

Interobserver Agreement in the Interpretation of Nugent Scoring Method for Diagnosis of Bacterial Vaginosis in a Tertiary Care Hospital in South India

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ABSTRACT

Background : Bacterial vaginosis is one of the most common causes of abnormal vaginal discharge in women of reproductive age. Diagnosis is confirmed clinically using Amsel's criteria which vary from patient to patient. Thus, grading the Gram-stained vaginal smears, using a criteria proposed by Nugent et al is used for diagnosis of BV.

Aim and Objectives : To examine interobserver agreement on interpretation of Nugent scoring method in the diagnosis of Bacterial vaginosis in a tertiary care hospital in south India.

Materials and methods: This prospective study was conducted in a tertiary care hospital in South India from January to June 2013. After getting informed consent and ethical committee approval, high vaginal swabs collected from 220 married, sexually active symptomatic women between 18-49 yrs were subjected to Gram stain and Nugent's scoring done by 3 independent qualified microbiologists. The results were graded as 0-3 (normal), 4-6 (intermediate) and 7-10 (bacterial vaginosis) independently by each observer and compared by ANOVA statistical analysis.

Results: Complete agreement was obtained for 168 slides by all three examiners. The prevalence of Bacterial vaginosis in our population was 14.5%. The inter-observer reproducibility of Nugent score was excellent in this study (76%). Since the interpretation of Gram-stained smears correlate with the clinical diagnosis and high level of interobserver agreement has been demonstrated and statistically significant, we believe the Gram-stained smear alone, without culture, can be used to evaluate vaginal swabs for bacterial vaginosis

Conclusion: Nugent scoring system appears to be a reliable and convenient method for laboratory evaluation of bacterial vaginosis.

Key words: Bacterial vaginosis, Nugent's scoring, Amsel's criteria, interobserver agreement.

INTRODUCTION:

Bacterial vaginosis is reported to be one of the most common causes of abnormal vaginal discharge or vaginal symptoms in women of reproductive age¹. This is characterized by symptoms of vaginal malodour and a slight to moderate increase of white discharge which appears homogenous. Bacterial vaginosis (BV),

characterized by a change in vaginal microbial flora where *Lactobacillus* is replaced by a mixed anaerobic flora and *Gardnerella vaginalis*, is considered to be the most common form of vaginal infection in the reproductive age^{2,3}. Complications of untreated BV include adverse pregnancy outcomes, upper genital tract infections, urinary tract infection and cervical intraepithelial neoplasia^{4,5}. Diagnosis is confirmed clinically using

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Amsel's criteria⁶, but may not be adequate, as approximately 50 per cent of BV patients may be asymptomatic. Diagnosis of Bacterial vaginosis by Amsel's criteria is by the presence of any three of the following four criteria - characteristic thin, homogenous vaginal discharge, vaginal pH greater than 4.5, release of a fishy amine odour on addition of 10% KOH (whiff test), and demonstration of clue cells in more than 20% of the total cell population. The Amsel's criteria are dependent on clinical signs which cannot be quantified and standardized and are neither sensitive nor specific and varies from patient to patient. Thus, grading the Gram-stained vaginal smears, using a Nugent's criteria proposed by Nugent et al⁷ has been used for diagnosis of BV. In 1991, Nugent et al. suggested a modification of Spiegel's method of scoring Gram-stained vaginal smears for the diagnosis of bacterial vaginosis. The score, calculated by assessing the presence of *Lactobacillus* morphotypes, small Gram-negative / Gram-variable rods and curved Gram-variable rods can range from 0 to 10 with a score of 7 to 10 being consistent with bacterial vaginosis. Nugent's score has been favoured because of its superior reproducibility and sensitivity. Nevertheless, evaluation of smears is also subjective and requires an experienced slide reader. In a developing country with limited resources such as India, where highly trained skilled manual labor comes at a premium, diagnosis of bacterial vaginosis by Nugent's score would place a great strain on available resources⁸.

AIM & OBJECTIVES:

The aim of this study was to examine interobserver agreement on interpretation of gram stained smears using Nugent scoring in the diagnosis of Bacterial vaginosis.

MATERIALS AND METHODS:

This prospective study was conducted in a tertiary care hospital in South India from January to June 2013. After getting ethical committee approval and informed consent, high vaginal swabs collected from 220 married, sexually active women between 18-49 yrs of age with symptoms of vaginal discharge, genital itching, genital burning were subjected to Gram staining and Nugent's scoring was done by 3 independent observers, all being qualified microbiologists. Each of the three observers scored and interpreted the slides independently using the Nugent method⁷. Gram-stained slides were examined under oil immersion. Each microbiologist was asked to quantitate the number of large gram-positive bacilli (*Lactobacillus* morphotypes), small gram-variable bacilli (*Gardnerella* morphotypes), curved gram-negative bacilli (*Mobiluncus* morphotypes), and all other bacteria, yeast cells, or Trichomonads on a scale of 1 to 4 according to the method of Spiegel et al. The scores used were 1+ (<1 cell per field), 2+ (1 to 5 cells per field), 3+ (6 to 30 cells per field), and 4+ (>30 cells per field) (7, 10). They were also asked to quantify the number of polymorphonuclear neutrophils (pus cells) using the same rating scale.

