Prevalence of Bacterial Vaginosis in Females of Child-Bearing Age and Utility of pH and Whiff Test in Diagnosis

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Abstract

Objectives: To determine the prevalence of bacterial vaginosis among females of reproductive age and assessment of the effectiveness of pH and the Whiff test in its diagnosis.

Materials and Methods: This was a cross-sectional study. A total of 100 subjects were included, coming with a complaint of vaginal discharge. The bacterial vaginosis was diagnosed by using the Nugent scoring system, Whiff and pH tests. The prevalence was calculated by descriptive statistics and using the Chi-square test and results were shown in percentages and mean with standard deviation.

Results: The estimated prevalence of bacterial vaginosis among the local population of females of child-bearing age was found to be 39%. The mean age of females with bacterial vaginosis was 33.33±10.46 years. The Whiff test was positive in 89.74% cases, while pH was elevated in 94.87% females with bacterial vaginosis.

Conclusion: Bacterial vaginosis is a frequent cause of vaginal discharge in females of reproductive age in Pakistan. The diagnosis can be easily made by using Whiff and pH tests even at resource-poor settings.

Keywords: Bacterial vaginosis, Child-bearing age, pH test, Whiff test.
Introduction

Vaginal discharge is the most common torment complaint among females of reproductive age which can lead to physical and psychological stress among them. Bacterial vaginosis (BV) is one of the most profound reasons for vaginal discharge. It is a polymicrobial disease characterized by replacement of normal vaginal lactobacilli with several other bacteria including, Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma urealyticum, Mobiluncus species, and anaerobic Gram-negative rods belonging to the species of Prevotella, Porphyromonas, Bacteroides and Peptostreptococcus.1,2 BV is a diversified disease in terms of its clinical signs and symptoms, which can be asymptomatic at one end and being extremely exasperating at other ends. About 60% of cases of BV are symptomatic with presenting complaints of vaginal malodor along with white and viscous discharge often accompanied by pruritus, dyspareunia, and lower back pain. It is estimated that around 20-30% of females of reproductive age, attending sexually transmitted disease clinics have bacterial vaginosis is worldwide.3 This prevalence has reached up to 50-60% in high-risk populations.4 The prevalence of BV in India ranges between 13.33%-32.8%.5 An Iranian study has estimated the prevalence of BV among non-pregnant women population, which was found to be 28%.6 The BV burden among Pakistani females with preterm labour was found to be 21%.7 The exact pathology behind BV is not clear. It is suggested that disturbance in the ecology of the normal vagina, which is efficiently maintained by Lactobacillus species, leads to the development of BV.8 According to the conceptual model proposed by Schwebke9 in 2014, the main culprit for BV pathogenesis is Gardnerella vaginalis. The organism metabolically generates amino acids which in turn convert into volatile amines. These amines are conveniently utilized by other anaerobes in the vagina, thus lowering pH and facilitating the growth of Gardnerella vaginalis.10 Consequently, partially or totally replacing commensal Lactobacillus. The BV associated bacteria produce mucin degrading enzymes, which eventually degrade normal vaginal mucin gel, resulting in homogenous viscous discharge and characteristic ‘fishy’ odor. The accurate diagnosis and prompt treatment of BV are crucial as it increases the risk of preterm birth, endometritis, cervicitis, ectopic pregnancy, infertility and acquisition of sexually transmitted diseases including HIV.

The Nugent scoring system and Amsel criteria are widely accepted methods for the diagnosis of BV. The Amsel criteria include typical vaginal discharge, pH > 4.5, positive Whiff test, and the presence of clue cells. Three out of four findings confirm the BV diagnosis.10 The Amsel criteria are highly dependent on clinical signs and symptoms and standardization of the method is not easily achievable. The Nugent scoring system relies on the presence of clue cells and is considered as a single most sensitive and reliable criterion in the scoring method. In developing countries, the paucity of quality microbiological laboratory settings and skilled staff, at primary health care units is commonly seen. The clinicians usually rely on their clinical expertise for diagnosing BV which is sometimes not up to the mark. The Whiff test is one of the pivotal findings in Amsel criteria. The pH levels of the vagina are measured by using colorimetric pH sticks or pH papers. In this study, the prevalence of BV was estimated in the patients coming with the history of vaginal discharge by using simple yet handy, pH and Whiff tests.

