ORIGINAL ARTICLE

Diagnosis of Bacterial Vaginosis in Women in Labour

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Abstract

A prospective study on 120 women in labour was conducted to determine the reliability of of different methods like clinical criteria, gram staining and vaginal culture for diagnosing bacterial vaginosis(BV). To determine the laboratory methods that best predicted the BV we calculated sensitivity, specificity, predictive value of a positive and negative test for clinical criteria, gram stain criteria of Nugent and vaginal cultures compared with gram stain criteria of Spiegel. BV was diagnosed in 23.3% of women in labour by Spiegel criteria. Sensitivity and specificity of Amsel criteria was 60.7% and 97.8% respectively. Culture of vaginal specimens yielded 58.8% predictive value of a positive test except for Mobiluncus species. Gram stain evaluation of vaginal smears is a sensitive method for diagnosing BV. Amsel clinical criteria, which are more commonly used to diagnose BV, may lead to under diagnosis of BV.

Key Words

Bacterial vaginosis, Preterm labour, Anaerobes

Introduction

Bacterial vaginosis (BV) is a common vaginal infection that affects 12-32% of pregnant women (1). It is a polymicrobial condition characterized microbiologically by marked reduction in *Lactobacilli* with simultaneous increase in other microorganisms such as *Gardnerella vaginalis*, anaerobes and *Mycoplasma hominis* (2). The condition is although common but under diagnosed. This may be due to confusion over its microbial origin (3). Most of the women with this condition are asymptomatic, and BV is diagnosed more frequently in women with established preterm labour (PTL) or delivery and with preterm rupture of membranes suggesting that it may be associated with pregnancy abnormalities (4).

The diagnosis of BV has usually been based on three or more of the following clinical signs: a vaginal pH of more than 4.5, presence of clue cells in vaginal fluid, a milky homogeneous vaginal discharge, and the presence of amine (fishy) odour after the addition of 10% KOH, to the vaginal fluid (5). The laboratory-based tests for the diagnosis of BV include gram stain examination of vaginal smears, vaginal cultures, analysis of vaginal fluid for short chain fatty acids, assay for proline aminopeptidase and sialidase test (2). Early diagnosis and treatment of BV might be useful in some of the women with PTL and can only be achieved by some accurate, reproducible, inexpensive and accurate method. In the present study, we diagnosed BV by clinical criteria, evaluation of gram stained vaginal smear by both Spiegel and Nugent criteria and vaginal cultures. Interpretation of these signs can be difficult in labour patients, because of bleeding, presence of cervical mucus, which can result in elevated vaginal pH or may mask the amine odour

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(2, 6). Thus, in the present study sensitivity, specificity, positive and negative predictive values of different laboratory methods have been compared with gram_stained smear, which has been shown to be a sensitive and reliable method for diagnosing BV (7 - 9).

Material and Methods

The study was conducted in the Microbiology and Obstetrics department of a tertiary care hospital University College of Medical Sciences New Delhi. A total of 120 consecutive women in preterm labour were enrolled in the study after an informed consent (gestational age of 23-36 completed weeks). The women were considered to be in labour if two or more painful contractions occurred every 2 minutes for at least two hours (4). Those women who had diabetes mellitus, kidney or heart disease, preeclampsia, abruptio placentae, placenta praevia, preterm rupture of membranes, intrauterine growth retardation, known congenital malformation, cervical circlage and Rh immunization were excluded from the study. Any woman, who had taken antibiotics within preceding 4 weeks were not included in the study.

At the admission vaginal speculum was inserted without lubrication and the appearance of discharge (milky, floccular, purulent, curdy, homogeneous) was noted. The vaginal pH was determined by placing the discharge on pH paper [Indikrom papers (pH 3.5 - 6) (Qualigens fine chemicals)] (5). Three specimens were collected from posterior vaginal fornix with sterile cotton tipped swabs for saline and KOH mount, Gram staining and vaginal cultures. The saline wet mount was examined microscopically for *Trichomonas* and clue cells. The 10% KOH wet mount was examined for odour (normal, foul or amine odor) and microscopically for hyphae. One swab was rolled over a glass slide for microscopic evaluation of vaginal flora and the other swab was transported in modified stuarts medium for bacterial culture on the same day (2, 4, 5).

Microscopy

In the laboratory, vaginal smears were Gram stained and evaluated for BV by Spiegel criteria and Nugent criteria (10, 11). According to Spiegel criteria BV was present if *Lactobacillus* morphotypes were fewer than 5 per oil immersion field and if there were 5 or more *Gardnerella vaginalis* morphotypes together with 5 or more other morphotypes (gram positive cocci, small gram negative rods, curved gram variable rods or fusiforms) per oil immersion field. Nugent scoring system, weighted quantitation (0,1 to 4+) of the following morphotypes to yield score of 0-10 for each large gram positive rod (*Lactobacillus* morphotypes) weighted such that their absence yielded highest score, small gram negative to gram variable rods (*Gardnerella vaginalis* and *Bacteroides* spp. morphotypes) and curved gram variable rods (*Mobiluncus* spp. morphotypes).

