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Research Article

Comparison of laboratory investigative methods with clinical assessment criteria for the diagnosis of bacterial vaginosis

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Abstract: Bacterial vaginosis (BV) is the most common cause of vaginal discharge among women of childbearing age. .Its prevalence varies from 10 to 65% and is higher in sexually transmitted disease clinics. This study was carried out to compare the laboratory investigative methods with clinical assessment criteria for diagnosis of bacterial vaginosis. This prospective study included 200 vaginal swabs complaining of abnormal vaginal discharge. Amsels criteria, wet mount preparation and smears for Gram staining were prepared for Nugent criteria[5].Anaerobic cultures were done for isolation of anaerobic bacteria associated with BV. In results Total 128 patients showed bacterial vaginosis according to the amsel's criteria. Gram staining for Nugent scoring was examined by two different evaluators to see inter evaluator variations. The inter observer evaluation complete agreement was shown by 90.5 % i.e. in the 181 slides with agreement on slides, both the investigators scored the same group normal, intermediate or BV. While the interpretation of slides were in disagreement only in 9.5% of cases that too between intermediate and BV group. There is no disagreement in negative cases between both the observers. So interrater reproducibility was excellent between both the observers Out of 200 cases, 176 were culture positive for anaerobes other than lactobacilli. The Gram negative anaerobes out-numbered the Gram positive ones. In conclusion the prevalence of BV in developing countries are high So with limited resources countries such as India, diagnosis of BV , there is a great need for inexpensive diagnostic methods for bacterial vaginosis.
Keywords: Bacterial vaginosis, Amsel's criteria, Nugent scoring, Inter observer evaluation, anaerobes.

INTRODUCTION

Bacterial vaginosis (BV) is the most common cause of vaginal discharge among women of childbearing age. Although it is not a sexually transmitted disease, sexual activity is a risk factor for its acquisition [1]. The finding of bacterial vaginosis also in virginal females precludes its exclusive sexual transmission. Its prevalence varies from 10 to 65% and is higher in sexually transmitted disease clinics [2]. The condition is probably much more related to alterations in the vaginal ecology causing an increase in the local pH that result from a reduction in the hydrogen peroxide-producing lactobacilli. Lactobacilli help maintain the acidic pH of healthy vaginas and inhibit other anaerobic microorganisms. Normally, healthy vaginas have high concentrations of lactobacilli. In BV, the lactobacilli population is reduced greatly, while populations of various anaerobes and Gardnerella vaginalis are increased. The anaerobes implicated in BV include Mobiluncus spp., Prevotella spp., Bacteroides spp., Peptostreptococcus, Fusobacterium, and Eubacterium spp. Mycoplasma hominis and Ureaplasma urealyticum. Many serious obstetric and gynaecological sequelae and urinary tract infections are also more common in patients with BV. [2]Altered vaginal flora facilitates colonization with uropathogens leads to UTI. Most uropathogens originate in the rectal flora, colonize the peri urethral area and urethra, and ascend to the bladder [2]. If the organism is E.coli (UPEC), then recurrence of UTI occurs frequently .One in four women with UTI suffer from recurrences during pre-menopausal stage [3].

Many of the bacteria associated with BV are found in normal women, even though in smaller numbers; hence, the laboratory diagnosis of BV has been loaded with difficulty, with multiple methods described in the literature[1]. Diagnosis is best achieved by the use of Amsel's clinical criteria [4] and by assessment or scoring [5] of bacteria in a Gram-stained vaginal smear. Also anaerobic cultures plays important role in the isolation of the bacteria which can further help in appropriate treatment of the BV.