The results were graded as 0-3 (normal), 4-6 (intermediate) and 7-10 (bacterial vaginosis) independently by each observer and the results were then compared. If all the three observers had the same interpretation, it was the final diagnosis. In case of discrepancy, the slides were reviewed and a final diagnosis was made after thorough discussion amongst the three observers. If positive by two examiners, the result was taken as positive. If intermediate by two, positive by one also taken as positive. The results were statistically analysed using one way ANOVA test.

RESULTS:

The results are shown in Table 1, 2 and Figure 1. The interrater reproducibility was found to be excellent between the observers. F value between observer 1 and 2 is 312.752 and between observers 2 and 3 is 306.553 and the ratio is 1.02 which is significant. The P value is 0.000 which is also significant.

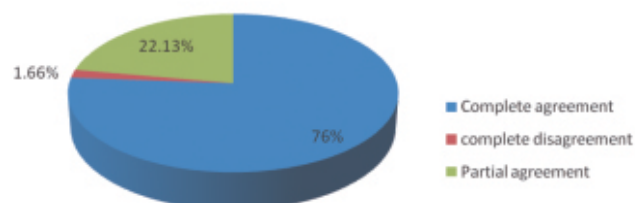
Table 1. Interobserver variation among three observers when interpreting gram stained smears by Nugent scoring method

S.No	Parameter	No of slides (%) n=220
1	Complete agreement ; i.e. interpreted by all three as	168 (76.36%)
	(i) Negative or normal vaginal state	125
	(ii) Intermediate grade	11
	(iii) Positive or BV	32
2	Complete disagreement; i.e. interpreted by all three as different	3(1.66%)
3	Partial agreement	49 (22.13%)
	(i) negative by 2, intermediate by 1	15
	(ii) intermediate by 2, negative by 1	15
	(iii) intermediate by 2, positive by 1	10
	(iv) positive by 2, negative by 1	1
	(v) positive by 2, intermediate by 1	8

Table 2. One way ANOVA Statistical analysis

		Sum of Squares	Df	Mean Square	F	Sig.
observer1 * observer2	Between Groups (Combined)	104.197	2	52.099	312.752	.000
	Within Groups	36.148	217	.167		
	Total	140.345	219			
observer3 * observer2	Between Groups (Combined)	100.780	2	50.390	306.553	.000
	Within Groups	35.670	217	.164		
	Total	136.450	219			

Figure-1. Interobserver variation among the observers.



DISCUSSION:

Culture is the gold standard method for diagnosis of most of bacterial diseases. But in bacterial vaginosis, the organisms involved cannot be isolated easily in the laboratory, it became increasingly more common to process these specimens by Gram stain alone. Nugent et al⁷ provided a more standardized scoring method of Gram stain interpretation for BV. But there are doubts regarding its interpretation by different observers⁹.

Out of 220 slides examined, complete agreement was obtained for 168 slides by all three examiners. The prevalence of Bacterial vaginosis in our population was 23% which correlates with studies by Sharma, Patel, Madhivanan, Rao.^{10,11,12,13} In another study from Haryana by Aggarwal AK¹⁴, India, bacterial vaginosis was diagnosed in a high percentage (48.5%) of rural women. Other studies by Balla in Delhi showed prevalence of 32.8%, by Uma S et al 45%, Mathew et al 38.5%, Renu Mathew et al 36.4%.^{15,16,17,18} This shows that prevalence of bacterial vaginosis varies widely among different areas and communities within the country. This variation in prevalence may be because of various reasons such as differences in economic status and educational background, study population and method used for diagnosis of bacterial vaginosis.

The inter-observer reproducibility of Nugent scores for the diagnosis of BV was statistically significant in this study (76%) which was similar to the studies by Srujana mohanty et al in which 76.2% of agreement was obtained and Joesoef MR et al in which 87.5% agreement was obtained.^{19,20,21}

The results of this study indicate that criteria for the

diagnosis of bacterial vaginosis by using the Gram stain can be reproduced reliably between different microbiologists.

The principal finding of this study was that the reproducibility of the Gram stain interpretations was very good, and showed substantial to near-perfect agreement.

There are several possible reasons for this high level of agreement. Our microbiologists have adhered to a quantitation scale under study conditions. Prior to commencing the study, a teaching session to review the microbiological characteristics of bacterial vaginosis was carried out for all the three microbiologists in the laboratory. Therefore, the high degree of consistency may reflect the fact that the features of bacterial vaginosis were fresh in the minds of them. Therefore the Nugent's scoring system appears to be a reliable and convenient method for the laboratory evaluation of the cases of bacterial vaginosis, as in similar studies which were done by R. Mathew et al, Chennai and Mohanty S, et al, New Delhi^{17,19}. An intermediate score between 4-6 may be found among women who is either recovering from bacterial vaginosis or on treatment or who may develop bacterial vaginosis subsequently. Such women therefore should be followed up to confirm the same.

The advantage of Nugent scoring of gram stained vaginal smears is that it can be used for mass screening of symptomatic women in the community for reproductive tract infections.

The limitations of this study are that only symptomatic patients were included. But studies state that Bacterial vaginosis also be present in asymptomatic patients.

CONCLUSION:

Since the interpretation of Gram-stained smears of vaginal swab specimens has been shown to correlate with the clinical diagnosis of bacterial vaginosis and because a high level of interobserver agreement has been demonstrated, we believe the Gram-stained smear alone, without culture, can be used to evaluate vaginal swab specimens for bacterial vaginosis. Nugent scoring system appears to be a reliable and convenient method for laboratory evaluation of bacterial vaginosis.

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