

Prevalence of bacterial vaginosis among women in Al-Diwaniya city by using Amsel's criteria and Nugent's scores

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الخلاصة

اجريت الدراسة الحالية في مدينة الديوانية للفترة من شهر كانون الاول 2012 ولغاية شهر كانون الاول 2013 من اجل التحري عن انتشار التهاب المهبل البكتيري باستخدام الصفات السريرية و نظام العد. اظهرت النتائج ان 6.25% فقط من النساء مصابات بالتهاب المهبل وفقا للصفات السريرية. كما اظهرت الدراسة ان حساسية صفات امسيل السريرية كانت واطنة (16.66%)، في حين كانت لها خصوصية عالية (95.69%). كان الاس الهيدروجيني للافراز المهبلي هو اكثر الصفات السريرية حساسية، بينما كان وجود الخلايا الطلائية المغطاة بالعصيات السالبة لصبغة غرام هو اكثر الصفات خصوصية، (100%) لكل منهما. وفقا لنظام العد Nugent's scores كانت 18 امرأة من ضمن المجموعة الاولى مصابات بالالتهاب البكتيري وهذا اعطى نسبة انتشار مساوية الى 16.07%، في حين كانت 93 امرأة بدون اصابة (بضمنها النساء اللتي لديهن فلورا مهبلية وسطية)، بالاضافة الى حالة واحدة، غير مسلجة سابقا في العراق، مصابة بالتهاب المهبل الحال للخلايا cytolytic vaginosis والتي تتميز بانخفاض الاس الهيدروجيني للافراز المهبلي و زيادة اعداد عصيات الحليب lactobacilli.

الكلمات المفتاحية: التهاب المهبل البكتيري، صفات امسيل السريرية، نظام العد لنوغنت

Abstract

This study has been conducted in Al-Diwaniya city through the period from December 2012 to December 2013, in order to investigate the prevalence of bacterial vaginosis, by using the clinical criteria and the scoring system. Results showed that only 6.25% had BV according to the clinical criteria. Accordingly, Amsel's criteria had low sensitivity (16.66%), but with high specificity (95.69%). The vaginal pH was the most sensitive criterion, while the most specific one was the clue cells (100% for both). According to Nugent's scores, 18 women in this group had BV (prevalence, 16.07%), 93 women were without this syndrome, and one women had unreported condition previously in Iraq; that is cytolytic vaginosis which is mainly characterize by low vaginal pH and an increase in numbers of lactobacilli.

Key words: Bacterial vaginosis, Amsel's criteria, Nugent's scores

Introduction

Bacterial vaginosis (BV) can simply be defined as a disturbance in the vaginal ecosystem in which the predominant lactobacilli are replaced by an overgrowth of vaginal commensal organisms (1). It may be transient or become persistent. BV is recognized as the most cause of abnormal vaginal discharge in women of childbearing age (2). The commonest presenting symptoms of women who have bacterial vaginosis is a malodorous vaginal discharge,

which is not associated with itching or irritation (1).

The reported prevalence of BV varies widely between different populations, it is affecting 10 to 37 percent of women (3). High prevalences of BV has reported in area of Sub-Saharan Africa (4,5) and among African-American women with percent of 51 (6). Some studies conducted in Iraq revealed that BV rates were between 28 and 37.5% among both pregnant and non-pregnant

women (7,8,9), while the percentage was up to 40% among infertile women (10).

Over the years a number of different methods have been used to diagnose bacterial vaginosis. In 1955 Gardner and Dukes described a close association between BV and the isolation of *G. vaginalis* from the vaginal discharge of women with this condition (11). However, as soon as it became clear that *G. vaginalis* can be recovered from about 36 to 50% of women without clinical signs of BV (12). In addition, culture of many of the other species associated with the condition was difficult and time consuming (13).

Problems with the use and interpretation of culture as a means of diagnosis led to the consideration of non-cultural methods, of these methods is the clinical criteria. The composite criteria for the diagnosis of BV were described by Amsel in 1983, the presence of at least three of four criteria are required for diagnosis of BV (14). These criteria are: a thin homogeneous vaginal discharge; a vaginal pH of more than 4.5; a positive KOH test; and presence of clue cells.

Gram staining for the scoring of morphotypes that associated with BV is another diagnostic procedure. Because the key feature in the diagnosis of BV is the absence of typical large Gram-positive bacilli (lactobacilli) and their replacement with Gram-variable or Gram-negative rods, Spiegel *et al.* tried to put this on a more systematic basis by using a scoring system of these morphotypes (15). According to this system, microbial morphotypes are quantitated under oil immersion as 1+ (< 1 per field), 2+ (1-5 per field), 3+ (6-30 per field), or 4+ (> 30 per field).