MATERIALS AND METHODS

This prospective study included 200 vaginal swabs received in the microbiology department during a time period of three years i.e. June 2011 to June 2014 from female patients of a reproductive age group who were attending the Obstetrics and Gynaecology departments of tertiary care hospital, as outpatients and inpatients complaining of abnormal vaginal discharge. Two vaginal swabs from each patient were collected from the lateral wall of vagina and transported to the laboratory. One swab in 3 ml of sterile thioglycollate broth for anaerobic culture, another swab was brought in 0.5 ml of saline for non culture methods. While taking the swab colour, consistency and odour of vaginal discharge was also noted. An Amsels criterion was also assessed [4]. The pH of vaginal discharge was measured directly by placing indicator paper for pH range of 4.0– 6.0 on the vaginal wall. An Amine test was performed. Patients not fulfilling the minimum of three out of four diagnostic criteria were considered normal. From one swab, wet mount preparation and smears for Gram staining were prepared, which were examined for the presence of clue cells, Pus cells and were read for morpho typing and scoring was done according to Nugent criteria [5]. Slide smears were examined by two independent observers to check inter observer variation. Each of the observers scored and interpreted the slides for diagnosis of bacterial vaginosis using the Nugent method. The second swab was processed by putting it into Robertson cooked meat media (RCM). Cultures were put up on brain heart infusion agar supplemented with haemin and vitamin K, L-cysteine, yeast extract with preliminary disks like metronidazole (5 µg), vancomycin (5 µg) and colistin (10 µg) sodium poly anethol sulphonate (SPS) discs for anaerobic incubation, Blood agar and Mac Conkey agar were put up for aerobic incubation. Incubation of brain heart infusion agar was done at 37°C for 48-72 h in anaerobic jar. Anaerobiasis was created by automated anaerobic system (Anoxomat). Aerobic plates were examined after 24 h. anaerobic plates were examined after 48-72 h and observed for any growth. Colony characteristics were noted and staining was done and those organisms which were sensitive to metronidazole were considered as anaerobes. However some colonies also showed resistance to metronidazole. These individual colonies were inspected and aero tolerance test was done for each of them. Those organisms which failed to grow aerobically after 24 h on blood agar are considered as anaerobes. Pure culture isolates were identified by standard biochemical methods [6, 7]. Antimicrobial susceptibility testing was done with various commonly used antimicrobial agents that are recommended by CLSI for anaerobes by the disc diffusion method [8].

RESULTS

The study was conducted on 200 high vaginal swabs ,all the four Amsel's criteria was defined in 76 patients and three out of four were defined in 52 patients , so total 128 patients showed bacterial vaginosis according to the amsel's criteria. But one or two criteria were fulfilled by rest of the patients.

According to the Nugent system Table 1 there was a complete agreement with 116 patients for BV score 7-10 while 48 samples diagnosed as intermediate with a Nugent score of 4 to 6 And 27 samples diagnosed as normal with a Nugent score of 0 to 3. Out of 200 cases, 176 were culture positive for anaerobes other than lactobacilli. In 24 samples there is only growth of lactobacilli. The Gram negative anaerobes out-numbered the Gram positive ones. The distribution of various isolated anaerobes is shown in Table 2. On wet mount as well as Gram staining we are able to see clue cells (Epithelial cells with gram variable coccobacilli on the surface). Comparison of Amsel's criteria, Nugent scoring and Anaerobes isolated in Bacterial vaginosis and non bacterial vaginosis cases shown in Table 3. All the patients having nugent scoring 7-10 have three or four amsel's criteria. But with amsel's criteria we were able to diagnose more 12 patients.

