Original Article

Comparison of Methods for Diagnosing Bacterial Vaginosis

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ABSTRACT

Objective: To determine the laboratory method that best predicts Bacterial Vaginosis.

Study Design: Descriptive Observational study

Place and Duration of Study: This study was conducted in the Department of Microbiology, Sindh Medical College (DUHS) and Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from Jan. 2005 to Feb. 2007.

Materials and Methods: A total of 150 randomly selected women were included in this study from OPD of various tertiary care hospitals and & family planning clinics of the city. In this study we compared and calculated the sensitivity, specificity and predictive value of positive and negative tests for wet mount, Gram stained vaginal smears and Gardnerella vaginalis (G.vaginalis) cultures with clinical sign Amsel's criteria (Gold standard), for the diagnosis of bacterial vaginosis(B.V).

Results: We diagnosed 54 (36%) cases of bacterial vaginosis by Gram's staining method, 61 (41.7%) cases by Wet Mount method, and 42 (28.0%) cases by Amsel's criteria and 47 (31.3%) cases by culturing.

Conclusions: Amsel's criteria were comparable with other laboratory tests for diagnosis of BV. Culture was laborious, expensive and least sensitive method.

Key Words: Bacterial vaginosis, Amsel's clinical criteria, Gram staining

INTRODUCTION

Bacterial vaginosis (BV) was initially recognized as a vaginal syndrome by Gardner and Duke[1]. They associated bacterial vaginosis with the isolation of Haemophilus vaginalis, later named it Corynebacterium vaginale and currently named Gardnerella vaginalis[2] . However, the microbiology of bacterial vaginosis is complex and involves organisms other than G.vaginalis. Large quantities of not only G.vaginalis but also anaerobic bacteria[3,4,5] and Mycoplasma hominis[3,4,6] can be recovered from women with bacterial vaginosis.

BV is the most common cause of malodourous vaginal discharge in females of child bearing age.[6,9,11].BV is characterized by alterations in vaginal flora. Normally, Lactobacilli constitute 95% of bacteria in the vagina, but in BV, Lactobacilli are absent or severely reduced and the concentration of other bacteria is increased by 10^2-10^4. The patho physiology of this syndrome is better understood, but little progress has occurred in identifying the casual factors.[8,12] Now BV is increasingly recognized as directly related to a number of serious obstetric and gynecological complications.[5,7,10]

G.vaginalis can be isolated from the vagina of 20% to 40% of women without bacterial vaginosis[1,4,6]. Symptoms of bacterial vaginosis are nonspecific, and has been associated with severe sequelae[13,14] and diagnosis should rely on confirmatory tests[15,16,17]

MATERIALS AND METHODS

A total of 150 randomly selected women were included in this study. They were grouped as:50 non-pregnant females complaining of discharge. 50 pregnant females, complaining of discharge.The third group included 50 Pregnant / Non-pregnant females not complaining of discharge (controls).The control groupwho had no sign and symptoms of vaginal discharge ,was examined and studied in an identical manner. They were matched for age and socioeconomic status. All females with history of previous abortion, preterm delivery or premature rupture of membrane and infertility were included. Females attending family planning clinic were also included. The exclusion criteria were females taking antibiotic, using vaginal douches, tablets or suppositories within the preceding 14 days. Women who had sexual intercourse within 24 hours were also excluded[18,19]

RESULTS

The results for the diagnosis of bacterial vaginosis by Amsel's criteria, culture, Wet Mount and Spiegel's criteria (Table 1). Statistical analysis showed that all the 4 methods could be used as a means for the diagnosis of bacterial vaginosis ($p<0.01$).

Table I: Shows different methods employed for the diagnosis of BV. In both the groups the wet mount for clue cells was more diagnostic 61(41.7).In group not complaining of vaginal discharge it was19 (38%) and in

group complainingit was42(43%). The second method was Gram's method which was 37(37%) in females complaining of discharge and17 (34%) in females not complaining of discharge. So over all it was present in 54(36%). The culture was least diagnostic and was only 31.3% diagnostic.