The principle of Spiegel's scoring system has been used by Nugent to develop a new scoring system which is known as "Nugent's score" (NS), where a 10-points scale was designed for assessment of vaginal flora (16). The final score is obtained by summation of

the individual points of the three categories. A score of 0-3 is representative of normal microflora, a score of 4-6 is regarded as intermediate and corresponds to a disturbed or altered microflora, and a score of 7-10 is consistent with BV.

This study was designed to detect the prevalence of bacterial vaginosis in Al-Diwaniya city by employment of both of the clinical criteria and the scoring system.

Subjects and Methods

A total of 112 women aged between 15-49 years, whom visiting the outpatient department in the Educational Hospital of Maternity and Pediatrics, in addition to some private clinics in Al-Diwaniya city, were enrolled in this study. Informed consent was obtained from all subjects, women using intrauterine contraceptive devices and those who used antibiotics or vaginal creams (during the last two weeks) were excluded. By assistance of clinicians, a sterile unlubricated speculum was inserted into the vagina and specimens were collected from the lateral vaginal wall and posterior fornix using two sterile cotton tipped swabs. Swabs were carefully removed to avoid contamination with microflora of the vulva and introitus.

An evaluation of the nature of the vaginal discharge was made by the clinician during pelvic examination. The vaginal pH has been determined directly with the use of narrow range (3.5-6) pH strips placed on the speculum after removing from vagina (17). A drop of 10% potassium hydroxide was placed on a glass slide and the first swab with vaginal fluid was stirred in the KOH drop and immediately evaluated for the presence of a fishy odour (18). Clue cells were detected during the examination of stained smears.

The second vaginal swab was used to prepare a dry vaginal smear by rolling it along the middle of a glass slide. The smear was air-dried and fixed with methanol then Gram stained (16).

The slides were examined under oil immersion objective 1000x magnification and evaluated for the following morphotypes: large Gram positive rods (*Lactobacillus* morphotypes), small Gram-variable rods (*G. vaginalis* morphotypes), small Gram negative rods (*Bacteroides* species morphotypes), small Gram-variable rods (*Mobiluncus* species morphotypes) and Gram positive cocci. Each morphotype was quantitated from 0 to 4+ with regard to the number of morphotypes per oil immersion field where 0, no morphotypes; 1+, less than 1 morphotype; 2+, 1 to 4 morphotypes; 3+, 5 to 30 morphotypes; and 4+, more than 30 morphotypes.

The final score is obtained by summation of the individual points of the three categories. A score of 0-3 is representative of normal microflora, a score of 4-6 is regarded as intermediate and corresponds to a disturbed or altered microflora, and a score of 7-10 is consistent with BV.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value were calculated in order to describe the performance of the diagnostic tests (19).

Results

Out of the 112 investigated women, only 7 (6.25%) were considered as patients with bacterial vaginosis since they matched three of Amsel's criteria. Amsel's criteria had high specificity (95.69%) and negative predictive value (85.57%) when compared with Nugent's scores as a gold standard, but they had low sensitivity (16.66%) and positive predictive value (42.85%).

The most sensitive clinical criterion was vaginal pH, which had a sensitivity of 100% (Table 1) since all patients with BV (diagnosed according to scoring system) had elevated pH between 4.8 and > 6.0. The specificity and PPV were low (40.86 and 24.65% respectively).

Table (1) Sensitivity, specificity, PPV, and NPV of Amsel's criteria and individual components in comparison to Nugent's scores as a gold standard

Amsel's criteria	No. positive	No. negative	Sensitivity%	Specificity%	PPV%	NPV%
pH	73	38	100	40.86	24.65	100
Clue cells	5	106	27.77	100	100	87.73
Discharge	17	94	11.11	83.87	11.76	82.97
Wiff test	46	65	44.44	59.13	21.05	84.41

The presence of clue cells in stained smears was the most specific criterion among the other clinical criteria (Figure 1), they had a specificity of 100% (Table 1) as they have not been detected in any subject without bacterial vaginosis. Clue cells have not been found in all or at least most of patients with

BV and as a result the test had poor sensitivity (27.77%).

The vaginal discharge also had a high specificity and NPV (83.87 and 82.97%

respectively), while sensitivity was very low at 11.11% (Table 1).

KOH test or wiff test for the detection of fishy odour associated with BV had moderate specificity (59.13%) and high NPV (84.61%) (Table 1), while the sensitivity and PPV were low (44.44 and 21.05% respectively).

According to NS, 18 out of 112 women had bacterial vaginosis, i.e. a prevalence of 16.07% (Figure 1). Ninety three women were without BV, including five subjects with intermediate flora (Figure 2) and the remaining 88 women had normal vaginal flora (Figure 3).

