



Evaluation and Comparison between Amsel's Criteria and Nugent's Score Methods in Diagnosis of Bacterial Vaginosis in Non-pregnant Women

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Authors' contributions

This work was carried out in collaboration between all authors. Author MH designed the study, wrote the protocol. Author NMJ wrote the proposal in English and wrote the first draft of the manuscript and performed many parts of analysis. Author FYH helped in the design and managed the literature searches, analyses of the study performed techniques and helped in analysis. Author ZM helped in design and performed many techniques the experimental processes. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Bacterial vaginosis (BV) is a poly-microbial syndrome. Amsel's criteria or Nugent's methods are usually used for its diagnosis. The present study was conducted to compare these two techniques regarding their reliability and possible preferences in practical use.

Study Design: A Cross-sectional research was designed. There was no time dimension while existing differences were identified and became base for grouping all cases involved in the study.

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Place and Duration of Study: Sabzevar, Iran, During 2012 to 2013.

Methodology: Four hundreds and sixteen (416) non-pregnant women with abnormal vaginal discharges were evaluated for the presence of BV using Amsel's criteria and Nugent's tests. All clinical symptoms and a standard screening questionnaire were prepared and collected for each person. For each case following examinations were practiced: pH, Whiff test, a test in which vaginal secretions are mixed with 10% KOH resulting in a fishy odor typical of bacterial vaginosis, and the presence of the clue cells on vaginal wet smear. Gram staining method was performed for Nugent's method.

Results: Nugent's score and Amsel's criteria tests showed that the prevalence of BV was 8.2% and 16.4% respectively. There was no perfect inter-rater agreement between both Amsel's and Nugent's score ($kappa = 0.58$) tests. Presence of the clue cells among different diagnostic values provided the highest degree of assurance.

Conclusion: Amsel's criteria method is a cheap and convenient means for BV diagnosis. However, Nugent's method is not always reliable due to the complexity of scoring and expertise requirements. Although we need more evidence, the results suggested that the combination of the pH and clue cells test relatively was the best practical and reliable choice in clinical set for BV diagnosis.

Keywords: Bacterial vaginosis; amsel's criteria; nugent's method; clue cells.

1. INTRODUCTION

Bacterial vaginosis (BV) is one of the most common vaginal infections associated with some disgusting symptoms such as vaginal discharges and consequent impact of women's life particularly at their reproductive age. It is usually asymptomatic and characterized by a disruption of the normal vaginal microflora which manifests in an abnormally vaginal malodor and a slight to moderate increase of white discharge [1,2]. Studies showed that the prevalence of BV ranged from 9% to 37% [3]. In other side some reports focused in problems and challenges facing to better diagnosis and cure approaches. So all historical, epidemiological, physiopathological, clinical and laboratorial evaluations have to be assess contiguously to find and rely on the best criteria to combat BV particularly in pregnant women [4]. In non-pregnant women BV is usually associated with infections in urinary tract, reproductive systems, gynecological surgeries, cervicitis and pelvic inflammatory diseases. It has been reported that gonorrhoea, chlamydial genital infection, trichomoniasis and some viral infections such as genital herpes and human immunodeficiency make women more susceptible to BV [5]. Bacterial vaginosis may also contribute to predispose spontaneous abortion in early pregnancy, pre-term birth, and post-partum endometritis [6].

Vaginal infections and subsequent diseases mainly diagnosed based on the clinical signs and symptoms. Clinicians have to develop treatment

plan according to such clinical findings and therefore, different therapeutic measures have been arisen today. This reality shows the importance of having reliable, practical, cost effective and standardized diagnostic techniques in clinical setting which provide effective treatment and more patients' satisfaction. There are two major methods for BV diagnosis, Amsel's and Nugent's score tests. Amsel's test was performed as a primary physician office-based diagnostic test. This test fulfills three of four following criteria: the presence of homogenous vaginal discharge, $pH < 4.5$, positive Whiff test and the presence of clue cells on the vaginal wet smear. However, Nugent's score test is widely used as a gold standard method. In this latter method a scoring Gram staining system was performed with a range of 0 to 10. The scores were depending on the presence or absence of morphotypes of different organisms such as *Gardnerella vaginalis* and *Mobiluncus Spp*. In this scoring system numbers between 7 and 10 may be considered as positive VB. Although Nugent's method is a suitable and reliable in epidemiological and screening settings, clinicians do not prefer it because it needs a very accurate interpretation and expertized and skillful personnel [7]. In a population with a high prevalence of BV, there is a positive correlation between the Amsel's and the Nugent's score test. This correlation has been also observed in both pregnant and non-pregnant women. Other methodology approaches have also been examined as Thais Marques [8] and colleagues compared flowchart of vaginal discharge and typical examinations in the clinical nursing

practice for BV diagnosis in pregnant women. Although they showed that flowchart was satisfactory to show presence of bacterial vaginosis, its sensitivity and specificity was not enough to be relied on for BV diagnosis. Particularly they could not identify specific infections such candidiasis and trichomoniasis and overall they need more reassessment. In a study in Bulgaria [9] three different tests have been simultaneously used to diagnose BV: scoring of Gram staining of vaginal smear, cultures, and polymerase chain reaction (PCR). The results showed that there was a greater concurrence with nearly 90% between Gram staining and PCR detection for BV compared with culture. They suggested that combination of Gram staining and PCR could be more reliable and repeatable for detecting vaginal discharge associated BV compared with culture test or any of those former tests alone. According to above, it is therefore logical to compare current techniques and their internal measures to find a single or appropriate combinations which lead to better and reliable results. We have to mention that we only compared Amsel's and Nugent's tests considering their limitations. The present study therefore, designed to evaluate and compare between these two major technical diagnostic methods to find which one or which combinations are more reliable and should be used for practical measures.

