Clinical characteristics of vaginal discharge in bacterial vaginosis diagnosed by Nugent's criteria

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Summary

Purpose of Investigation: To determine which clinical signs have an important contribution in bacterial vaginosis (BV) diagnosed by Nugent's criteria.

Methods: This was a cross-sectional study of 58 women undergoing vaginal examination at Hayashi women's clinic, Hyogo Prefecture. Characteristics of vaginal discharge (color, amount, odor, and pH level) and the number of clue cells were compared among normal, intermediate, and BV groups. BV was diagnosed by using Nugent's criteria.

Results: The incidence of BV in our study population was 25.9%. Compared to the non-BV group, women in the BV group were found more often to have yellowish color and a moderate amount of discharge (66.67% vs 33.33%, NS; 55.56% vs 44.44%, NS), odorous discharge (100% vs 0%, $p < 0.05$) and furthermore, pH level and the number of clue cells were significantly higher.

Conclusion: Odor, pH level and the number of clue cells in the vaginal discharge were helpful clinical signs for early detection of BV diagnosed by Nugent's criteria.

Key words: Bacterial vaginosis; Nugent's criteria; Clue cells; Gram staining; pH.

Background

Bacterial vaginosis (BV) is a polymicrobial syndrome. It is characterized by the replacement of normal vaginal Lactobacillus by Gardnerella vaginalis and anaerobic bacteria. Women with BV are at increased risk for genital infections and adverse pregnancy outcome. Several studies have documented associations of BV with preterm birth, chorioamnionitis, premature rupture of the membranes (PROM), and postoperative infections [1-7]. The incidence of BV in asymptomatic pregnant women resulted between 11.5% to 21.4% when BV was determined by using Nugent's criteria [5, 6, 8].

Nugent's criteria form a standardized method of gram-stained interpretation designed to evaluate the vaginal microflora to detect BV [9] with good to excellent inter-observer reliability [10]. Gram-stained vaginal smears themselves, compared with other laboratory methods for the diagnosis of BV, are the least expensive, require the least time to perform, are more widely available than other laboratory methods, and are better to identify women with clinical signs of BV from a cohort of women than other laboratory test [9, 11, 12].

On the other hand, previous studies and reports have documented the important role of some clinical signs, such as pH level and odorous discharge [13-15] and clue cells [16] in BV, however, they used other definitions in determining BV. There are still few reports evaluating the clinical signs, especially which sign is the best indicator, in BV diagnosed by Nugent's criteria. Thus, to evaluate which clinical signs are associated with BV diagnosed by Nugent's criteria, we examined the clinical characteristics of vaginal discharge in BV.

Subjects and Methods

A total of 58 healthy pregnant women with singleton pregnancies participated in this study at Hayashi women's clinic, Hyogo Prefecture, between January and February 2005 before 16 weeks of gestational age. All examinations and microbiologic procedures were performed according to the protocol. The protocol was approved by the institutional review board at Kobe University. For each woman, vaginal discharge was obtained for analysis. The reasons for exclusion from the study were history of sexually transmitted diseases and having taken antibiotic therapy before selection.

For the evaluation of vaginal discharge, a clean, unlubricated speculum was placed in the vagina, and then the physician examined the discharge color (white, yellow, or bloody), volume (scant, moderate or profuse), and odor (odorless or odorous). The presence of odor was determined by smelling the secretions on the withdrawn speculum. The vaginal pH was measured with pH-fix 3.6-6.1 (Azuwan, Japan). Sterile cotton swabs were used to obtain material from the posterior vaginal fornix for a vaginal smear.

Next, the vaginal discharge was put on slides for Gram's staining. The examiner was blind to the result of the clinical signs. The process of Gram's staining began with a thin, air-dried, heat-fix preparation on a glass slide that was flooded with crystal violet and allowed to sit for 30 seconds. The slide was then rinsed gently under running tap water and flooded with

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Gram's iodine for an additional 30 seconds. Then the preparation was decolorized by rinsing the slide with an acetone-ethanol solution for some seconds until all color was washed out. Finally, the slide was counterstained for 30 seconds with Pfeiffer's solution, rinsed, and air dried.

