

Diagnostic Value of Amsel Clinical Criteria Compared to Nugent Scoring System for the Diagnosis of Bacterial Vaginosis in Pregnant Women

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Abstract

Most clinicians prefer to apply more readily accessible clinical techniques for the diagnosis of bacterial vaginosis, which at the same time are time-conserving and highly accurate. The current cross-sectional study sought to evaluate the bacterial vaginosis diagnostic tests on 87 pregnant women referred to our center due to vaginal discharge using Amsel criteria and the Nugent scoring system. Our results showed that the Amsel criteria had a significantly higher sensitivity/specificity than the Nugent scoring system regarding the number of clue cells in the vaginal wet mount test. The specificity of a combination of the wet mount of clue cells in the vagina with other Amsel criteria along with the Nugent scoring system was much higher than both the Amsel criteria and Nugent scoring system. We observed that due to the high efficacy and the great time-conserving and economically-friendly nature of the Amsel criteria, the method is usually opted for by clinicians.

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Abbreviations

CIN: Cervical Intraepithelial Neoplasia; HIV: Human Immunodeficiency Virus; qRT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction; UTI: Urinary Tract Infection.

1. Introduction

The most common complaint of patients referring to gynecologic clinics, vaginitis [1], is believed to affect roughly 75% of all women in the world at least once in their lives [2]. Bacterial vaginosis, as a vaginal pathology, is a common complication in females during fertile life, with 11-51% of women of reproductive age. An alteration in the bacterial flora of the vagina, bacterial vaginosis can result in the loss of hydrogen peroxide generators and overgrowth of anaerobic bacteria and vaginal degradative bacteria [3]. The prevalence of asymptomatic infection is 50-75%. The symptomatic cases usually complain of homogeneous greyish white discharge and a fishy odor after intercourse or during menstruation [4]. Although bacterial vaginosis is known as a mild disease, it is associated with several obstetric and gynecological concerns [5].

In patients with bacterial vaginosis, the frequency of postoperative endometritis and cesarean section is six times higher. On the other hand, the incidence of pelvic inflammatory diseases triples after abortion, while following abdominal hysterectomy, the incidence of vaginal cuff cellulitis is raised by three to four-fold [6]. Bacterial vaginosis is associated with abnormal cytology of the cervix [7]. In pregnant women with the bacterial vaginosis, preterm delivery, amniotic fluid infection, low birth weight, and fetal infections are 50-100% more common. Also, different investigations have indicated the correlation of urinary tract infection (UTI) with vaginosis to such an extent that the infection with bacterial origin is suggested to be a risk factor for urinary tract infection [8]. Recent studies have shown that cervical intraepithelial neoplasia (CIN) is more common in women with bacterial vaginosis [9]. Vaginal microbiota has recently been suggested to be associated with the risk of CIN development [10]. Side effects of bacterial vaginosis include chorioamnionitis, amniotic fluid infections, premature rupture of membranes, low birth weight, high prevalence of post-abortion pelvic infections, post-hysterectomy vaginal cuff cellulitis, endometritis, UTI, CIN, predisposition to human immunodeficiency virus (HIV), and overall higher risk of abnormal pregnancy, infertility, and chronic pelvic pain [11]. The exact diagnosis of bacterial vaginosis is important due to adverse consequences during pregnancy and pelvic inflammatory diseases and an increased risk of infection with the HIV in subjects exposed to the virus.

Despite the fact that vaginitis can be easily treated, the rate of recurrence is so high, which may be due to the lack of accurate diagnosis of the type of vaginitis, resulting in dampened response to treatment and an increase in patients and community costs [11].

In this respect, the Nugent scoring and Amsel criteria are two methods widely adopted by clinicians for the detection of bacterial vaginosis [12]. The Nugent scoring, proposed by Nugent in 1991, is a Gram-staining scoring technique for vaginal swabs to measure the density of *Lactobacillus* and other species (*Gardnerella vaginalis*, *Prevotella* spp., and *mobiluncus* in various shapes) on a scale of 0-10, with the scores of 7-10 representing bacterial vaginosis.