Culture

Vaginal swabs were cultured both anaerobically and aerobically on the surface of freshly prepared brain heart infusion agar plate supplemented with vitamin K (0.5 mg/l) and Haemin (5mg/l), blood agar and chocolate agar plates. Additional Bacteroides Bile Esculin agar, Neomycin Vancomycin Chocolate agar plates were inoculated for anaerobic culture. Agar plates were examined after 48 hrs, 96 hrs and 7days and isolates were identified using standard microbiological techniques (12, 13).

Results

BV was diagnosed by finding three of the four clinical criteria in 15.8%, by Gram stain criteria of Spiegel in 23.3%, Nugent criteria in 17.5% and by *Gardnerella vaginalis* culture in 30.8% of women. (Table 1)The efficacy of each of the laboratory methods was compared with Gram stained criteria of Spiegel (Table 2). Amsel clinical criteria diagnosed BV in 17 of the 28 (89.4%) women who were positive by Gram stain. Considering Gram stain diagnosis of BV as the standard, clinical diagnosis had a sensitivity of 60.7%, specificity of 97.8%, positive predictive value of 89.4% and negative predictive value of 89.1%. These statistical measures when applied to individual components of clinical diagnosis are shown in Table 3. When individual clinical signs were compared, demonstration of clue cells in wet mount had a higher concordance than other tests.

Nugent scoring system diagnosed 21/28 smears as BV (score 7-10) while 7 smears positive by Spiegel criteria were classified in intermediate group (score 4 - 6). Out of these

7, 3 patients were positive for BV by culture and clinical criteria as well.

Each of the BV associated microorganism as shown in table 2 was more frequently isolated in women with BV

Table 1. Prevalance of Becterial vaginosis(BV) by different labortory methods.

Methods	BV	NOBV
Clinical Criteria	**n (%)**	**n (%)**
Homogenous vaginal discharge	19 (15.8)	101(84.1)
PH>4.7	74(61.6)	26(21.6)
Amine odour	6(5)	114(95)
Clue cells	18(15)	102(85)
Three of four	19(15.80	101(84.1)
Gram stain(Spiegel)	28(23.3)	92(76.6)
Gram Stain (Nugent)	21(17.5)	99(82.5)
Culture		
G.Vagnalis	37(30.8)	83(69.1)
Bacteroides sp.	18(15)	102(84.1)
Peptostreptococcus sp.	27(22.5)	93(77.5)
Mobiluncus sp.	3(2.5)	117(97.5)
Anaerobes*	20(16.6)	100(83.3)

N=120, n= number, %= percentage.
Parenthesis indicates percentage.
*Presence of any anaerobe alone or in combination.

Table 2. Efficiency of gram stain in diagnosing BV Compared with other methods.

Methods	BV in Gram stain Present	Absent
Clinical Criteria	**n (%)**	**n (%)**
Homogenous vaginal discharge(n=19)	16(84.2)	3(15.7)
PH>4.7(n=74)	24(32.4)	50(67.6)
Amine oduor (n=6)	6(100)	-
Clue cells (n=18)	18(100)	-
Three of four (n=19)	17(89.4)	2(10.5)
Nugent		
BV (n=21)	21(100)	-
Intermediate(N=13)	7(53.8)	6(46.1)
Culture		
Gardenerella vaginalis(n=37)	18(48.6)	19(51.3)
Bacteroides sp.(n=18)	11(61.1)	7(38.8)
Peptostreptococcus sp.(n=27)	13(48.1)	14(51.9)
Mobiluncus sp. (n-=3)	3(100)	-
Anaerobes* (n=24)	20(58.8)	14(41.2)

N=120, n= number, %= percentage
Parenthesis indicates percentage.
*Presence of any anaerobe alone or in combination.

Table 3. Correlation of diagonosis of bacterial vaginosis by Gram stain.

Methods	SENS	SP	PPV	NPV
Clinical Criteria				
Homogenous vaginal discharge	57.1	96.7	84.2	89.1
PH>4.7	85.7	45.6	32.4	91.3
Amine odor	21.4	100	100	80.7
Clue cells	64.2	100	100	90.1
Three of four	60.7	97.8	89.4	89.1
Gram stain(Spiegel)	75	100	100	92.9
Culture				
B.Vagnalis	64.2	79.3	48.6	87.9
Bacteroides sp.	39.2	92.3	61.1	83.8
Peptostreptococcus sp.	46.4	84.7	48.1	83.8
Mobiluncus sp.	10.7	100	100	78.6
Anaerobes[a]	71.4	84.7	58.8	98.6

Sens: Sensitivity, sp: Specificity
ppv: Positive predictive value, npv: Negative predictive value
Data are given as percentages
Gram stain was used as standard

compared to women without it ($P<0.01$ for each of the comparisons by Fisher exact test). Lactobacilli were isolated less frequently from women with BV. All the aerobic and facultative anaerobes (*Eschereschia coli*, *Group B Streptococci*, *Enterococcus* sp., *diphtheroids*, *Micrococci*, and *coagulase negative Staphylococcus*) were isolated with equal frequency in women with and without the condition.