The specimen was evaluated for microbiological examination. Clue cells were counted with 400x magnification. At least five fields were evaluated and the rate number of clue cells was documented for further analysis. A 1000x magnification with immersion oil was used for counting the number of Lactobacillus and other microorganisms (Nugent's criteria). At least five fields were evaluated and the rate number of each morphotype was documented for further analysis.

Nugent's scoring criteria was used for the diagnosis of BV. It was based on the total number of large gram-positive rods (Lactobacillus morphotypes), the number of small gram-variable and gram-negative rods (Gardnerella vaginalis, Bacteroides, and Prevotella morphotypes) and curved gram-negative rods (Mobiluncus morphotypes). A score of 0-3 is representative of normal microflora, a score of 4-6 is designated intermediate and corresponds to disturbed or altered microflora, and a score of 7-10 is consistent with BV microflora [9, 12].

Statistical Analysis

The results were displayed as frequencies, percentages, mean and standard deviation (SD). The Student's t-test and chi-square test were used for comparison between groups and 95% of confidence intervals (CI) were calculated. A probability (i.e., p value) of < 0.05 was considered significant.

Results

The mean gestational age of the women included at enrollment was 8.5 weeks ± 3.2 weeks (mean ± SD), ranging from four weeks to 16 weeks of gestational age. Patients with and without bacterial vaginosis were similar with regard to gestational age. Of the 58 smears examined using Nugent's criteria, 22 (37.9%) were graded as normal, 21 (36.2%) as intermediate and 15 (25.9%) as BV (Figure 1).

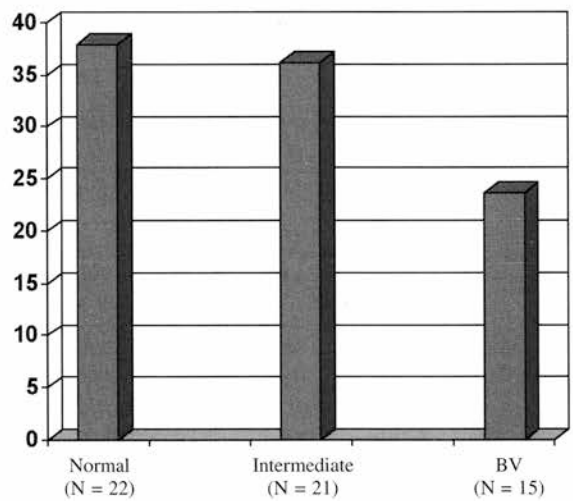

Figure 1. — Incidence of BV assessed by Nugent'S Criteria.

Clinical examination of the vaginal discharge showed that most of the patients had a white color, slight amount and odorless discharge. When these clinical signs were compared among the three groups, significant differences were observed for discharge odor (Table 1). Furthermore, pH level and the number of clue cells were analyzed. Three patients with bloody discharge were excluded in the analysis of pH level. As seen in Table 2, pH level and the number of clue cells were significantly higher in women in the BV group compared to women in the normal and intermediate groups.

As this study showed that the mean pH level in BV patients was 4.96, we tried to do a comparison based on a pH level at 5.00 which resulted in 11 patients with BV (78.57%) with a pH ≥ 5.00 and 64.70% of women with a pH ≥ 5.00 that were found to have BV (Table 3).

