Original Article

Comparison of the Methods of Diagnosis of Bacterial Vaginosis

Microbiology Section

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ABSTRACT

Background and Objective: Although Nugent's criterion is considered as the gold standard, routinely a combination of various methods is used for the diagnosis of bacterial vaginosis. In the present study we compared culture, Spiegel's criteria and Amsel's criteria with Nugent's method for the diagnosis of bacterial vaginosis.

Materials and Methods: Five hundred and twenty seven women who attended the Government Maternity Hospital and a tertiary care centre in south India for antenatal care or forany other complaint formed the study population. Diagnosis of bacterial vaginosis was done by culture, Amsel's, Nugent's and Spiegel's criteria. The positive predictive value, the negative predictive value and the sensitivity and specificity of these methods,in comparison with Nugent's criteria, by considering it as the gold standard, were calculated. Statistical analysis was done by using the Chi Square test or the Fisher's exact test as was appropriate.

Results: In comparison with Nugent's criteria, the positive predictive value, negative predictive value and the sensitivity and specificity of Amsel's criteria were 80.4%, 94.8%, 78% and 95.6% and that of Spiegel's criteria were 77.5%, 100%, 100% and 93.2%. The culture was 51% sensitive and 88.7% specific, the positive predictive value was 85.5% and the negative predictive value was 58%. We diagnosed 100 (19%) casesof bacterial vaginosisby Nugent's method,129 (24%) casesby Spiegel's method,97 (18%) cases by Amsel's criteria and 88 (16.7%) cases by culturing.

Conclusion: Amsel's and Spiegel's criteria were comparable with Nugent's criteria for the diagnosis of bacterial vaginosis. Diagnosis of bacterial vaginosis by culture was least sensitive method.

Key Words: Amsel's Criteria, Bacterial Vaginosis, Methods of Diagnosis, Nugent's Criteria, Spiegel's Criteria

KEY MESSAGE

- Amsel's and Spiegel's criteria were comparable with Nugent's criteria for the diagnosis of bacterial vaginosis.
- Diagnosis of bacterial vaginosis by culture was least sensitive method.

INTRODUCTION

Bacterial vaginosis is a common clinical condition in women of reproductive age[1]. It represents a unique and complex change in the flora of the vagina, which is characterized by a reduction in the prevalence and the numbers of lactobacilli and an increase in the concentration of *Gardnerella vaginalis* and resident anaerobic bacteria [1]. Most of the women are asymptomatic, but some women with bacterial vaginosis have a foul smelling, thin, homogeneous, frothy, vaginal discharge [1], [2]. In addition to a nuisance infection, bacterial vaginosis can lead to a variety of obstetric and gynaecological complications such as preterm birth and pelvic inflammatory disease (PID) [1], [2]. As it is just an overgrowth of the normal flora of the vagina without inflammation, there is no single best method for the diagnosis of bacterial vaginosis [1], [2]. Most often, multiple criteria are used forthe diagnosis of bacterial vaginosis. One of the methods of diagnosis is the Amsel's composite criteria which includes clinical diagnosis and a few simple laboratory tests [3].

Bacterial vaginosis can also be diagnosed by Spiegel's and Nugent's criteria [4]. Both these criteria are based on the evaluation of the normal flora in the gram stained smears of the vaginal discharge. In the present study we have compared culture, Amsel's criteria and Spiegel's criteria with Nugent's criteria considering it as gold standard for diagnosis of bacterial vaginosis [5],[6].

MATERIAL AND METHODS

Five hundred and twenty seven women who attended two hospitals in south India for antenatal care or for the insertion or the removal of intrauterine contraceptive devices, with complaints of discharge, abdominal pain or any other complaint, formed the study population. This study had the approval of the institutional ethics committee.

Married women between the ages of 21-35 years and women with or without complaints of vaginal discharge were included. Women who were menstruating at the time of the specimen collection and women who were on medication for any bacterial, fungal, parasitic or viral infections for up to one month prior to the specimen collection, were excluded. A detailed clinical history of each woman was taken and their vaginal swabs were collected. The vaginal swabs were used for gram staining, for the determination

of the pH of the vagina and for the whiff test and culture.Diagnosis of bacterial vaginosis was done by Nugent's criteria, Amsel's criteria, Spiegel's criteria 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 Nugent's criteria by considering it as the gold standard. Statistical analysis was done by using the Chi Square test or the Fisher's exact test as was appropriate.