Materials and Methods

The study was conducted at the Department of Microbiology, Basic Medical Sciences Institute in collaboration with the Department of Gynecology and Obstetrics, Jinnah Post Graduate Medical Centre, Karachi. A total of 100 non-pregnant women, presenting with complaints of vaginal discharge, were enrolled in the study. The consent was taken from the patients and the procedure was thoroughly explained. All collected information was kept highly confidential. Patients were asked about their signs and symptoms of vaginal discharge, via a pre-designed questionnaire. Patients with ongoing menstruation, HIV infection and who had used oral and topical antibiotics during the last two weeks were excluded from the study. Specimens were collected under the supervision of an experienced gynecologist. An un-lubricated Cusco’s vaginal speculum was inserted into the vagina. The color, odor and amount of discharge were evaluated. Samples were taken by using sterile cotton swabs from the posterior fornix and lateral vaginal walls. The pH was tested by pH paper (Merck, Darmstadt, Germany) and the Whiff test was performed by using 10% KOH. The test relies on the production of fishy odor on the addition of 10% potassium hydroxide (KOH) on the vaginal smear. Vaginal fluid smears were made and evaluated for the Nugent score and
presence of clue cells. The final diagnosis of bacterial vaginosis was made by assessing clinical symptoms and the Nugent score. Each bacterial morphotype was quantified under an oil immersion objective (100x) by using the scheme shown in (Table 1). Large Gram-positive rods were taken as *lactobacillus* morphotypes; small Gram-negative to Gram-variable rods was considered as *Gardnerella vaginalis* and *Bacteroides* spp, curved Gram variable rods were considered as *Mobiluncus* spp.

**Table 1: Nugent scoring of Gram-stained smear for bacterial vaginosis**

| Bacterial morphotype             | Number/oil immersion field | Nugent score* |
|----------------------------------|----------------------------|---------------|
| Gram positive rods               |                            |               |
| >30                              | 0                          |               |
| 5-30                             | 1                          |               |
| 1-4                              | 2                          |               |
| <1                               | 3                          |               |
| 0                                | 4                          |               |
| Curved Gram-negative rods        |                            |               |
| >5                               | 2                          |               |
| <1-4                             | 1                          |               |
| 0                                | 1                          |               |
| Coccobacilli and pleomorphic rods|                            |               |
| >30                              | 4                          |               |
| 5-30                             | 3                          |               |
| 1-4                              | 2                          |               |
| <1                               | 1                          |               |
| 0                                | 0                          |               |

*Total score: 0-3 Normal, 4-6 Intermediate, 7-10 Bacterial vaginosis

Data were analyzed by using statistical package for social sciences version 20. Descriptive statistics and chi-square tests were applied.

**Result**

A total of 100 women with a history of vaginal discharge were enrolled in the study. The mean age of patients with the vaginal discharge was found to be 34±11.4 years. A Majority (91%) of cases was married and 70% of them belonged to lower socio-economic group. The most common complaint was lower abdominal pain (Table 2). Apart from bacterial vaginosis, other causes of vaginal discharge were vaginal candidiasis, trichomoniasis, physiological discharge and mixed infection (non-bacterial vaginosis group). The mean age of females with bacterial vaginosis was found to be 33.33±10.46 years (Figure 1). The estimated prevalence of bacterial vaginosis by Nugent scoring system was found to be 39% (Figure 1). The Whiff test was positive in 89.7% cases of bacterial vaginosis, while 94.8% of patients of bacterial vaginosis yielded pH above 4.5, the *p*-value indicating a positive correlation between bacterial vaginosis, positive Whiff and pH tests, in comparison to non-bacterial vaginosis (Table 3).