2. MATERIALS AND METHODS

It was a cross-sectional study conducted on 416 non-pregnant women with vaginal discharges. They were admitted in the outpatient departments of hospitals in the city of Sabzevar, north east Iran. The study was approved by the ethical research committee at Sabzevar University of medical sciences as a mandatory process. We explained the main important goals of the study and potential ethical issues to the patients and obtained their written signed consensus forms. All samples were examined and their clinical symptoms and some demographic features were recorded using a standard screening questionnaire. The discharge of the lateral walls and the posterior fornix of the vagina were collected on a dried sterile cotton wool tipped swabs. One of the swabs was pressed briefly against an indicator paper (Merck, Germany) to measure the pH of the sample (with a range of 4.0 to 6.0). The swab was then mixed with two drops of normal saline in a glass tube. Another swab with 10% KOH was used to perform Whiff test to check whether

fishy odor is present or not. All swabs were also evaluated for *Lactobacillus* and *G. vaginalis* (Gram-variable rods) and were then scored using Nugent's method. For each person swabs were also collected for Amsel's criteria test. Thus, the excessive vaginal discharge, positive Whiff test and all pH under 4.5 were examined. The presence of the clue cells on the vaginal wet smears was also examined. To compare two methods, specificity, sensitivity, negative and positive predictive values, *Kappa* measure of agreement and odd ratio (OR) were recorded (By measuring the odds ratio association between an exposure and an outcome can be interpreted). Amsel's criteria were evaluated separately and in combination when the confidence value of 90% was regarded.

3. RESULTS

A total of 416 non-pregnant women were included in the study. The average of the age was 32 ± 9 years. Four age groups were designated as follows: 15-24 (20%), 25-34 (40.7%), 35-45 (27.5%) and women over 45 years old (11.8%). The number of married women was 388 (93.3%) of those 348 were parous (83.7%). Each group's percentage compared to the total sample size is shown in brackets.

The prevalence of BV by Nugent's score methods was 8.2% (34 persons), whereas Amsel's criteria method revealed 16.8% (70 persons) positive cases. The difference between these two test was significantly different ($P < 0.005$). The mean vaginal pH was $4.79\% \pm 0.89$ for both tests.

Amsel's criteria showed that the sensitivity and specificity were 82.4% and 89% respectively. The positive and negative predictive values were also recorded as 40% and 98.3% respectively. Table 1 shows the positive and negative predictive values for each criterion of Amsel's method and in comparison to the Nugent's score results and some combinations. The OR of Nugent's score was 37.8 times greater compared to the Amsel's criteria ($p < 0.001$).

Table 1 also shows *Kappa* measure of agreement for various diagnostic values in Amsel's criteria and pH for all vaginal discharges. The results showed that the highest degree of assurance, in average, was referred to the Amsel's criteria in whole with 89.7% and 82.4% specificity along with the presence of the clue cells with sensitivity of the 89.7% and specificity

Table 1. Diagnostic values of the Amsel's criteria and each of the criterion individually and in combination

Tests alone and combinations	Sensitivity	Specificity	PPV	NPV	Kapa	Odd ratios
Amsel's criteria as a whole	82.4	89	40	98.3	48.1	37.8(14.7,96.5)*
Clue cells	89.7	82.9	28.6	99.1	36.5	42.1(12.4,143.4)*
pH	84.8	42.7	11.7	96.9	7.1	4.2(1.6,11.0)*
Homogenous discharges	67.6	38.5	9	93	1.6	1.31(.620,2.767)
Whiff test	38.2	89.5	24.5	94.2	22.1	5.27(2.5,11.3)*
Clue cells & pH	82.1	88.9	35.9	98.5	44.6	36.8(13.3, 102.1)*
Clue cells & whiff test	44.8	97.1	54.2	95.8	45.6	27.2(10.6,70.2)*
Clue cells & homogenous discharges	55.2	91	32	96.4	34.6	12.5(5.5,28.1)*
pH & Whiff	34.9	89.7	25.5	94.3	23.3	5.7(2.6,12.3)*
Whiff test & homogenous discharges	17.6	94.2	21.4	92.7	13	3.5(1.3,9.3)*
Whiff test & homogenous discharges	57.6	62.9	12.3	94.3	7.6	2.3(1.12 , 4.47)*
Clue cells & whiff test & pH	46.4	97.3	56.5	96	47.6	31(11.7,82.1)*
Clue cells & whiff test & homogenous discharges	20.7	98.4	50	94.2	26.2	16.1(4.8,53.9)*
Clue cells & pH & homogenous discharges	50	93.7	37.8	96.1	38.1	14.9(6.4,35)*

of the 82.9% when they were compared with other tests. Although Whiff test showed 89.5% specificity, its sensitivity was as low as 38.2%. The lowest specificity was for the homogenous vaginal discharge with 38.5% compared to the same values for in single or combinations tests.