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 [3].

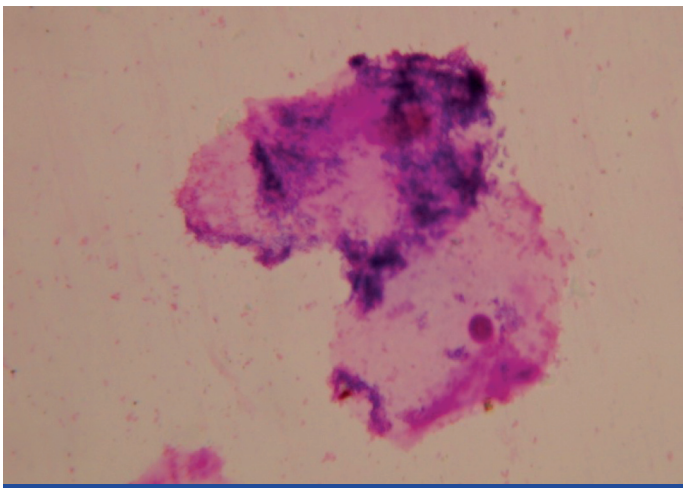
Vaginal pH determination: Vaginal secretions or discharge was collected from the lateral vaginal walls with a cotton swab and this was then transferred onto a strip of pH paper (Qualigens Fine Chemicals, India). This was compared with a standardized colourimetric reference chart to estimate the actual pH [4,7].

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 [8].

Clue cells: The vaginal discharge was smeared on clean glass slides, air dried, heat fixed and stained by Gram's method by using an acetone alcohol (1:1) mixture as a decolouriser and dilute carbol fuchsin as the counter stain. The vaginal epithelial cells which were completely covered by the gram variable coccobacilli so that their edges which normally have a sharply defined cell border became indistinct or stippled, were considered as the clue cells [9] [Table/Fig-1].

DIAGNOSIS BY CULTURE

The vaginal swabs were inoculated on appropriate culture media and incubated at 37°C for 24 to 48 hrs. For the isolation of aerobes and facultative anaerobes, Columbia blood agarand Mac Conkey's agar were used [10]. For the isolation of *G.vaginalis*, Columbia human blood bilayer agar with Tween 80 and a *G.vaginalis* selective supplement were used [11]. These plates were incubated in a candle jar with a piece of wet, sterile cotton placed in it, to provide a humid environment. For anaerobes, Columbia laked human blood agar with a neomycin supplement was used [12]. These plates



[Table/Fig-1]: Photograph of gram stained smear of vaginal discharge showing clue cells

*Score	lactobacillus morphotypes	Gvaginalis and Bacteroidesspp. morphotypes	Curved gram variable rods
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

[Table/Fig-2]: Nugent's method of diagnosis of bacterial vaginosis
* For each smear whatever the organism and their numbers seen scores were given. These scores were added up to yield a final score of 0 to 7 or more. The criterion for bacterial vaginosis was a score of 7 or higher; a score of 4 to 6 was considered intermediate, and a score of 0 to 3 was considered normal

were incubated in an Anaero Hi Gas Pack TM anaerobic jar (Hi Media Laboratories, Pvt. Ltd., Mumbai, India). Aerobes, facultative anaerobes and obligate anaerobes were identified by their colony morphologies, gram staining and standard biochemical reactions [10], [12]. All media, reagents and discs were obtained from Hi Media Laboratories, Pvt. Ltd., Mumbai. 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 [2].

DIAGNOSIS BY NUGENT'S CRITERIA

Each bacterial morphotype was quantitated under an oil immersion objective (l000 x) by using the following scheme: 1+, <1 per field; 2+, 1 to 5 per field; 3+, 6 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 in [Table/ Fig-2]. These scores were added up to yield a final score of 0 to 7 or more. The criterion for bacterial vaginosis was a score of 7 or higher; a score of 4 to 6 was considered as intermediate, and a score of 0 to 3 was considered as normal [3],[4],[5].

DIAGNOSIS BY SPIEGEL'S CRITERIA

When the gram staining showed predominance (3 to 4+) of the lactobacillus morphotype with or without the *Gardnerella* morphotype, 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].