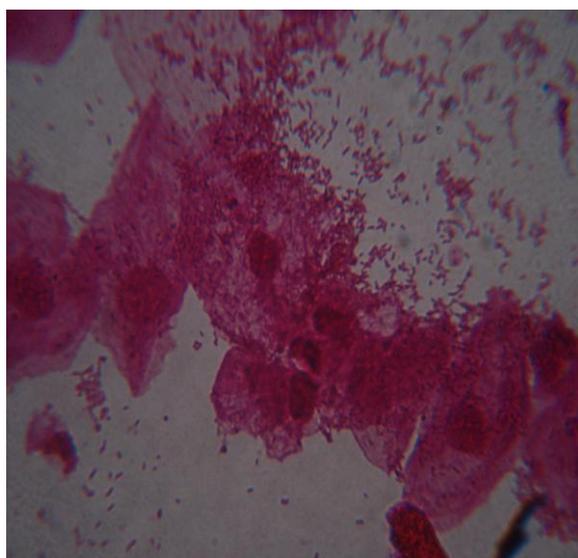


Figure (1) Vaginal smear from a patient with bacterial vaginosis shows clue cells (1000x)

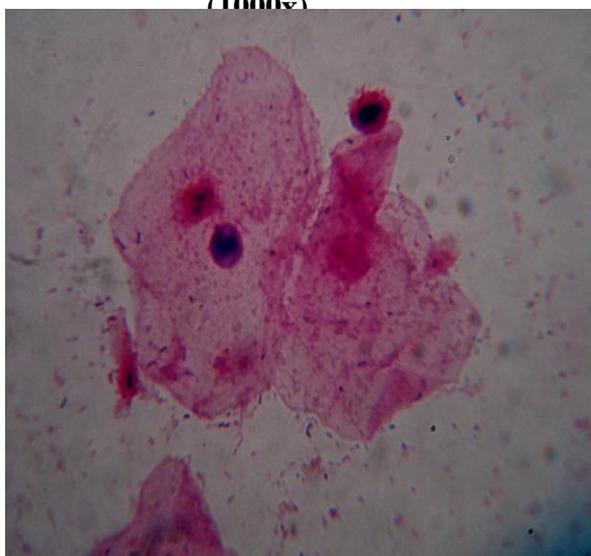


Figure (2) Vaginal smear from a women with intermediate flora (1000x)

One women, out of 112, had a condition known as cytolytic vaginosis. According to the national survey of literatures, this is the first report for such a case in Iraq. The reported case was pregnant women (14 weeks gestation), she had five parities and one previous abortion. She was complaining from itching and abnormal white yellowish discharge, which had a pH of 3.5. On Gram stained slid there was high abundance of lactobacilli, little numbers of white blood cells, and lysed epithelial cells (Figure 4).

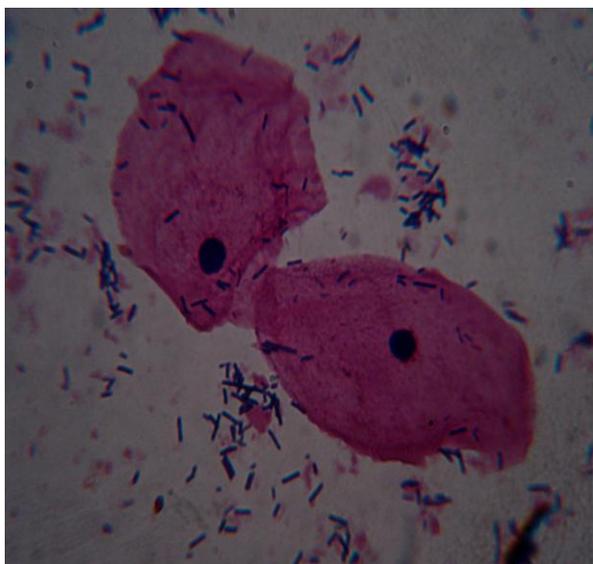


Figure (3) Vaginal smear from a women with normal flora (1000x)

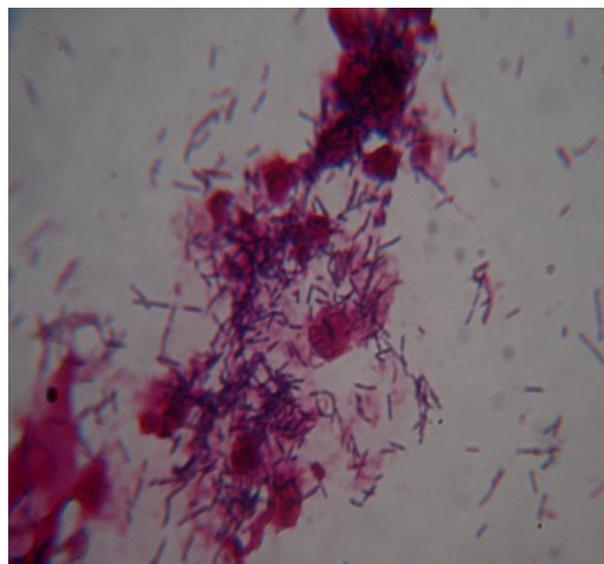


Figure (4) Vaginal smear from a patient with cytolytic vaginosis (1000x)

Discussion

According to the clinical criteria, only 6.25% of women were considered as patients with bacterial vaginosis, Accordance results have been reported by Sha *et al.* (20).