Table-1: Nugent scoring system

	Organism Morpho type Per High Power Field		
Score	Lactobacillus (Parallel-sided, Gram positive rods)	Gardnerella/Bacteroides (tiny, Gram variable coccobacilli and rounded, pleomorphic, Gram negative rods with vacuoles)	Mobiluncus (curved, Gram negative rods)
0	>30	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	
4	0	>30	

Nugent scoring system
Bacterial vaginosis- 7-10
Intermediate- 4-6
Normal - 0-3

Table-2: Anaerobes isolated from samples

Anaerobes	Total	BV	NBV
Peptostreptococcus	52	38	14
Peptococcus	20	18	2
Bacteroides	70	59	11
Mobiluncus	1	1	0
Prevotella	8	8	0
Velloneilla	2	2	0
Porphyromonas	3	3	0
Eubacterium	20	20	0
Total	176	149	27

Table-3: Comparison of Amsel's criteria, Nugent scoring and culture for Bacterial vaginosis and Non bacterial vaginosis cases

	Amsel's criteria	Nugent scoring	Culture
Bacterial vaginosis	128(64%)	116 (58%)	149 (74.5)
Non bacterial vaginosis	72	27	27 (13.5%)

DISCUSSION

Bacterial vaginosis (BV) is the most common cause of vaginal discharge among women of childbearing age. Normally, healthy vaginas have high concentrations of lactobacilli. In BV, the lactobacilli population is reduced greatly, while populations of various anaerobes and Gardnerella vaginalis are increased. The anaerobes implicated in BV include Mobiluncus spp., Prevotella spp., Bacteroides spp., Peptostreptococcus, Fusobacterium, and Eubacterium spp. Mycoplasma hominis and Ureaplasma urealyticum have also been implicated [2].

In our study out of 200 samples, 128 (64%) samples defined amsel's criteria [2] All the four Amsel's criteria was defined in 76 patients and three out of four were defined in 52 patients. Clue cells have the highest sensitivity and specificity. Sensitivity of pH and whiff test is 51.18%, 65.63% and specificity 100% respectively. Vaginal discharge has lowest sensitivity and specificity. Study done by Hemlata *et al.;* [9] and jyothi *et al.;* [10] showed the pH and whiff test when combined showed better sensitivity and specificity and vaginal discharge had the lowest specificity.

Out of 200 samples subjected to nugent scoring using Gram stain examination, the inter observer evaluation complete agreement was shown by 90.5 % i.e. in the 181 slides with agreement on slides, all investigators scored the same group normal, intermediate or BV. While the interpretation of slides were in disagreement only in 9.5% of cases that too between intermediate and BV group. So using Nugent score for the intermediate group is more difficult in our study that was supported by another study done by Chawla *et al.;* [11]. There is no disagreement in negative cases between both the observers. So interrater reproducibility was excellent between both the observers. Study done by Mohanty *et al.;* [12] also shown the excellent interrater reproducibility of Nugent scoring system for the diagnosis of BV.

176 anaerobes were cultured. Gram negative anaerobes outnumbered Gram positive anaerobes. Overall Bacteroides species and peptostreptococcus species predominate. A study done by Aggarwal *et al.;* [13] showed the predominance of the same bacteria in BV cases. In study done by Krohn *et al.;* [14] bacteroides outnumbers Gram positive cocci. In the study done by Sumati *et al.;* showed the comparable results as in our study [15].

Because the prevalence of BV in developing countries are high So with limited resources countries such as India, diagnosis of BV , there is a great need for inexpensive diagnostic methods that are reliable and unifies clinical and microbiological parameters to make it more sensitive while retaining its specificity. The diagnosis usually made on clinical grounds i.e, Amsel's composite clinical criteria alone which may be misleading because of low sensitivity of the test.

Nugent scoring system appears to be reliable, convenient and cost effective method for laboratory evaluation of cases of bacterial vaginosis when combined with amsel's clinical criteria, because of the various factors that might lead to discrepant results that too in intermediate cases in Nugent scoring system. Also anaerobes are important pathogens in the causation of BV along with other aerobic bacteria. So anaerobic cultures plays important role in the isolation of the bacteria which can further help in appropriate treatment of the BV. So the microscopic methods such as Nugent scoring system and culture methods for the diagnosis and appropriate treatment of bacterial vaginosis could be used to complement or confirms clinical evaluation of the patient with abnormal vaginal discharge.

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