Due to the considerable sensitivity of this method, it has been recognized as a golden standard for bacterial vaginosis [13]. In 1938, Amsel et al. introduced a benchmark for the diagnosis of bacterial vaginosis. They proposed four criteria, the presence of three of which was sufficient for making a confirmed diagnosis of bacterial vaginosis: 1) increased homogeneous, smelly, and white brown vaginal discharge; 2) a change in vaginal pH from acidic to alkaline, with a value higher than 4.5; 3) release of amine odor once 10% potassium hydroxide is added to vaginal secretions; and 4) clue cells presence on wet mount examination of vaginal discharge [14]

In 2012, a study by Taj and colleagues examined the Amsel criteria and microbiological methods for the detection of bacterial vaginosis. They found that Amsel's criteria had a high diagnostic value for identifying bacterial vaginosis [15]. In a study conducted in 2013, Rangari and colleagues reviewed the Nugent Scoring

System. They showed that the Nugent Scoring System had a high sensitivity for the detection of bacterial vaginosis, while Amsel's criteria were less sensitive and more specific. However, the Amsel's criteria may be less advantageous in terms of feasibility due to the prerequisite of staining techniques [12]. Based on an investigation of quantitative reverse transcription polymerase chain reaction (qRT-PCR), there is an excellent agreement between the Amsel criteria and the Nugent scoring system for the clinical detection of bacterial vaginosis [16].

Today, with a higher emphasis on evaluation of clinical symptoms, most bacterial vaginosis are rapidly diagnosed and treated. For accurate diagnosis of bacterial vaginosis, the use of laboratory methods and microscopic examinations, such as the preparation of wet mount and the determination of the acidity of vaginal discharge, is necessary; otherwise, the results of the examination will not be reliable. The most important disadvantages of the Nugent scoring system, which is the golden standard for the detection of bacterial vaginosis, are the time-consuming and costly nature of this method. Accordingly, the results may not be reported fast enough to help with the diagnosis of bacterial vaginosis. Thus, most clinicians prefer to use precise clinical criteria [17]. Regarding the high prevalence of bacterial vaginosis, the importance of timely diagnosis and treatment, and the fact that there may not always be access to laboratory evaluation, this study sought to explore the Amsel criteria in comparison with the golden standard Nugent, separately and in combination, in the diagnosis of pregnant women' bacterial vaginosis.

2. Materials and Methods

This cross-sectional investigation was conducted to evaluate the diagnostic tests for bacterial vaginosis in 87 pregnant women who had complaints of vaginal discharge and/or burning or itching sensation and were referred to Ali ibn Abitalib Hospital Zahedan. The criteria for subjects to be included in the study were pregnant women with a gestational age of under 28 weeks who complained of vaginal discharge and/or burning or itching sensation. The exclusion criteria included: 1) vaginal bleeding; 2) history of the vaginal shower in the last 48 hours, 3) history of antibiotic use in the last two weeks or vaginal medicine within three days; and 4) sexual intercourse within the last 24 hours. Eighty-seven pregnant women under 28 weeks of gestation, who complained of vaginal secretions, were examined. A sterile speculum was placed into the vagina following the examination of the vulva. Vagina and cervix were examined for inflammation and redness, as well as any abnormal findings considering the form, color, consistency, and smell. Three slides were prepared for each patient's discharge. For this purpose, samples were taken from the lateral wall and posterior fornix of the vagina using a sterile swab that was then transferred onto three glass slides. The air-dried Gram-stained slides were microscopically (1000 x) examined in the laboratory by a pathologist. Meanwhile, the Nugent scoring system was adopted for the evaluation of bacterial vaginosis, and the results were provided within two days. Scores > 7 were considered bacterial vaginosis (Table 1). The second slide was microscopically explored for clue cells by adding 1-2 drops of normal saline. The last slide was investigated for amine odor by the Whiff test.