4. DISCUSSION

Utilizing a proper clinical diagnostic method for BV still is one of the most challengeable and problematic clinical facts [8]. The reason is the complexity of BV due to its polymicrobial nature [2], difficulties in interpretations in diagnosis of some specimen, the cost effective and time consuming issues. Also some studies establishes that young women are more vulnerable to have BV [9] compared to other ages so the importance of this health alarming fact may kin us to find the more reliable way for diagnosis BV in women particularly at their reproductive age. Although many advanced molecular laboratory methods such as PCR, rapid nucleic acid hybridization test and proline amino peptidase activity, particularly for research purposes, have been available and developed [10,11,12], routine clinical use of those are not yet reasonable and effective enough in many ways such as cost-effective and time consuming matters. Moreover, recently various point-of-care tests based on different combinations of microbial products, presence of RNA and sensor arrays are available. But, these methods are also expensive and more importantly not yet

approved to be more sensitive or specific than traditional standardized methods. Considering the above facts, Amsel's criteria and Nugent's score methods still are the best standard choices. They are widely used in both industrialized and developing countries because they are the most viable, economical and practical tests compared to those mentioned methods.

Our results showed that the prevalence of BV was 8.2% when we used Nugent's method. However, in other studies in different regions of Iran different rates were reported when they used the same method. For example, it was 16.2%, 18%, 28.5%, and 37.7% in Zanjan, Hormozgan, Hamadan and Kerman respectively [13,14,15,16]. In other hand, when we used and referred to the Amsel's criteria the prevalence of the disease was 16.8% which was significantly higher than that when we used Nugent's method. Similar to our findings Chaljareenont and colleagues in Thailand reported the same results [17] indicating that Amsel's test could be preferred choice. We had the sensitivity and specificity of Amsel's criteria at 82.4% and 89% respectively. Therefore it seems to be the best average rate. Quite similar although there are some other studies which reported different values, they were not very far from over results which make a better point to prefer Amsel's criteria test. The mentioned studies showed that the specificity was 83.6%, 96%, 98% and 94% and the sensitivity was 84.4%, 92%, 51.4% and

70% respectively [18,19,20,21]. In some cases we saw higher sensitivity than our results but, we may reason that this fact was observed because many women who participated in our study were asymptomatic.

It has been recommended that we should have minimum 3 to 5 vaginal swabs [19] for an approved result while in reality the fact is that most clinicians only order a single swab routinely in hospitals or even in private clinics. Amsel's criteria test undesirability is because of taking more than single swab is unpleasant and time consuming to both women and laboratories [22,23,24]. Nevertheless, regarding the reliability of Amsel's method almost all researchers perform this method for the diagnosis purposes. In the present study the positive and negative predictive values for this test were 40% and 98.35% respectively. These values particularly the positive predictive value was less compared with some other studies in the different regions of Iran [22,24].

We found that the presence of the clue cells on the wet smears had the highest specificity, (82.9%), sensitivity (89.7%), and positive and negative predictive values respectively compared with Amsel's criteria. This was consistent with many other studies [22,14,25]. Our results showed that performing at least one combination of two criteria instead of a single value measurement would have a better outcome regarding sensitivity and specificity. For example, measuring of pH and the presence of the clue cells for a sample at the same time could make the diagnosis more sensitive and specific [17,23] in comparison to other combinations. Although other combinations showed a reasonable good specificity, the sensitivity was substantially low compared with the clue cells and pH combination (Table 1). Therefore, based on our results we may suggest that choosing combination of the clue cells and pH is a better, desirable and more reliable when practical and cost effective considerations cannot be ruled out and are important for any national health system. However, it is worth to say that detection of the clue cells is the most complicated criterion which requires expert clinicians. It is also expensive method and is not widely accepted as the best indicator for BV [17,26,27]. Therefore, further investigations requires to clarify which combinations, among Amsel's criteria, would be the best for diagnosis purposes. There was a lower positive predictive value in clinical diagnostic tests in Iran compared to these values

worldwide and in addition we should notice that a vast majority of gynecologists are females. Therefore, we may search through a comprehensive study with appropriate sample size to answer if any gender may influence in diagnosis BV as we have studies in which some degrees of differences have been shown in acquiring technical and conceptual skills between two genders [28].

5. CONCLUSION

Overall although many clinicians and researches prefer Amsel's test, we did not find strong reason for replacement of this method with other techniques to cover possible weaknesses. We suggest that combination of clue cells and pH is more reliable compared to other examined criteria amongst methods. This justifies that further investigations with bigger size samples requires to clarify which combinations, among Amsel's criteria, would be the best for diagnosis purposes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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