**Table 2: Demographic profiles of patients enrolled in the study**

| Characteristics                              | Study subjects (n=100) |
|---------------------------------------------|-----------------------|
| **Age in years**                            | Mean ±SD              |
| **Marital status**                          |                       |
| Married                                     | 91%                   |
| Unmarried                                   | 9%                    |
| **Socio economic status**                   |                       |
| High                                        | 3%                    |
| Middle                                      | 27%                   |
| Low                                         | 70%                   |
| **Lower abdominal pain**                    | 56%                   |
| **Vaginal spotting**                        | 22%                   |
| **Dyspareunia**                             | 22%                   |
Figure 1: Prevalence of various causes of vaginal discharge

Figure 2: Mean ages of patients with the cause of vaginal discharge

Table 3: Correlation between bacterial vaginosis and positive pH and Whiff test¹

| Test           | Bacterial vaginosis (%) | Vaginal candidiasis (%) | Trichomoniasis (%) | Physiological Discharge (%) | Mixed Infection (%) | P-value |
|----------------|-------------------------|-------------------------|--------------------|-----------------------------|---------------------|---------|
| pH test        |                         |                         |                    |                             |                     |         |
| pH>4.5         | 37(94.87)               | 12(60)                  | 8(72.72)           | 20(95.23)                   | 7(63.63)            | 0.01    |
| pH<4.5         | 2 (5.12)                | 8(40)                   | 3(27.27)           | 1(4.76)                     | 4(36.36)            |         |
| Total          | 39 (100)                | 20(100)                 | 11(100)            | 21(100)                     | 11(100)             |         |
| Whiff test     |                         |                         |                    |                             |                     |         |
| positive       | 35(89.74)               | 2(20)                   | 9(81.81)           | 0                            | 6(54.54)            | 0.01    |
| negative       | 4(10.25)                | 18(80)                  | 2(18.18)           | 21(100)                     | 5(45.45)            |         |
| Total          | 39(100)                 | 20(100)                 | 11(100)            | 21(100)                     | 11(100)             |         |

P-value Calculated by Chi-square test
P-value <0.05 was considered significant

Discussion

Accurate diagnosis of bacterial vaginosis only on clinical grounds is quite challenging. Not only, it is a matter of one’s clinical knowledge and expertise but it also requires quality microbiological settings. The alternative methods for diagnosing bacterial vaginosis are polymerase chain reaction, rapid nucleic acid hybridization test, proline aminopeptidase activity, and point of care test.¹⁶ These tests are expensive and cost arm and leg to the patients belonging to the developing countries like Pakistan. Keeping these considerations, the pH and Whiff tests remain the most practical, rapid and economical options for diagnosing bacterial vaginosis, even at primary care units.

In our study, the mean age of females presenting with the vaginal discharge was found to be 34 ±11.26 years, which indicates that females of childbearing age are more prone to develop bacterial vaginosis. Most of the women (91%) with discharge were married. This is because of the frequent sexual practices and usage of
hormones or intrauterine contraceptive devices. Similarly (70%) of the affected females belonged to lower socio-economic strata, which indicates the delayed seeking of medical aid due to financial strains. The prevalence of bacterial vaginosis among women, presenting with vaginal discharge, was found to be 39%. The finding was in agreement with Islam et al, who reported bacterial vaginosis in 44% cases. A study by Aslam et al at Jinnah hospital Lahore reported 18.7% of cases of bacterial vaginosis. The prevalence was calculated by the Nugent scoring system, considering its gold standard in the study. The results of Whiff and pH tests were compared between bacterial vaginosis and non-bacterial vaginosis in terms of percentages; the p-value was statistically significant. The Whiff test was positive in 89.7% of cases of bacterial vaginosis, while pH test showed raised pH in 94.87% patients. Thomsan et al predicted the diagnosis of bacterial vaginosis in 94% cases by the Whiff test alone. The Whiff test being subjective is dependent upon one’s sense of olfaction. Therefore, their interpretations can vary from person to person. Similarly, Gutman et al reported 93% positivity of the Whiff test in bacterial vaginosis. They also reported that 89% of females having bacterial vaginosis had raised pH. These findings are in accordance with our results. The p-value calculated by the Chi-square test clearly indicated that these tests possess greater significance in diagnosing bacterial vaginosis from other causes of vaginal discharge. In this study, the pH test appeared to be the better method for diagnosing bacterial vaginosis in comparison with the Whiff test even at the bedside. Practicing these simple and inexpensive methods along, with the expert insight of the patient’s clinical picture, the clinician can reach up to accurate diagnosis and eventually successful treatment of this troublesome condition. They are not only budget-friendly diagnostic methods; also they do not require specialized microbiologic commodities.

**Conclusion**

Early detection of bacterial vaginosis among females of child-bearing age is highly advocated due to its deleterious effects on subsequent pregnancies. This can be efficiently achieved by using the Whiff and pH test.

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