The vaginal discharge acidity was measured with pH paper; for this purpose, pH paper was placed for one minute on the side of the vagina (without being exposed to the alkaline surface of the cervix). Bacterial vaginosis was diagnosed based on the following criteria: the presence of a minimum of three Amsel criteria, positive whiff test, vaginal pH > 4.5, homogeneous gray-white vaginal discharge, and observation of clue cells. The gold standard to diagnose bacterial vaginosis in the current study was the Nugent scoring system. As a result, the sensitivity, the Amsel's specific features have been studied individually and in combination with the Nugent scoring system.

Moreover, 95% CI and a P-value < 0.05 were considered statistically significant. Figure 1 represents the study procedure.

Table 1. Nugent's scoring system

| Score | Organism morphotype/high power field | | |
|-------|--|--|---|
| | Lactobacillus (parallel-sided, <i>Gr</i> ⁺ rods) | Gardnerella/Bacteroides (tiny, <i>Gr</i> variable cocci/bacilli and rounded, pleomorphic, <i>Gr</i> ⁻ rods with vacuoles) | Mobiluncus (curved, <i>Gr</i> ⁻ rods) |
| 0 | > 30 | 0 | 0 |
| 1 | 5-30 | < 1 | 1-5 |
| 2 | 1-45 | 1-4 | > 5 |
| 3 | < 1 | 5-30 | |
| 4 | 0 | > 30 | |

