaginal secretions are mixed with 10% KOH resulting in a fishy odor typical of bacterial vaginosis)and the presence of the clue cells on vaginal wet smear. Gram staining was performed forNugent's method.The present study included 362 cases of abnormal vaginal discharge.Prevalence of BV was 48%.Age group 24-29 years was most affected. Amsel's criteria detected 170/362 whereas Nugent score identified 173/362 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 78.72%, 92.35%, 75.51% and 93.54%.The prevalence rate of BV in present study high. With limited resources in developing countries like ours,there is a great need for inexpensive diagnostic methods for bacterialvaginosis.Amsel'scriteria is as good as Nugent's scoring in diagnosis of BVand it is simple, easy, cost effective, fast and reliable, andcan be done in OPD which can be used for precise and fast treatment.

KeywordsBacterial vaginosis, Amsel's criteria, Nugents scoring, Clue cells, Whiff test, Vaginal Discharge

INTRODUCTION

Bacterial vaginosis (BV) is the most common cause of vaginitis in women of reproductive age group [1]. It the most common infection encountered in the Gynaecological outpatient setting. More than 90 million cases of bacterial vaginosis are reported worldwide per annum. According to the mid-term review of National AIDS control Programme III the prevalence of Bacterial vaginosis in adult population ranges from 17.8% to 63.7% in India [2].

BV is a poly-microbial synergistic infection characterized by complex changes in the normal vaginal flora attributed to reduction in the prevalence of Lactobacilli and an increase in the concentration of pathogenic organisms [1,3]. In the healthy vagina, lactobacilli, inhibit the growth of other microorganisms through certain properties such as adhesive ability, production of acids, bacteriocins, hydrogen peroxide and bio surfactants and their ability to compete for mannose and glycoprotein receptors [1].

The commonest pathogenic organisms causing vaginosis are facultative anaerobic bacteria like Gardnerallavaginalis and Mycoplasma, anaerobic Gram-negative rods such as Prevotellabivia, P. intermedia, Bacteroides spp, anaerobic Gram-positive rods like Mobiluncusmorphotypes and anaerobic Gram-positive cocci like Peptostreptococcus pp[1]. The aetiology of BV is probably multi-factorial and the factors initiating the shift are unclear. The local pH of the vagina is increased mostly due to the reduction in the number of H_2O_2producing lactobacilli making it more susceptible for growth of pathogenic organisms [4,5,6].

BV may be symptomatic or asymptomatic.Symptomatic womencommonly present

with increased vaginal discharge that is characteristicallyfoul smelling, thin, grey and homogeneous [3]. Bacterial vaginosis can lead to a variety of obstetric and gynaecological complications such as endometritis, Salpingitis, Pelvic inflammatory disease(PID) or complications of pregnancy such as premature rupture of membranes, preterm labour and spontaneous abortions[1], chorioamnionitis, postpartum endometritis, cuff cellulitis & vault infections after hysterectomy[4,5,6].

Most often, multiple criteria are used for the diagnosis of bacterial vaginosis. One of the methods of diagnosis is the Amsel's composite criteria which includes clinical diagnosisand a few simple laboratory tests. 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 [3].

In a developing country with limited resources such as India, where highly trained skilled manual labour comes at a premium, 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 know the prevalence of bacterial vaginosis but also to compare Amsel's criteria with the Nugent scoring.

MATERIALS AND METHODS

Study setting & duration:

The study was conducted from February 2015 till November 2015 at Department ofMicrobiology and Department of OBG, at Tertiary care hospital in Hyderabad.Approval of institutional ethical committee was taken for this study.

Study design:

Prospective, Cross sectional study

Study population:

Total number of patients with vaginal discharge attending the Gynaecology OP in the duration of 9 months. Exclusion criteria included vaginal bleeding, pregnancy, vaginal or cervical mass and patients on antibiotics.After obtaining written informed consent from the subjects, a detailed clinical and personal history regarding age, symptoms, character and quantity of discharge, odour, and pruritus were taken from the patients[8].