Table No.1: Methods used for Diagnosis of Bacterial Vaginosis (n=150)

Patients	Amsel's Criteria (Gold standard)	Wet Mount	Gram's Staining	Culture (HBT)
Not complaining vaginal discharged (n=50)	08 (16.0%)	19 (38.0%)	17 (34.0%)	16 (32.0%)
Complaining vaginal Discharge (n=100)	34 (34.0%)	42 (43.0%)	37 (37.0%)	31 (31.0%)
Total (n=150)	42 (28.0%)	61 (41.7%)	54 (36.0%)	47 (31.3%)

Table 2: Shows the comparison of the sensitivity, specificity, positive and negative predictive value of wet mount, gram staining and culture as compare to Amsel's criteria. According to this the positive predictive value, negative predictive value and the sensitivity and specificity of Wet Mount were 62.3%, 95.5%, 90.5% and 78.7% respectively. Those of Gram's staining were 72.2%, 96.9%, 92.9% and 86.1% respectively. The culture was 64.3% sensitive and 81.5% specific, the positive predictive value was 57.4% and the negative predictive value was 85.4%.

Table No. 2: Sensitivity, Specificity, Positive and Negative Predictive value of wet mount, gram staining and Cultures as compare to amsel's criteria

	Wet mount	Gram's staining	Culture
Sensitivity	90.5%	92.9%	64.3%
Specificity	78.7%	86.1%	81.5%
Positive Predictive Value	62.3%	72.2%	57.4%
Negative Predictive Value	95.5%	96.9%	85.4%

Table 3: Shows if the four diagnostic methods were compared for the reliability, time consumption and approximate cost per test and labor to perform these tests. The Amsel's criteria was easy to perform and cheap method as compared to culture method which is time consuming, costly and quite labor intensive.

Table No.3: Reliability, Time Consumption and Approximate Cost of the Test Method for Detection of BV

Test Method	Reliability		Time Consumption	Cost Per Test	Labor
	Sensitivity (%)	Specificity (%)			
Amsel's	Standard or Reference Method		3 Min	Cheap	Very easy to perform
Wet Mount	90.5	78.7	5 Min	Cheap	Easy to perform
Gram's Staining	92.9	86.1	10-15 Min	Cheap	Easy but requires experience
Culture	64.3	81.5	24-72 Hrs	Costly	Laborious

A thin, homogenous, foul smelling discharge that is adherent to the vaginal walls is characteristic of B.V. The discharge should not be confused with cervical mucus, which is characteristically clear, indicating the absence of an inflammatory response A milk like consistency that is distinctly nonfloccular, nongranular, nonstingy and not clumped is most characteristic. The discharge is clear to grey in color but has occasionally been reported as green, yellow or even white[1,4,18] The volume of discharge varied from scanty, moderate to profuse. These criteria were used to define a normal or abnormal discharge (bacterial vaginosis) in all subsequent results and analysis .[18]

A detailed clinical history of each woman was taken and their two high vaginal swabs were collected. One swab was suspended in a sterile tube containing 0.5 ml of sterile physiological saline and second swab was suspended in Stratus transport medium to be used for culture. The vaginal swabs were used for gram staining, for the determination of the pH of the vagina and for the Whiff test. Diagnosis of bacterial vaginosis was done by Amsel's criteria, Wet Mount Gram staining and by culture. The parameters that are necessary to decide the efficacy of the diagnostic tests, namely positive predictive value, negative predictive value and sensitivity and specificity were calculated in comparison with Amsel's criteria by considering it as the gold standard. Statistical analysis was done by using the Chi Square test .In all statistical analysis,only P values<0.05 were considered to be significant.

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 gram stained vaginal discharge smears and a positive whiff test. According to Amsel, if 3 of the 4 criteria are positive, the patient has bacterial vaginosis [4,20]

Vaginal pH determination:
Vaginal secretion or discharge was collected from the lateral vaginal walls with a cotton swab and this was then transferred onto a strip of pH paper . This was compared with a standardized colorimetric reference chart to estimate the actual pH [21] .

Whiff test:
A drop of vaginal discharge was mixed with a drop of 10% potassium hydroxide which was taken on a slide. A fishy smell indicated a positive test [22]

Processing of sample:
The time period between collection of sample and inoculation was restricted to1 hour.

Wet Mount Examination:
One drop of sterilized saline suspension was applied on a glass slide and covered with slip. It was examine microscopically for clue cells (vaginal epithelial cells with characteristic stippled or granulated appearance) that is the vaginal epithelial cells with indistinct cell border obscured by the large number of coccobacilli . [19,23]

Lactobacilli were recorded as the predominant flora on wet mount if long morphologic types were judged to be the predominant form.

