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Utility of Vaginal pH as Point of Care Test for Detection of Bacterial Vaginosis

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ABSTRACT

Background & objectives: Bacterial vaginosis (BV) is a Reproductive tract infection (RTI) among young sexually active women with high prevalence. It is associated with complications related to pregnancy and an increased risk of acquiring STDs. This poses a need for cost-effective detection of BV in low resource settings. Hence, we propose to study the utility of vaginal pH determination for the detection of BV.

Material & Methods: This is a single center, 1 year cross-sectional study. Swabs were collected from 250 non-pregnant women attending the out-patient department of Obstetrics and Gynecology with vaginal discharge as a predominant symptom with or without backache and abdominal pain. Vaginal pH determination, Gram stain, wet mount, Whiff test, and Amsel's criteria were used for BV detection.

Results: 250 study participants with vaginal discharge suggestive of BV were analyzed. Vaginal pH was significantly higher in women with BV with the mean pH being 6.2. Vaginal pH >4.5 had a sensitivity of 85% and specificity of 66% to detect BV. The Whiff test had the least sensitivity. Clue cells and Amsel's criteria of ≥ 3 were significant for BV. A combination of pH and Whiff test performed better had high sensitivity and specificity.

Conclusions: A combination of pH determination and the Whiff test serves as a low-cost alternative in resource-poor settings for detection of BV. Though Clue cells and Amsel's criteria ≥ 3 were most sensitive and specific, they cannot be used in low resource settings. Vaginal pH alone can be used to detect BV in areas of low prevalence.

KEYWORDS: Vaginal pH, Bacterial Vaginosis, Point of care test

INTRODUCTION

Reproductive tract infections (RTIs) are a frequent cause of OPD attendance in gynecology reporting with symptoms like abdominal pain, backache, vaginal discharge, burning micturition, itching over genitalia, and dyspareunia [1, 2]. About 60% women complain of vaginal discharge, otherwise known as leucorrhoea [2]. Leucorrhoea is excessive watery/thick, white/yellow/green, purulent vaginal discharge, non-blood stained, may or may not be associated with obvious local pathology [3, 4]. It may be physiological or pathological. The most frequent causes of pathological vaginal discharge which cover almost 95% of cases are Bacterial Vaginosis, Vulvovaginal candidiasis, Trichomoniasis, and Pelvic inflammatory disease (PID) [5–7]. The vaginal microbiome harbours lactobacilli which maintain a low vaginal pH (<4.5). Vaginal dysbiosis causes scarce lactobacilli which alter vaginal pH leading to abundant growth of anaerobes like Gardnerella vaginalis, Mycoplasma hominis, Urea plasmaurealyticum and anaerobes viz Prevotella, Mobilincus and Bacteroides which form an infected biofilm [8, 9]. Vaginal dysbiosis is found to vary in different regions of the world causing variations in its prevalence depending on the geographic location, socio-economic status and ethnicity.Bacterial Vaginosis(BV)commonly presents as a malodourous discharge per vagina that is thin, grey or milky in women of reproductive age. It is one of the commonest RTIs with high prevalence in young sexually active women. Among non-pregnant women, the prevalence of BV is around 20- 47 % in developing countries. Apart from being a source of constant distress, BV causes adverse pregnancy outcomes like miscarriages, stillbirth births,preterm births, premature rupture of membranes, chorioamnionitis, ectopic pregnancy etc [10, 11]. Other complications like vault cellulitis after hysterectomy, endometritis following caserean section or abortion, infertility, chronic pelvic pain are also associated with BV [11]. It is known to increase the risk of sexually transmitted diseases like gonorrhea, trichomoniasis, and infections with Human Papilloma Virus (HPV), Herpes Simplex Virus-2 (HSV-2), and Human Immunodeficiency Virus

(HIV) Infection. Recurrence of BV is reported in approximately about 52% of women even after complete treatment requiring repeated antibiotic therapy [12, 13].