Specimen collection was done in a well-lit room. Patients were asked to lie on the examination table in lithotomy position. An unlubricated Cusco's vaginal speculum was introduced into the subjects vagina under aseptic conditions, nature of the discharge, condition of vagina and cervix were noted [3]**.** 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[3].

Vaginal pH determination:

pH of the vagina was tested using a pH paper (Qualigens Fine Chemicals, India) by dipping it in the secretions pooled in the posterior 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 xs & 40 x magnifications within 10 mins. The vaginal epithelial cells which were coated with coco-bacillary 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. The presence of any motile trichomonads, budding yeast cells and pseudohyphae were also noted.

DIAGNOSIS BY NUGENT'S CRITERIA

The vaginal discharge was smeared on clean glass slides, air dried, heat fixed and stained by Gram's staining[3]. Each bacterial morpho-type was quantitated under an oil immersion objective (l00x) by using the following scheme: 1+, <1 per field; 2+, 1 to 4 per field; 3+, 5 to 30 per field; 4+, >30 per field. Large Gram-positive rods were taken as lactobacillus morphotypes; small Gram-negative to Gram-variable rods were considered as G.vaginalis and Bacteroides spp. morphotypes; curved Gram variable rods were considered as Mobiluncus spp. morphotypes.

The scoring was done as shown:

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

Organism Morpho type	Number/oil immersion field	Score
Lactobacillus - like (parallel sided, gram positive rods)	>30	0
	5-30	1
	1-4	2
	<1	3
	0	4
Mobiluncus- like (curved, gram negative rods)	>5	2
	<1-4	1
	0	0
Gardnerella/bacteroides - like (tiny, gram variable coccobacilli and pleomorphic rods with vacuoles)	>30	4
	5-30	3
	1-4	2
	<1	1
	0	0

Total score:-0-3 Normal; 4-6 Intermediate, repeat test later; 7-10 Bacterial vaginosis.

RESULTS

A total of 362 patients in reproductive age group with complaints of vaginal discharge were examined for diagnosis of Bacterial vaginosis. Among these, 173 patients (48%) were diagnosed to be affected with BV by Nugent scoring and 170 by Amsel's criteria.

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.

Table 2: Amsel's criteria

S.no.	VARIABLE	PRESENT
1	Vaginal Discharge	173(100%)
2	Clue cells	121(69.9%)
3	Whiff test	170(98.26%)
4	pH>4.5	117 (67.6%)

Based on Amsels Criteria, 170 cases were labelled to have BV

Table 3: Nugent's scoring

S.no.	SCORE	NUMBER OF CASES
1	0-3	96(26.51%)
2	4-6	93(25.69%)
3	7-10	173(47.7%)
	TOTAL	362

Based on Nugents Criteria, 173 cases were labelled to have BV

In comparison with Nugent's criteria, the sensitivity, specificity, positive predictive value and negative predictive value of Amsel's criteria were 78.72%, 92.35%, 75.51% and 93.54%. Statistical analysis showed that both methods could be used as a means for the diagnosis of Bacterial vaginosis ($p< 0.01$).

Table 4: Comparison of diagnosis of Bacterial Vaginosis by Amsel's criteria with Nugent's scoring as gold standard.

Methods of diagnosis		Diagnosis of BV by Nugents scoring			P-value
		Nugents score > 7 n=173	Nugents score 0-6 n=189	Total n=362	
Amsel's criteria	Bacterial vaginosis	159	11	170	<0.01
	Normal	14	178	192	

DISCUSSION

Bacterial vaginosis is the most common cause of vaginal discharge among women in reproductive age with a prevalence of 16-69% depending on the population studied [9]. Culture is the gold standard method for diagnosis of most of thebacterial diseases; however, culture cannot become the gold standard for diagnosis of bacterial vaginosis as the organismswhich are involved in bacterial vaginosis cannot be isolated in thelaboratory easily and as normal women also have this flora in their vagina in small numbers [3].