Gr: Gram, *Gr*⁺: Gram-positive, *Gr*⁻: Gram-negative

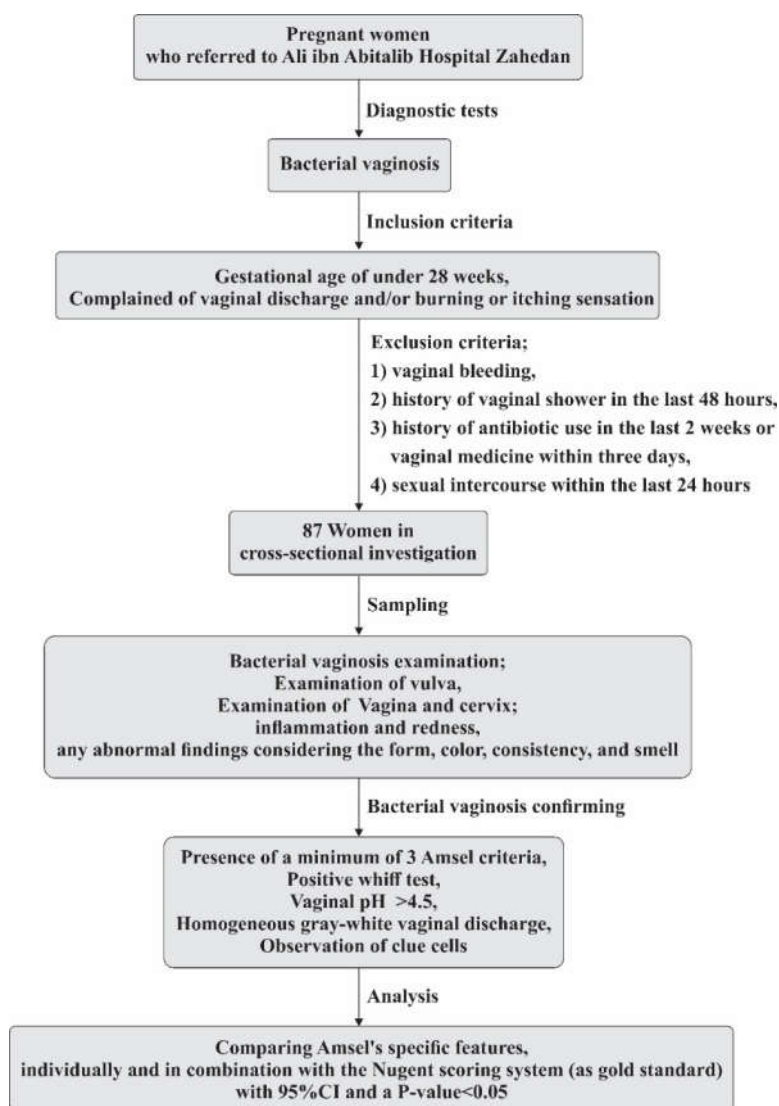


Figure 1. Study procedure

3. Results and Discussion

This study investigated 87 pregnant women, the demographic characteristics of whom are shown in Table 2. The subjects' mean age (years) and pregnancy age (weeks) were 27.48 ± 5.56 (range: 20) and 20.92 ± 7.69 , respectively.

Table 2. Frequency distribution of age, pregnancy age, among pregnant women who were the main complaints of vaginal discharge, burning or itching, and bacterial vaginosis

| | Average | Standard deviation | Middle | Range |
|---------------|---------|--------------------|--------|-------|
| age | 27.48 | 5.56 | 28 | 20 |
| Pregnancy age | 20.92 | 7.69 | 20 | 32 |

The sensitivity and specificity of Amsel's criteria in combination with the Nugent scoring system were measured (Table 3). Comparison of one Amsel criterion with Nugent scoring system: As listed in Table 3, the clue cells' presence on vaginal wet mount confers greater sensitivity (97%) and specificity (100%) than that of Amsel's criteria compared with the Nugent scoring system. Other important histopathological signs, including homogeneous greyish discharge, $\text{pH} \geq 4.5$, and whiff test, are associated with 50%, 47%, and 30.5% sensitivity, respectively. These findings, in the same order, confer specificity of 68.6%, 56%, and 47.5% in the diagnosis of bacterial vaginosis, respectively. Taken together, the whiff test and homogeneous discharge deliver the lowest sensitivity and specificity, respectively.

Table 3. Amsel's criteria and Nugent scoring comparison for the diagnosis of bacterial vaginosis in pregnant women who were the main complaints of vaginal discharge, burning or itching, and bacterial vaginosis

| | | Nugent scoring | | | SN (%) | SP (%) |
|-----------------------------|-------|----------------|----------|-------|--------|--------|
| | | Positive | Negative | Total | | |
| Vaginal pH | + | 17 | 22 | 39 | 47 | 56 |
| | - | 19 | 29 | 48 | | |
| | Total | 36 | 51 | 87 | | |
| Clue cells | + | 35 | 0 | 35 | 97 | 100 |
| | - | 1 | 51 | 52 | | |
| | Total | 36 | 51 | 87 | | |
| Amsel's criteria whiff test | + | 11 | 16 | 27 | 30.5 | 68.6 |
| | - | 25 | 35 | 60 | | |
| | Total | 36 | 51 | 87 | | |
| Gray-White Discharge | + | 18 | 27 | 45 | 50 | 47.5 |
| | - | 18 | 24 | 42 | | |
| | Total | 36 | 51 | 87 | | |

SN: Sensitivity, SP: Specificity, +: Positive, -: Negative

Comparison of two combined Amsel's criteria with Nugent scoring system: Table 4 shows that the clue cells presence along with other Amsel's criteria compared with Nugent scoring system had the highest specificity among all Amsel's criteria with Nugent scoring system. Homogeneous discharge and $\text{pH} \geq 4.5$ compared with clue cells on the vaginal wet mount had considerable sensitivity (100%), and whiff test / Clue cell had the second highest level of sensitivity (91%) for detection of bacterial vaginosis. Overall, a positive record of clue cells along with Amsel's criteria (Vaginal pH / Clue cells and Clue cell / Gray-White Discharge) had the highest specificity (100%) and sensitivity (100%) compared with the Nugent scoring system.