Table 1. — *Color, amount and odor of vaginal discharge in the three groups (normal, intermediate and BV).*

Discharge	Normal (N = 22)	Intermediate (N = 21)	BV (N = 15)	p value
Color:				
White	21 (42.9%)	18 (36.7%)	10 (20.4%)	0.129
Yellow	0 (0%)	2 (33.33%)	4 (66.67%)	
Bloody	1 (33.3%)	1 (33.3%)	1 (33.3%)	
Amount:				
Scant	21 (42.9%)	18 (36.7%)	10 (20.4%)	0.058
Moderate	1 (11.1%)	3 (33.3%)	5 (55.6%)	
Profuse	0 (0%)	0 (0%)	0 (0%)	
Odor:				
Odorless	22 (40%)	21 (38.2%)	12 (21.8%)	0.010
Odorous	0 (0%)	0 (0%)	3 (100%)	
			N (%)	

Table 2. — *pH level and clue cells of vaginal discharge among three groups (normal, intermediate and BV).*

	Normal (N = 22)	Intermediate (N = 21)	BV (N = 15)
pH	4.26 ± 0.59	4.52 ± 0.51	4.96 ± 0.35*
Clue cells	0 ± 0	0.18 ± 0.83	1.45 ± 2.23*

Mean ± SD; *p < 0.05 vs normal and intermediate.

Table 3. — *Comparison of BV incidence based on pH level at 5.0.*

pH level	Normal (N = 22)	Intermediate (N = 21)	BV (N = 15)
pH < 5.0	85.7%	85%	21.4%
pH ≥ 5.0	14.3%	15%	78.6%

Discussion

The incidence of BV in this study as assessed by Nugent's criteria (25.9%) was higher than that in most studies. A study report with a population of Japanese pregnant women [8] showed that the prevalence of BV as diagnosed by Nugent's criteria between January and December of 2000 was significantly higher than that between January and December of 1995 (21.3% vs 15.1%, $p < 0.001$) [8]. This study may suggest that the prevalence of BV is increasing year by year.

In this study pregnant women in their first trimester of pregnancy participated. A different result in the incidence