Proper diagnosis of bacterial vaginosis is challenging. In addition to scientific considerations, choosing a method for laboratory diagnosis requires consideration of complexity, cost, and the frequency of un-interpretable specimens. Nevertheless, some alternative diagnostic methods have been developed, such as the polymerase chain reaction (PCR), rapid nucleic acid hybridization test, proline amino peptidase activity. More recently, several point-of-care tests based on various combinations of microbial products, presence of RNA, or more complex laboratory instrumentation such as sensor arrays, have also been introduced for the diagnosis of bacterial vaginosis. However, most of these are expensive and their sensitivities and specificities donot offer a huge advantage over the classical methods. Given these considerations, methods like Amsel and Nugent's scoring[13]remain the most practical, viable and economical options for diagnosing bacterial vaginosis, especially in developing countries [16].

Most often, multiple criteria are used for the diagnosis of bacterial vaginosis. One of the methods of diagnosis is the Amsel's composite criteria which includes clinical diagnosisand a few simple laboratory tests. 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 [3].We conducted a study on 362 cases complaining of vaginal discharge. Of these, 173(48%) were diagnosed as BV. Similar prevalence rates were found in other studies: (41.5%by Nawani *et al.*[8]and 53% by Tiyyagura *et al.* [10].

BV was most common in the 24-29years age group. The disease occurs mainly in young women in the reproductive age group which also correlates with other studies done earlier.[8] Changes in structure and composition of vaginal ecosystem maybe influenced by age, infections, methods of birth control by using contraceptives, frequency of sexual activitiesand number of sexual partners. These featuresare most likely seen in women of reproductive age group [8].

The most common symptoms of BV were vaginal discharge and malodour- found in all the 173 cases followed by itching and dysuria. These were also the findings of studies done by Falagas *et al.;*in 2007 [11]. Among the 362 patients 170(47%) werediagnosed having bacterial vaginosis by Amsel Criteria, i.e. 173(48%) patients had vaginal discharge, 170(47%) had a positive whiff test, 163(45%) had clue cells and 159(44%) had ph>4.5., and 173 (48%) were diagnosed by Nugent's Scoring. These similar resultswerereported by Gratco*et al.;*[12].

Nawani *et al.*[10] also reported than Amsel's criteria are as good as Nugent's scoring and it is simple, easy, and cost effective and fast and reliable, andcan be done in OPD which can be used for precise and fast treatment.A Study by K. Pavani [17] showed the prevalence of Bacterial vaginosis as 24.01% by Amsel's criteria, 23.03% by Nugent scoring and that Amsel's method was found to be 78.72% sensitive and 92.35% specific as compared to Nugent's method . These are comparable to our study.

A study by Udaya Laxmi *et al.*[3]which involved comparison of Amsel, Spiegel's criteria and culture with Nugent as gold standard showed that culture is the least sensitive method. Enrique *et al.*[19]showed that sensitivity and specificity of the Nugent's score compared to the Amsel's criteria were 97% and 98%, respectively. Schwebke *et al.*[20]showed that vaginal Nugent's score was more sensitive than Amsel's criteria for diagnosis of BV. But, based on Amsel's criteria, 90 precent of women with BV can be diagnosed correctly as reported in the studies by Amsel R *et al.;*[21] and Thomason JL *et al.;*[22]

As the prevalence of BV in developingcountries are high, countries with limited resources have a great need forinexpensive diagnostic methods that are reliable andunifies clinical and microbiological parameters to makeit more sensitive while retaining its specificity.

CONCLUSION

The prevalence of BV was 48%. Amsel's criteria were comparable with Nugent's criteria for the diagnosis of bacterial vaginosisand it is simple, easy, and cost effective and fast and reliable, andcan be done in OPD which can be used for precise and fast treatment. Nugent score is specific but needs microbiology expertise. 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.