The WHO (World Health Organization)recommends an algorithm for the management of vaginal discharge. It is based on a clinical assessment to identify cervicovaginal infections and initiate syndromic management. It is however found to have low sensitivity, low Positive Predictive Value leading to over treatment [14]. Several genomic studies have demonstrated that in many of healthy women, there is a lack of lactobacilli colonies and other bacteria maintain the acidic pH of the vagina. In such women using Nugent's score may not be appropriate. Moreover, diagnostic tests for BV namely Amsel's criteria and Nugent's score need qualified personnel, are cumbersome and time-consuming [15, 16]. There is a need to evaluate low-cost, rapid diagnostic tests for BV owing to the high prevalence and burden of health problems it can cause due to delayed detection and initiation of treatment. Hence, we conducted this study to evaluate the utility of pH strips for the diagnosis of BV in our population.

MATERIAL AND METHODS

Study design- This was single-centre, 1 year prospective cross-sectional study conducted at a tertiary care teaching hospital. Our hospital is a major referral center in North Karnataka. The study was conducted from January 01, 2016 to December 31, 2016.

Study setting- This study was conducted in Department of Obstetrics and Gynecology and Department of Microbiology attached to KAHER'S Jawaharlal Nehru Medical College, Belagavi. Non-pregnant women between the age group of 18-45yrs (reproductive age group) attending Out Patient Department (OPD) of Obstetrics and Gynecology, with symptoms of excessive watery/thick, white/yellow vaginal discharge with or without associated itching, backache or abdominal pain were included in this study. The study was approved by the institutional ethics committee (MDC/DOME/385).

The sample size was calculated using the formula n= 4pq/ $d^2$where, n= sample size, p= 30 (prevalence), q= 70 (100-p), d= absolute error = 20%. Power of test at 80% with 5% significance. The sample size determined was 233.

A total of 562 women with vaginal discharge were screened, 280 women satisfying the inclusion criteria were included in the study.

Inclusion Criteria were Married/ sexually active women in the reproductive age group (18-45 years) with excessive vaginal discharge. Exclusion Criteria were Women with active vaginal bleeding, pregnancy, unmarried, history of intercourse in the last 48hrs, postnatal, post-hysterectomy status, post-menopausal, women on hormonal therapy/Oral Contraceptive Pills (OCPs), Intrauterine Contraceptive Device (IUCDs), women currently on antibiotics or who had history of antibiotic intake within the last 2 weeks, women with genital prolapse, women with a history of procedures done on the cervix (viz Trachelectomy, cervical amputation, LEEP, cryotherapy) and women with cervical lesions or vaginal growth were excluded.

Data collection: Informed consent was taken. The demographic and clinical details were collected using pre-tested proforma.

Sample collection:Per-speculum examination was done by a gynecologist under aseptic conditions. The nature of the vaginal discharge was noted. Three high vaginal swabs/vaginal secretion were collected from posterior and lateral fornices for determining pH, Wet mount, Gram staining. The pH estimation was done in OPD using standard pH strips with range of pH from 3.5 to 9. The sample was processed in the hospital laboratory for Gram staining and wet mount.

Diagnosis of BV: Gram-staining with Nugent's score >6 was used to diagnose BV. The clinical composite criteria reported by Amsel et al for diagnosing bacterial vaginosis was also evaluated were thin homogeneous discharge, vaginal pH greater than 4.5, positive Whiff test ,release of amine odor after addition of 10% KOH, and clue cells on microscopic evaluation. The presence of any three of the four Amsel's criteria confirms BV [13, 14].

RESULT

250 samples were analyzed after excluding the ones with vaginal commensals, Trichomoniasis and Candida on gram staining and microscopy. Continuous data were compared with t-test and categorical data compared with the chi-square test. Sensitivity, specificity, NPV, and PPV with the categorical variable were calculated with a 2x2 contingency table, and with continuous, variable ROC curve was plotted. Statistical analysis was done using Graph pad prism 9 and a p-value of <0.05 was considered significant.

Demographic characteristics of study participants-

The demographic data including the age, literacy, parity and socio-economic status is represented inTable 1. The mean age in our study was 31.22± 7.9yrs. 30% of women belonged to the age group of 18-25 years. A proportion of 30.4% had primary education.30.8%belonged to the middle class. The median parity was 2. No significant difference was noted in the age, education, and socioeconomic status of women with and without BV.62.2% of women had undergone a permanent sterilization procedure.