Discussion

The importance of recognizing and treating BV in various clinical settings is increasingly recognized. Treatment with antibiotics might be helpful in some cases of idiopathic preterm labour but at present knowledge and diagnostic methods are not sufficient in recommending antibiotic therapy in routine clinical practice (5). BV may be misdiagnosed by using conventional methods like clinical criteria, as the components are subjective and dependent on the acuity of clinician and available equipments (2, 3). In our study, BV was diagnosed in only 15.8% of women by clinical criteria in contrast to 28% by gram stain method which was similar to Krohn etal who diagnosed BV in 21% of pregnant women by clinical criteria and in 12% by Gram stained smears (6). The subjective nature inherent in the evaluation of clinical

criteria may result in significant under diagnosis of BV in some centers and in some patient groups. In labour patients these criteria are difficult to interpret because of bleeding and increased vaginal discharge. Analysis of sensitivity and specificity of individual components used in Amsels diagnosis of BV revealed that identification of clue cells by light microscopy has best sensitivity, specificity, positive and negative predictive value of all the four criteria.

Evaluation of gram stained smears by Nugent criteria had excellent specificity and predictive value of positive test but lower sensitivity of 75%suggests that some of the true positives for the syndrome of BV were classified in intermediate group. An alternative explanation can be that Spiegel criteria diagnosed some false positive patients as BV but, the finding of BV in three of the seven patients by clinical criteria and culture favors Spiegel method of diagnosing BV.In the present study, *G. vaginalis, Bacteroides, Peptostreptococcus, Mobiluncus* were significantly associated with BV but were isolated in only 30.8%, 15%, 22.5% & 2.5% of women respectively. The lower frequency of isolation of these organisms reflects the difficulty in isolating these microorganisms from routine genital cultures. None of the single microorganism associated with BV (*G. vaginalis, Bacteroides, Peptostreptococcus*) had a good sensitivity and positive predictive value except for *Mobiluncus* which had a 100% specificity and positive predictive value but a very low sensitivity. Thus, the isolation of any one microorganism does not reliably predict women with BV. However, presence of multiple organisms improves the sensitivity of diagnosis with no advantage on positive predictive value. Thus, the value of vaginal cultures for any of these microorganisms is doubtful for identifying women with BV. Our finding support the conclusion that Gram stained vaginal smears identifies women with BV better than any of the laboratory tests. Gram stain based diagnosis is reliable, reproducible, least expensive,less time consuming and widely available.We believe that Gram stained smear alone, without culture, can be used to evaluate vaginal swab specimens for BV.

References

1. Mastrobattista JM, Bishop KD, Newton RE. Wet smear compared with Gram stain diagnosis of Bacterial vaginosis in asymptomatic pregnant women. *Obstet Gynaecol* 2000; 96: 504-06.
2. Hillier SL. Diagnostic microbiology of bacterial vaginosis. *Am J Obstet Gynecol* 1993; 169: 455-59.
3. O'Dowd TC, West RR, Winterburn PJ, Hewlins MJ. Evaluation of a rapid diagnostic test for bacterial vaginosis. *Br J Obstet Gynaecol* 1996; 103 (4): 366-70.
4. Holst E, Goffeng AR, Andersch B. Bacterial vaginosis and vaginal microorgansims in idiopathic premature labour and association with pregnancy come. *J Clin Microbiol* 1994; 32: 176-86.
5. Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach D, Holmes KK. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983; 74: 14-22.
6. Krohn MA, Hillier SL, Eschenbach DA. Comparison of methods for diagnosing bacterial vaginosis among pregnant women. *J Clin Microbiol* 1989; 27: 1266-71.
7. Tam MT, Yungbluth M, Myles T. Gram stain method shows better sensitivity than clinical criteria for detection of bacterial vaginosis in surveillance of pregnant, low income women in clinical setting. *Infect Dis Obstet Gynecol* 1998; 6 (5): 204-48.
8. Schwebke JR, Hillier SL, Sobel JD, McGregor JA, Sweet RL. Validity of the vaginal gram stain for the diagnosis of bacterial vaginosis. *Obstet Gynecol.* 1996; 88: 573-36.
9. Mazulli T, Simor AE, Low DE. Reproducibility of interpretation of Gram stained vaginal smears for the diagnosis of Bacterial vaginosi. *J Clin Microbiol* 1996; 28 (7): 1506-12.
10. Spiegel CA, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct Gram stain of vaginal fluid. *J Clin Microbiol* 1983; 18: 170-77.
11. Nugent RP, Krohn MJ, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J Clin Microbiol* 1991; 29: 1730-31.
12. Sutter VL, Citron DM, Eldstein MAC, Finegold SM. Wadsworth anaerobic bacteriology manual. 4th ed. Belmont, California: Star Publishing; 1985.
13. Baron EJ, Peterson LR, Finegold SM. Processing clinical specimens for anaerobic bacteria : Isolation and identification procedures. In: Bailey and Scotts Diagnostic Microbiology 10th ed. St. Louis, CV Mosby Co. 1998; pp 697-713.