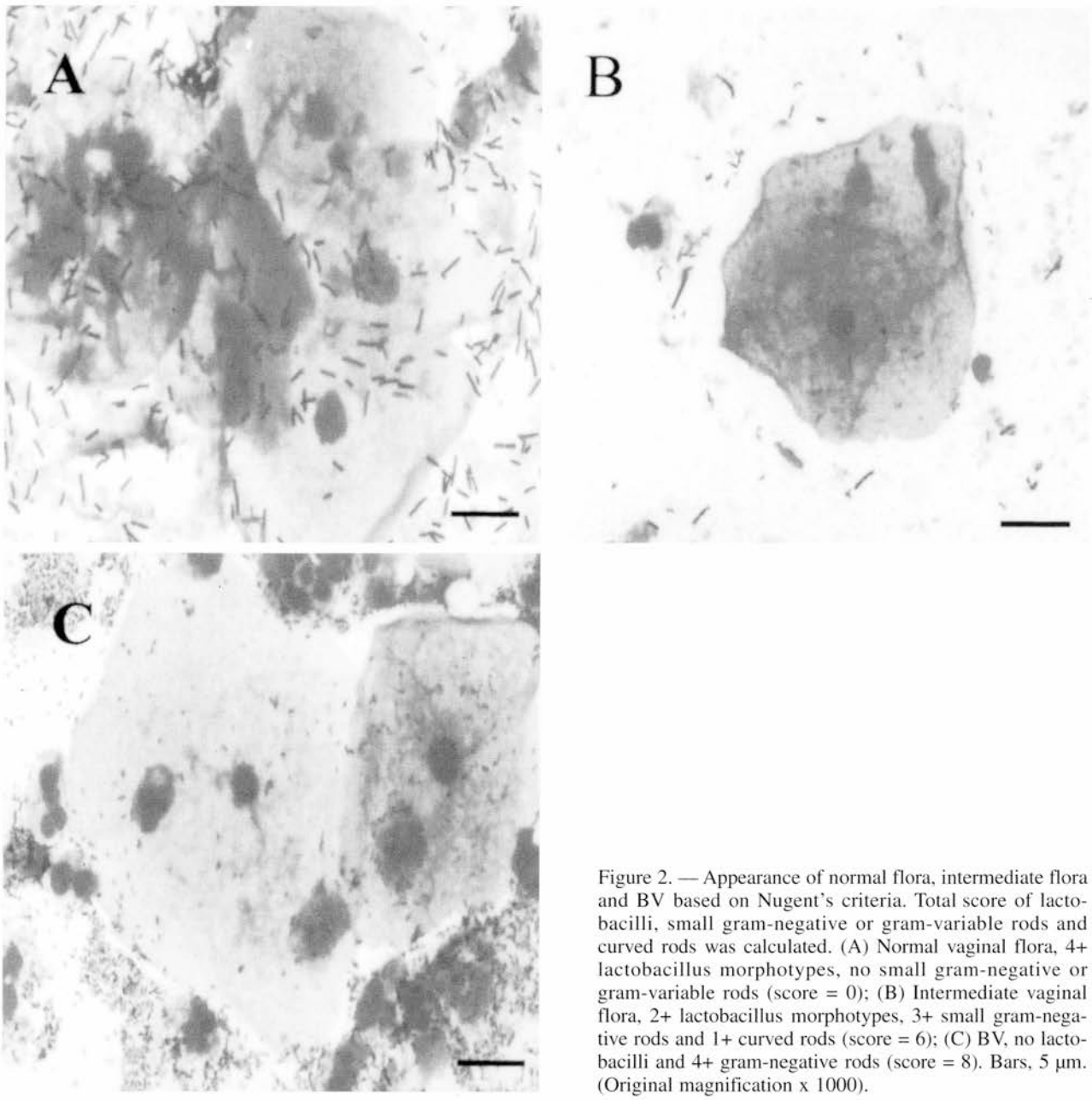

Figure 2. — Appearance of normal flora, intermediate flora and BV based on Nugent's criteria. Total score of lactobacilli, small gram-negative or gram-variable rods and curved rods was calculated. (A) Normal vaginal flora, 4+ lactobacillus morphotypes, no small gram-negative or gram-variable rods (score = 0); (B) Intermediate vaginal flora, 2+ lactobacillus morphotypes, 3+ small gram-negative rods and 1+ curved rods (score = 6); (C) BV, no lactobacilli and 4+ gram-negative rods (score = 8). Bars, 5 μm. (Original magnification x 1000).

of BV during pregnancy, especially in each trimester, was recognized. Riduan *et al.* [6] reported that the rate of BV was 17% in the first trimester (16-20 weeks) and 15% in the second trimester (28-32 weeks). Eliyan & Rund [17] reported that women with BV in the second trimester tended to remain BV positive in the third trimester and those women with intermediate flora had a significant chance of progressing to BV [17]. However, the gestational age at which BV was screened for and diagnosed did not influence the increased risk of preterm birth [18, 19].

Yellowish color and a moderate amount of discharge were found more often in the BV group compared with the non-BV group (66.67% vs 33.33%, NS; 55.56% vs 44.44%, NS). This result is consistent with the study by Garner and Dukes [15] that suggested the volume of discharge from Haemophilus vaginalis infections (now Gardnerella vaginalis) varied from scanty to profuse, but was usually moderate [15]. Furthermore, they also stated that the volume of normal vaginal secretions is largely dependent on the hormonal status of individual patients and the color was usually described as white. Thus, a yellowish color of discharge indicates abnormality. However, almost 30 years later, in their study, Amsel *et al.* [13] stated that no reference to color or amount of discharge was made in their study as these factors are difficult to assess in a standard manner [13]. Furthermore, it is mentioned that during pregnancy, vaginal secretions increase and are thick and white [20, 21].

Figure 3. — Typical picture of clue cells. Note the indistinct margins and a granular appearance resulting from the adherence of numerous small bacteria. Bars, 5 μm (original magnification x 400).