RESULTS

The results of the diagnosis of bacterial vaginosis done by Amsel's criteria, culture, Nugent's criteria and Spiegel's criteria are shown in [Table/Fig-3]. We diagnosed 100 (19%) cases of bacterial vaginosis by Nugent's method,129 (24%) cases by Spiegel's method, 97 (18%) cases by Amsel's criteria and 88 (16.7%) cases by culture. In comparison with Nugent's criteria, the positive predictive value, negative predictive value and the sensitivity and specificity of Amsel's criteria were 80.4%, 94.8%, 78% and 95.6% and that of Spiegel's criteria were 77.5%, 100%, 100% and 93.2%. The culture was 51% sensitive and 88.7% specific, the positive predictive value was 85.5% and the negative predictive value was 58%. Statistical analysis showed that all the 4 methods could be used as a means for the diagnosis of bacterial vaginosis ($p< 0.01$)

Methods of diagnosis		Diagnosis of bacterial vaginosis by Nugent's criteria (Results)			P-value
		Nugent's score > 7 n = 100	Nugent's score (0 – 6) n = 427	Total n = 527	
Amsel's criteria	Bacterial vaginosis	78	19	97	< 0.01
	Normal	22	408	430	
Spiegel's criteria	Bacterial vaginosis	100	29	129	< 0.01
	Normal	0	398	398	
Culture	Bacterial vaginosis	51	37	88	< 0.01
	Normal	49	290	339	

[Table/Fig-3]: Comparison of diagnosis of bacterial vaginosis by culture, Amsel's and Spiegel's criteria with the gold standard Nugent's criteria

DISCUSSION

We conducted a study on 100 cases of bacterial vaginosis which were diagnosed by the gold standard method of Nugent's criteria [5],[6]. It classifies gram stained vaginal smears into normal, intermediate and bacterial vaginosis based on the gram stain scoring system. The standardized score had an improved inter center reliability as compared to Spiegel's criteria which divided thegram stained vaginal smears into only 2 categories,namely, normal or bacterial vaginosis [4],[5],[6]. In a previous study where women with intermediate flora were followed up for 3 months, some of them developed bacterial vaginosis, some continued to have an intermediate vaginal flora and some reverted to the normal flora patterns[13]. So, it is evident that women with intermediate flora must be considered separately. Hence, Spiegel's criteria which divides women into only 2 categories, namely, bacterial vaginosis and normal flora, is not as popular as Nugent's method. There are many studies which have tried to formulate better gram stain scoring systems, but these are not as popular as Nugent's method of the diagnosis of bacterial vaginosis[14],[15].

Previous studies have shown that the diagnosis of bacterial vaginosis by Amsel's criteria wasless sensitive than the gram stain interpretation[3],[16]. This low sensitivity may be because many cases of bacterial vaginosis are asymptomatic. In the present study, Amsel's method was found to be 78% sensitive and 95.6% specific as compared to Nugent's method. The diagnosis by Amsel's criteria requires a minimum of 3 to 5 vaginal swabs from each patient[3],[16]. It has been observed that routinely, only a single swab was sent to the laboratory to rule out bacterial vaginosis in the hospitals where the study was carried out. This might be the reason why the diagnosis of bacterial vaginosis by Amsel's criteria was unpopular at these places. But Amsel's method is very popular as a means of diagnosis of bacterial vaginosis in every research paper on bacterial vaginosis [3],[4],[6].

Culture is the gold standard method for diagnosis of most of the bacterial diseases; however, culture cannot become the gold standard for diagnosis of bacterial vaginosis as the organisms which are involved in bacterial vaginosis cannot be isolated in the laboratory easily and as normal women also have this flora in their vagina in small numbers.

The rate of bacterial vaginosis, when diagnosed by Nugent's scoring system, was 19%. Indian studies which were conducted on the general population, have shown a similar prevalence[2],[17]. Hospital based studies tend to over report the cases of bacterial vaginosis, as they invariably collect vaginal swabs from women with vaginal discharges. Even if this wasa hospital based study, it simulated the general population due to the large sample size. Our study population consisted of women who attended these hospitals for the routine antenatal check up or some other routine problems, irrespective of whether they had abnormal discharges or not. Most of the women were asymptomatic or had not noticed the abnormal discharge or foul smell. This might be the reason why under routine circumstances, bacterial vaginosis goes undiagnosed[18],[19].

CONCLUSION

Amsel's and Spiegel's criteria were comparable with Nugent's criteria for the diagnosis of bacterial vaginosis. Diagnosis of bacterial vaginosis by culture was least sensitive method.

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