Clue cells:
The vaginal discharge was smeared on clean glass slides, air dried, heat fixed and stained by Gram's method. The vaginal epithelial cells which were completely covered by the gram variable coco bacilli so that their edges which normally have a sharply defined cell border became indistinct or stippled, were considered as the clue cells [24].

Diagnosis by culture:
The vaginal swabs were inoculated on selective differential Human Blood Bilayer agar medium with Tween 80 (HBT) culture media and incubated at 37˚C for 24 to 48 hrs in a candle jar to provide 5-10% co $_2$.(totten et al 1982) * Aerobes, facultative anaerobes and obligate anaerobes were identified by their colony morphologies, gram staining and standard biochemical reactions .[25,26] . Those women of whom the culture showed predominant growth of G.vaginalis or an anaerobe or both were considered as positive for bacterial vaginosis by culture

Diagnosis by Spiegel's criteria:
When the gram staining showed predominance (3 to 4+) of the lactobacillus morph type with or without the Gardnerella morph type, it was interpreted as normal. When the gram staining showed a mixed flora consisting of gram-positive, gram negative, or gram-variable bacteria and the lactobacillus morphotype was decreased or absent (0 to 2+), the gram staining was interpreted as consistent with bacterial vaginosis.[4, 21]

DISCUSSION

The goal of this study was to evaluate and correlate several clinical and microbiologic criteria that have been used for the diagnosis of BV. We were particularly interested in the diagnostic values of simple observations and procedure that could be carried out in the physician's office, and in the correlation between such office procedures and less costly and more readily available microbiologic test for BV.

In prior reports, individual laboratory methods of diagnosing bacterial vaginosis have been compared with clinical signs[27,18,28,29] However, multiple laboratory methods have not been compared with a single cohort of women. In the present study, we determined the vaginal flora of patients with bacterial vaginosis diagnosed by clinical signs, wet mount, gram stained vaginal smears and culture to document that each diagnostic method was associated with similar vaginal flora.

Results of this study agree with that of Amsel that majority of women who participated in the study remained free of any definite symptoms. [18,7]

In this study, each of Amsel's clinical criteria (homogenous discharge, positive whiff test, vaginal pH>4.5 and clue cells) were strongly correlated with wet mount, gram's staining and culture findings.

Donders [29] conducted a study to assess vaginal flora on wet mount and gram stained specimens in all cases. They found that wet mount is quick to perform but Gram stain is performed more in routine. There was easier recognition of lacto bacillary morph types on a wet mount than on gram stains which results in the loss of lactobacilli by the process of fixation or gram staining and recommended wet mount is cheaper and easier to perform for microscopy of vaginal smears rather than Gram staining. In this study it was also seen that wet mount was (41.7%) and gram stain(36%) positive in total cases.

In study by [30,31] it was highlighted that Gram staining is gaining acceptance as diagnostic test of choice. It is simple for the physician who only has to smear a glass slide and allow it to air dry.

This study confirmed the common and established finding that gram method of staining is simple, inexpensive, sensitive, specific and reproducible way to diagnose[32]

Our study reinforces the finding that vaginal cultures have the positive predictive value and is less than 60%.So cultures are not recommended[33,19,] Vaginal cultures for G. vaginlis is not often the primary

laboratory test. The usefulness of these cultures is doubtful. In our study G.vaginalis could be recovered from 31.3% of women. So the incidental finding of G.vaginalis from a routine cultures should not be used unless clinical signs and/ or Gram staining shows its presence[33]

In this study the presence of other organisms was not noted,as this study was oriented towards detection of G.vaginalis, and should not be interpreted as a study of the complete normal flora.This study also agrees with previous reports of [34,35] that G.vaginalis can be found in vaginal secretions from some asymptomatic women.

This study agrees with[35] that isolation and identification in routine laboratory is both time consuming and difficult. So diagnosis most usually be made on the basis of Amsel's criteria and on the characteristic microscopic appearance of wet mout and Gram stained smears of the discharge [35,10]

CONCLUSION

The importance of an accurate, reproducible, and inexpensive laboratory method to diagnose bacterial vaginosis has increased with the recent association.Use of Amsel's clinical criteria and Gram staining especially in primary care unit and laboratories is recommended.

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