This present study suggests no significant differences in discharge color and amount among normal, intermediate and BV groups, thus these clinical signs are not good indicators for BV diagnosed by Nugent's criteria. However, as we consider the p value for vaginal discharge color and amount, more attention should be focused on the amount of vaginal discharge. Further studies with larger numbers are needed to confirm this result. In a clinical setting, it is better to confirm the result of a moderate amount of discharge with discharge odor.

On the other hand, odorous discharge was found in the BV group but not in the normal and intermediate groups (100% vs 0%, $p < 0.05$). BV is characterized by the replacement of Lactobacillus with other microorganisms. A decreased number of Lactobacillus accompanied by an increased number of other microorganisms raise the pH level and the production of amine and ammonia that emit the amine odor. These microorganisms also produce bacterial toxin that damage the upper layer of the vaginal tract adherent to the vaginal epithelial cells. Malodorous vaginal discharge is a good indicator as a clinical sign of BV.

The pH level of vaginal discharge in the BV group was significantly different from that of the normal and intermediate groups. Moreover, more than 75% of women with a pH level at 5.00 or more had BV. The pH level of normal vaginal fluid is usually in the range of 3.8 to 4.5 [13, 14, 22]. Lactobacillus is thought to be responsible for maintaining an acid pH in the vagina by metabolism of the glucose generated by the metabolism of glycogen and also may influence bacterial adherence to vaginal epithelial cells [14, 23]. During pregnancy, the acidic discharge provides the mother and fetus with some protection against infection. Perinatally, and between menarche and menopause, increased levels of estrogen cause large amounts of glycogen to be deposited in the vaginal epithelium. During these periods, the anerobic metabolism of glycogen, by the epithelial cells themselves and/or by vaginal flora, causes the vagina to become acidic (pH ~ 4.0) [24]. Our findings are consistent with previous reports that pH level is higher in BV [13, 15, 16, 23]; pH level is one of the important clinical signs of vaginal discharge in BV.

In this study, no clue cells were found in normal patients. Furthermore, 90.90% of patients with clue cells were found to have BV. Clue cells are very often observed in BV. Typical clue cells have indistinct margins and a granular appearance resulting from the adherence of numerous, small bacteria [25] as can be seen in Figure 3. The bacteria include Gardnerella vaginalis, Bacteroides, and Mobiluncus [14]. This study suggests a way to detect clue cells by using gram-stained smears from the same slide because then the number of Lactobacillus and other microorganisms to diagnose BV can be further investigated by using Nugent's criteria. Also, instead of using criteria in detecting BV where at least 20 % of the epithelial cells are clue cells, this study used the rate number of clue cells from five fields that were evaluated. Further study is needed to confirm this different procedure in detecting BV based on Nugent's criteria.

In this study, we decided to divide the subjects into three groups of normal, intermediate and BV (Figure 2) in considering the sample size. Although Nugent *et al.* [12] suggested that the score can be used to assess the degree of alteration in vaginal flora as a continuum rather than as a forced dichotomy, several studies used criteria of normal, intermediate and BV to investigate many aspects of BV, such as risk factors, effect of treatment and pregnancy outcome. An exception, a study by Hauth *et al.* [26] that compared three score groups of score 0-6, score 7-8, and score 9-10 to predict preterm birth, instead of score 0-3, score 4-6, and score 7-10 resulted in women with a vaginal pH of 5.0 or greater or a vaginal pH of 4.5 or greater and a gram-stain score of 9 to 10 had significantly increased preterm births at < 37, < 35, and 32 weeks' gestation and/or a birth weight less than 2500 g or less than 1500 g [26]. The different method of Nugent's score grouping may contribute to the different result. Thus, further study is still needed to confirm it.

In conclusion, the incidence of BV was 25.9% in this study suggesting the significant contribution of clinical signs in diagnosing BV by Nugent's criteria. Except for the color and amount of discharge, odor, pH level and the number of clue cells were good indicators as clinical signs of vaginal discharge in BV.

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