Although they can performed somewhat easily and in short time, Amsel's criteria have several disadvantages. Firstly, *Lactobacillus* species, the keyword in any definition of BV, are completely ignored in these composite criteria. Secondly, Amsel's criteria are either subjective (discharge and amine odour) or potentially difficult to judge (clue cells). Thirdly, Amsel's criteria are combination of clinical and laboratorial observations, i.e. discharge and pH are observed clinically while fishy odour and clue cells are tested in the laboratory, thus there is a needing for both a clinician and a technician at the same time.

Vaginal pH had a sensitivity of 100%, pH of vaginal discharge can be raised in response to several factors or during different situations.

Vaginal pH may elevate above 4.5 at the time of menstruation (1). In addition, semen have a pH between 7 and 8 (1) and during sexual intercourse the vaginal pH may be rise as a result of the effect of seminal fluid. Other causes of increased vaginal pH may include infections such as trichomoniasis, atrophic vaginitis (3), and desquamative inflammatory vaginitis (19), also it was found that 25% of women with a pH above 4.7 have had coccoid aerobic vaginitis (21).

pH may rise if the sample contains blood or if it was close to cervix, where cervical mucus has alkaline pH (1). In addition, douching also increases the vaginal pH (22).

The most specific criterion was the presence of clue cells in the stained smears. The microscopic analysis of clue cells is sometimes difficult, and this criterion was applied differently from mere existence to occurrence on 20% of the epithelial cells (23).

About 16.12% of women without BV, according to Nugent's scores, had abnormal discharge. Abnormal discharge is associated with other infections rather than BV such as trichomoniasis and candidiasis (24). On the other hand appearance of vaginal fluid may be altered by several factors including sexual intercourse and douching (13,17).

Wiff test had specificity and sensitivity of 59.13 and 44.44% respectively, this test, like other Amsel's criteria, is also subjective and depends on the investigator variation in the ability to detect the characteristic amine odour. Infection with *T. vaginalis* may give positive result for wiff test (25). Also false positive KOH tests can occur in women whose have had sexual intercourse recently (26). In addition, when wiff test give a positive result, as soon as sample become without amine odour due to volatility of amines quickly and completely (1).

According to the scoring system, the prevalence of BV was 16.07%. In some studies being conducted in Iraq the prevalence of bacterial vaginosis ranged from 28.6% (7) to 40.3% (10) by using Amsel's criteria as a diagnostic tool, and from 37.5% (9) to 68.7% (27) using Nugent's scores.

Differences in the methods used to detect BV status and the demographic differences (pregnancy state, fertility, presence of other infection) within subjects enrolled in these studies and our study may reflect on the variation in BV prevalence. However, in the current study the prevalence of BV was significantly higher among non-pregnant women. Agreeable results have been obtained by Al-Fadul (27), where BV rate was 86.9% in non-pregnant women vice versa 13.1% in those pregnant.

Bacterial vaginosis occurrence during pregnancy has an importance since it was shown to be associated with several obstetric sequelae such as premature rupture of membranes, preterm labour, still births, abortion, postpartum infections, and low weight infants (28,29).

Examination of Gram-stained smear has revealed that one pregnant women, out of the

112 investigated women, had cytolytic vaginosis. Hu *et al.* (30) reported that the percentage of pregnant women that have had CV was 81.80%. This may be as a result of the increased level of glucose during pregnancy, hence an increase in the numbers of lactobacilli.

Cytolytic vaginosis is sometimes misdiagnosed as candidiasis because it produces symptoms similar to that of VVC (31). However, on microscopic examination, large amounts of lactobacilli and fragmented or lysed epithelial cells are seen (32).

Cytolytic vaginosis may be confused with BV if the dominant *Lactobacillus* species is *L. iners* because it stains Gram negative and its cell morphology is rather coccobacillar than bacillar (33). The exact mechanisms leading to fragmentation or cytolysis of vaginal epithelia are not known (34).

In conclusion, the clinical criteria are subjective and has a low discriminatory value for the diagnosis of bacterial vaginosis, on the other hand the scoring system provide a more reliable diagnostic tool.

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