Table 4: Comparison of combine two Amsel criteria along with Nugent scoring system for the diagnosis of bacterial vaginosis among pregnant women

| | | Nugent scoring | | | SN (%) | SP (%) |
|----------------------------------|-------|----------------|----------|-------|--------|--------|
| | | Positive | Negative | Total | | |
| Vaginal pH/ whiff test | + | 6 | 12 | 18 | 30 | 67 |
| | - | 14 | 25 | 39 | | |
| | Total | 20 | 37 | 57 | | |
| Vaginal pH/ Clue cells | + | 16 | 0 | 16 | 100 | 100 |
| | - | 0 | 29 | 29 | | |
| | Total | 16 | 29 | 45 | | |
| Vaginal pH/ Gray-White Discharge | + | 5 | 13 | 18 | 45 | 53 |
| | - | 6 | 15 | 21 | | |
| | Total | 11 | 28 | 39 | | |
| whiff test/ Clue cell | + | 11 | 0 | 11 | 91 | 100 |
| | - | 1 | 35 | 36 | | |
| | Total | 12 | 35 | 47 | | |
| whiff test/ Gray-White Discharge | + | 6 | 12 | 18 | 26 | 62 |
| | - | 13 | 20 | 33 | | |
| | Total | 19 | 32 | 51 | | |
| Clue cell/ Gray-White Discharge | + | 17 | 0 | 17 | 100 | 100 |
| | - | 0 | 24 | 24 | | |
| | Total | 17 | 24 | 41 | | |

SN: Sensitivity, SP: Specificity, +: Positive, -: Negative

Briefly, in this study, Amsel's criteria and Nugent scoring were compared to one another in the diagnosis of bacterial vaginosis in terms of sensitivity and specificity. The presence of clue cells on vaginal wet mount confers the greatest sensitivity and specificity of Amsel's criteria compared with the Nugent scoring system. Homogeneous greyish discharge, $\text{pH} \geq 4.5$, and whiff test rank 2nd-4th in terms of sensitivity. In the case of specificity, whiff test, $\text{pH} \geq 4.5$, and homogeneous discharge deliver the highest specificity, respectively. Moreover, the comparison of combined Amsel criteria (Vaginal pH / Clue cells and Clue cell / Gray-White Discharge) with the Nugent scoring system had the highest specificity (100%) and sensitivity (100%), was the best Amsel's criterion compared with the Nugent scoring system for bacterial Vaginosis diagnosis. Based on our findings, the presence of the clue cells in vaginal secretions has a high diagnostic value for bacterial vaginosis. Vaginal squamous epithelial cells (or clue cells) with distinct morphology are usually surrounded by anaerobic Gram-variable *coccobacilli* (i.e., *G. vaginalis*) and other bacterial vaginosis agents [14]. In an older investigation, the high specificity (93%) and sensitivity (86%) of clue cells have been reported in the diagnosis of bacterial vaginosis [18]. One study reported clue cells in the vaginal discharge of 93% of patients with bacterial vaginosis [17]. Similarly, another report confirmed the high sensitivity and specificity and high diagnostic value of clue cells in the identification of bacterial vaginosis [19]. In the present study, we report a 97% sensitivity and 100% specificity for clue cells compared with the Nugent scoring system and 100% sensitivity and 100% specificity for combined Vaginal pH / Clue cells and Clue cell / Gray-White Discharge compared with the Nugent scoring system in the diagnosis of bacterial vaginosis.

Vaginal acidity ($\text{pH} \geq 4.5$), another criterion of the Amsel's criteria, exhibited high sensitivity (97%), albeit a low specificity (26%) [18]. Given the fact that vaginal acidity is typically between 3.8 and 4.2, the pH of the vagina depends on vaginal microflora activity [20]. However, in addition to bacterial vaginosis, other factors, including cervical secretions, trichomonas vaginalis infection, contact with sperm, and the use of lubricant gels, may increase vaginal acidity. Therefore, the combination of the vaginal pH test with other criteria can increase the accuracy of the test in the diagnosis of bacterial vaginosis [17]. Our results showed that the vaginal acidity test in comparison

with the Nugent scoring system was characterized by a low specificity (56%) and sensitivity (47%), but in the comparison of combined vaginal pH / Clue cells with the Nugent scoring system, the highest sensitivity (100%) and specificity (100%) was reported. Many factors, including vaginal infection along with cervical mucus infection, may affect this criterion. Hemalatha et al. suggested that detection of a vaginal pH > 4.5 by pH strips and pH Glove confers sensitivity of 72% and 79% and specificity of 60% and 53%, respectively, for diagnosis of bacterial vaginosis. They stipulated that the whiff test along, with the pH test, might impair the sensitivity but enhance specificity [21].