REFERENCES

1. Sobel JD; Bacterial vaginosis. Annu Rev Med 2000; 51:349-56.

2. International Institute forPopulation Sciences (IIPS) and ORC Macro. 2000. National Family Health Survey (NFHS-2), Mumbai, India; 1998–99: 307-360.
3. Laxmi U,]Bhat G, Kotigadd S, Shenoy S; Comparison of the Methods of Diagnosis of Bacterial Vaginosis. Journal of Clinical and Diagnostic Research, 2011; 5:498-501.
4. Money D; The laboratory diagnosis of bacterial vaginosis. The Canadian Journal of Infectious Diseases & Medical Microbiology, 2005; 16(2): 77–79.
5. Hill, Gale B; The microbiology of bacterial vaginosis. American journal of obstetrics and gynecology 169.2 (1993): 450-454.
6. Spiegel CA; Bacterial vaginosis. ClinMicrobiol Rev 1991;4:485-502.
7. Madhivanan P, Krupp K, Chandrasekaran V, Karat C, Arun A, Cohen CR, *et al.;* Prevalence and correlates of Bacterial vaginosis among young of reproductive age in Mysore. Indian J Med Microbiology. 2008; 26: 132-7.
8. SaradaTiyyagura, MadhuriTaranikanti, Swathi Ala, Dinesh Raj Mathur; Bacterial Vaginosis in Indian Women in the Reproductive Age Group. Int J of Biomed Res, 2012; 3(8) 371–373.
9. National AIDS Control Programme III. Report on Midterm review of STI services, December 2009.
10. Manju Nawani , Sujatha R; Diagnosis And Prevalence Of Bacterial Vaginosis In A Teritiary Care Centre At Kanpur", Jemds, 2013; 2(22) 3959–3962.
11. Falagas ME, Betsi GI, Athanasiou S; Probiotics for the treatment of women with bacterial vaginosis. Clin. Microbiol. Infect. 2007; 13:657-664.
12. Grataco AE, Figueras F, Barranco M, Ros R, Andreu AA, Pedro L*et al.;* Prevalence of bacterial vaginosis and correlation of clinical to Gram stain diagnostic criteria in low risk pregnant women. European Journal of Epidemiology 1999; 15: 913-6.
13. Nugent RP, Krohn MA, Hillier SL; Reliability of diagnosis of BV is improved by standardised method of Gram stain interpretation. J ClinMicrobiol 1991; 29(2): 297-301.
14. Chaijareenont K, Sirimai K, Boriboonhirunsarn D, Kiriwat O; Accuracy of Nugent's score and each of Amsel criteria in the diagnosis of bacterial vaginosis. J Med Assoc Thai 2004; 87: 1270-1274.
15. Gratacós E, Figueras F, Barranco M, Ros R, Andreu A, AlonsoPL, *et al.;* Prevalence of bacterial vaginosis and correlation of clinical to Gram stain diagnostic criteria in low risk pregnant women. Eur J Epidemiol 1999;15: 9136.
16. Verstraelen H, Verhelst R; Bacterial vaginosis: an update on diagnosis and treatment. Expert Rev Anti Infect Ther 2009; 7: 1109-1124.
17. Pavani K, Saileela K; "Diagnosis of bacterial vaginosis in reproductive age group in tertiary health care hospital in south India: Comparison of clinical and microbiological criteria". Journal of Evolution of Medical and Dental Sciences 2013; 2(35): 6611-6615.
18. Muthusamy S, langovan SE; Comparison of Amsel's Criteria, Nugent Score and Culture for the Diagnosis of Bacterial VaginosisNational Journal of Laboratory Medicine. 2016; 5(1): 37-40.
19. Coppolillo EF, Perazzi BE, Famiglietti AM, ElisehtMG, Vay CA, Barata AD; Diagnosis of bacterialvaginosis during pregnancy. J Lower Genital Tract Dis2003; 2: 117-21.
20. Schwebke JR, Hillier SL, Sobel JD, McGregor JA, Sweet RL; Validity of the vaginal Gram stain for thediagnosis of bacterial vaginosis. ObstetGynecol 1996;88: 573-6.
21. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D,Holmes KK; Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983; 74:14-22.
22. Thomason JL, Gelbart SM, Anderson RJ, Walt AK, Osypowski PJ, Broekhuizen FF; Statistical evaluation of diagnostic criteria for bacterial vaginosis. Am J ObstetGynecol1990; 162:155-60.