Vaginal discharge characteristics in women with and without BV

The nature of vaginal discharge was compared in women with and without BV. Table 2 represents the characteristics of vaginal discharge in both groups. Among 250 women, 221 (88%) had a thin, grey, homogenous discharge.29 (12%) of them had a thick, white, non-offensive discharge. Thin

Variables	Groups	
	BV (n=156)	**No BV (n=94)**
Age (Mean ±SD)*	30.9± 8.03	31.73±7.6
Literacy (No. & %)$		
Illiterate	8 (5.1)	7 (7.4)
Primary	46 (29.48)	30 (30.8)
Middle school	27 (17.3)	14 (14.8)
Secondary	35 (22.43)	16 (17.02)
Graduate	40 (25.61)	27 (28.73)
Median Parity	2	2
Socio-economic status		
Upper class	08	09
Upper middle class	38	39
Middle class	30	26
Lower middle class	11	09
Lower class	40	40

* p = 0.43, $ p=0.0009

Table 1: Demographic details of participants

homogeneous discharge was significantly associated with BV. A total of 141 (56.4 %) women had vaginal pH >4.5. The mean pH being 6.2. The difference in vaginal pH between women with and without BV was significant (p<0.001). Vaginal odor was not a predominant complaint in women with BV in our study. The presence of clue cells and positive whiff test were significant (p<0.001) for detection of BV. Amsel's criteria(≥ 3) was significant (P<0.001) for BV.

Comparison of the various tests for detection of BV

Sensitivity, specificity, 95% CIs, of individual tests and combination of tests for detection of BV, when compared with Nugent's score are shown in Table 3. Vaginal pH >4.5 had a sensitivity of 85% and specificity of 66% . The presence of clue cells had a sensitivity of 95% and specificity of 90% among the individual criteria. This test performed better individually for the detection of BV. Amsel's criteria had the highest sensitivity of 98% and specificity of 80% and an NPV of 99%. Among the combination criteria, Clue cells and pH >4.5 had the highest sensitivity and specificity. pH test when combined with positive Whiff test had 40% and 75% sensitivity and specificity, respectively.

To identify the sensitivity and specificity of pH (<4.5), Receiver operating curve (ROC) was plotted as shown in Figure 1 which shows that vaginal pH test was found to be significant (p=<0.0001) with the area under the curve (AUC) of 0.85.

Variables	Groups		P value
	BV (n=156)	No BV (n=94)	
Nature of discharge			
Thin homogenous	134 (85.8)	87 (92.5)	0.11
Thick white	7 (7.4)	22 (14.1)	
Vaginal odour	60 (38.4)	30 (31.9)	0.027
Diagnostic tests			
Positive Whiff test	40 (42.55)	2 (1.2)	<0.001
Clue cells present	87 (92.5)	58 (37.1)	<0.001
Amsels criteria (≥3)	93 (98.9)	30 (19.2)	<0.001
pH (>4.5)	90 (95.7)	51 (32.6)	

Table 2: Characteristics of vaginal discharge among participants

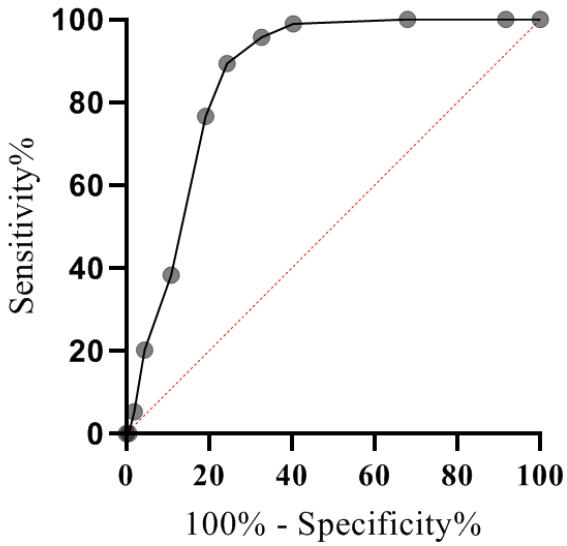

Figure 1: Area under the curve (AUC) was found to be 0.85 and test was significant (p=<0.0001)