The whiff test is another Amsel criterion for the diagnosis of bacterial vaginosis. Our study reported a 30.5% sensitivity (as lowest sensitivity) and 68.8% specificity for the whiff test. Hallen et al. (in 1987) reported a sensitivity of 33.9% and a specificity of 86.9% for the whiff test. In their study, they evaluated the clinical criteria for those who were present in clinics for sexually transmitted diseases. They reported that in these patients, the positive results of the whiff test were about 95% [22]. The researchers concluded that if the Gram staining technique was not available, the Amsel criteria could be applied for the rapid detection of bacterial vaginosis [23].

A diagnosis of bacterial vaginosis is clinically made based on the presence of homogeneous thin gray/white discharge, along with other important criteria mentioned earlier [24]. Homogeneous greyish discharge is the second sensitive and fourth specific criterion in the diagnosis of bacterial vaginosis. In 2010, Ling et al. showed that homogeneous white-brown vaginal discharge is present in more than 85% of women with bacterial vaginosis [25]. Having explored the effects of metronidazole and thyme in bacterial vaginosis, Simbar *et al.* reported a similar prevalence of homogeneous white and brown vaginal discharge in both groups [26].

In a study by Rangari and his colleagues in 2013, the Nugent scoring system showed a high sensitivity for the diagnosis of bacterial vaginosis, but Amsel's criteria were less sensitive, albeit more specific. They reported a low diagnostic value for the Amsel's criteria in the absence of Nugent's criteria (staining methods) [12]. A study by Bhat et al. in 2012 reported a specificity (96.5%) and sensitivity (78%) for the Amsel criteria compared to the Nugent scoring system [27]. Taj et al., who examined the prevalence of bacterial vaginosis by two techniques, Amsel's criteria and Gram staining, showed positive cases of 62% and 72%, respectively [15]. Given the fact that Gram staining is a precise and reliable test for the detection of bacterial vaginosis, Gram staining is required to confirm the Amsel's criteria results. Mengistie et al. compared different identification methods for bacterial vaginosis and Amsel clinical criteria and reported a sensitivity of 85.7% and 98.0% compared to the Nugent scoring system. They showed that clue cells were identified as the best Amsel's criteria with the highest sensitivity and specificity as well as the whiff test and acidity, respectively, with the lowest specificity and sensitivity [23].

Bacterial vaginosis in pregnant patients in their first trimester is treated with 0.75% metronidazole gel applied locally every night for seven nights in total. As for patients in their second trimester, metronidazole 500 mg tablets can be prescribed twice a day for seven days [28]. In the case of recurrent disease, metronidazole 0.75% gel can be used for seven days [29], the use of which, in the case of more severe disease, should be extended to 4 to 6 months twice a week [30]. The present investigation is limited in that it did not consider recurrence, which is prevalent in 30% of pregnant women with vaginal infections [31].

4. Conclusions

In the present study, the comparison of Amsel criteria with the Nugent scoring system showed high sensitivity and specificity in the identification of bacterial vaginosis. On the other hand, among the Amsel criteria, the clue cells

in the wet smear had the greatest sensitivity and specificity. Since this technique is efficient, inexpensive, time-saving, and rapid (requiring less equipment), it is usually the preferred option. However, if not available, the Nugent scoring system can be of great value in detecting bacterial vaginosis.

Conflicts of Interest

The authors declare no conflicts of interest.

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