Test	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
Whiff test	0.42 (0.33-0.52)	0.68 (0.65-0.73)	0.95 (0.84-0.99)	0.74 (0.67-0.79)
Clue cells	0.92 (0.85-0.96)	0.90 (0.85-0.95)	0.60 (0.51-0.67)	0.93 (0.86-0.96)
Amsel's criteria	0.98 (0.94-0.99)	0.80 (0.73-0.86)	0.75 (0.67-0.82)	0.99 (0.95-0.99)
p H strip >4.5	0.85 (0.80-0.90)	0.66 (0.58-0.73)	0.63 (0.55-0.71)	0.96 (0.90-0.98)
p H & Whiff test	0.94(0.83-0.99)	0.98(0.93-0.99)	0.94(0.83-0.99)	0.98(0.93-0.99)
p H & Clue cells	0.95(0.88-0.98)	0.98(0.91-0.99)	0.98(0.94-0.99)	0.73(0.60-0.78)
pH & Amsel's($\geq$ 3)	0.75(0.67-0.82)	0.99(0.94-0.99)	0.98(0.94-0.99)	0.78(0.70-0.84)

CI- confidence interval, PPV- positive predictive value, NPV- negative predictive value

Table 3: Strengths of various diagnostics methods of BV as compared to Nugent's Score.

DISCUSSION

BV is the commonest RTI in women of reproductive age group [12]. The WHO protocol on syndromic management of vaginal discharge based on clinical examination is effective but leads to over treatment [13]. We found thatnature of vaginal discharge to detect BV was only 48% sensitive to diagnose. As there could be other factors causing physiological variation of discharge, using the nature of discharge alone can lead to unnecessary medication or treatment with inappropriate drugs. Both situations can be harmful Hence, more objective tests are required to establish the diagnosis [14, 15].

In our study, pH determination was 85% sensitive and 66% specificwith NPV of 96% to detect BV as compared to the study done by Hemalata et al [16]. The high sensitivity could be because we excluded women who have cervical erosion,intermediate flora on gram staining, and those who had a history of coitus in the last 48hrs to eliminate false elevations in pH caused by semen and mucus.pH >4.5 could also mean change in vaginal microbiome with lesser lactobacilli which is permissive to growth of atypical bacteria. We found better correlation of pH and BV in pH estimates of >6.

It was observed that Whiff test had lesser sensitivity but was more specific as seen in Table 3. This could be due to need for good sense of smell to detect the amine odor. The combined pH and Whiff test were more specific for the detection of BV which was similar to a study by Posner et al. In low-resource settings, this serves as an inexpensive method of diagnosis of BV [17]. This combined test is easy to learn and interpret as not much skill is involved. Hence, it is a valuable tool in low-resource and community settings for management of BV.

Clue cells were the single most reliable indicators of BV in our study as shown in Table 3 which concurs with most studies [18]. Though it is a simple method, the need for a microscope, trained personnel on-site, and being time-consuming are its major limitations [17, 18]. This can be practical in hospital set-ups but not in low-resource or community settings.

The sensitivity and specificity of Amsel's criteria ($\geq$3) were comparable with most other studies. Combined use of pH and Amsel's improved detection to almost 98% in our study. This finding eliminates need for microbiological culture for confirmation of BV.

LIMITATIONS OF THE STUDY

Since this study was done in women who already had symptoms of vaginal discharge, the findings cannot be extrapolated to asymptomatic women with BV. Larger population based-studies would be needed to study the vaginal microbiome in our population and test the validity of vaginal pH changes in them.

CONCLUSION-

Vaginal pH determination of >4.5 is a very quick, cost-effective point of care test and sensitive test for the diagnosis of BV. It can be for rapid diagnosis of BV. Combining vaginal pH with the Whiff test is a low-cost alternative to other diagnostic tests for BV. Higher the pH, better is the detection of BV. In areas of low prevalence, vaginal pH alone can serve as a tool for detection for BV.

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Conflict of interest- There is no conflict of interest among the authors with regards to the study.

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