Use of Vaginal pH in Diagnosis of Infections and Its Association With Reproductive Manifestations

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Increase in vaginal secretion pH is an indicator of bacterial vaginosis (BV), but is yet to be in use as a diagnostic tool by clinicians. Similarly, no reports are available on the effect of cervical chlamydia infection and different reproductive manifestations on vaginal secretion pH. This study evaluated the use of vaginal pH for screening of BV, the effect of *Chlamydia trachomatis* (*C. trachomatis*) infection, and different reproductive manifestations on vaginal pH of women attending the gynecology outpatient department of a general hospital. Vaginal pH was recorded while diagnosing infections in 358 women, among which 45 were with repeated spontaneous abortion, 79 with

infertility, 185 had sign and symptoms of lower genital tract infection, and 49 had no history or symptom of any complications or infections. Normal vaginal pH, BV, and *C. trachomatis* infection were observed in 72.6, 21.5, and 10.1% of women, respectively. BV and *C. trachomatis* were observed in 78.6 and 4.1% of women, respectively, with high vaginal pH; 12.3% of women with normal vaginal pH had *C. trachomatis* infection. *C. trachomatis* infection or different reproductive manifestations do not lead to change in vaginal pH but high vaginal pH correlated with BV and should be used as a simple tool for its diagnosis. J. Clin. Lab. Anal. 22:375–379, 2008. © 2008 Wiley-Liss, Inc.

Key words: pH; infection; reproductive manifestation

INTRODUCTION

The pH of vaginal secretion varies between 3.8 and 4.5 and it remains within this range in healthy women even during the menstrual cycle (1). A pH of 4.5 or less signifies the absence of vaginitis, whereas a pH of more than 4.5 is classified as vaginitis as the presence of bacterial vaginosis (BV) leads to high pH of vaginal secretion. It has been reported that the use of vaginal secretion pH to screen BV has a greater sensitivity than its use to screen other causes of vaginitis (2). Besides pH, clinical signs and symptoms diagnose vaginitis on the basis of presence of vaginal inflammation with or without discharge. Several other factors are known to disturb this pH. Antibiotic treatment (1), presence of microorganisms, inflammatory reactions (3), and even intercourse (1) lead to the elevated pH. In postmenopausal women the pH of vaginal secretion is increased, which is attributed to the decreasing level of estrogen (4). However, subjects with evidence of vaginitis were not always asymptomatic (5–9). In these women clinical signs and symptoms alone are not appropriate for

screening of vaginitis and pH of vaginal secretion is an important factor for diagnosis. Though several methods are available for diagnosis of BV (10), most of them remain as research tools and are not available in clinical settings. Researchers have now adopted Gram stain for diagnosis of BV for its high sensitivity (11,12). In a low prevalence area as well as in resource poor settings it is not practical to have microscopic examination along with other specific diagnostics for diagnosis of vaginitis. In these situations, pH combined with clinical signs and symptoms can be used as an initial screening test for vaginitis (2). However, this has yet to become a practice among the clinicians to measure the vaginal secretion pH to detect BV. People usually delay or fail to seek

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appropriate treatment for reproductive tract infections (RTIs)/sexually transmitted infections (STIs). This at a later stage allows the manifestation of different clinical symptoms. BV, a cause of elevated pH, is a sexually associated infection; if not treated in time it can increase the risk of acquiring other infections and complications such as upper genital tract infection, miscarriage, and infertility (13–15), which are also observed with *Chlamydia trachomatis* (*C. trachomatis*) infection (16,17). Though free treatment for BV is available in public health-care facility, in India, we do not have any report that indicates the use of vaginal pH for diagnosis of BV. This is not because it is not accessible, but the clinicians never give a thought to use it as a diagnostic tool. Through this study, we have attempted to establish that measurement of vaginal pH can lead to diagnosis of BV. Simultaneously we studied the effect of *C. trachomatis* infection on vaginal pH and association of different reproductive manifestations if any with vaginal pH of women attending the gynecology outpatient department (OPD) of a general hospital.

SUBJECTS AND METHODS

Subjects enrolled ($n = 358$) in the study were women attending the gynecology OPD of King Edward Memorial (KEM) Hospital, Parel, Mumbai. Nondiabetic women without a history of sexual act on the previous night, without any medication, were only considered for the study. Written, informed consent as sanctioned by the institutional ethical committees to participate in the study was obtained from each woman before enrollment. Each woman was asked about their reproductive health history, including history of any STIs and current gynecologic symptoms, on a standard clinic form before a pelvic examination. Pelvic examination was done to record clinical signs of infection, collection of vaginal smear, measurement of vaginal pH for diagnosis of BV, and also for collection of endocervical swab for *C. trachomatis* detection. Vaginal secretion was collected with a swab from the posterior fornix after inserting the speculum. A smear for Gram's stain was immediately prepared by rolling the swab on the surface of the microscopic glass slide. The material on the slide was fixed by passing the slide rapidly through the flame of a burner three times. Gram's stain was done using commercially available stain (Qualigen Fine Chemicals, Glaxo India Limited, Mumbai, India). After staining, the slide was examined at $1,000\times$ magnification with the use of immersion oil. The vaginal flora was recorded according to Nugent's score and the presence of clue cells was noted (18). In brief, for Nugent's score calculation, each Gram-stained smear was evaluated for different morphotypes under oil

immersion ($1,000\times$ magnification). Each morphotype was quantified from 1 to 4 with regard to the number of morphotypes per oil immersion field. The absence of lactobacillus morphotype yields the highest score of 4, whereas the presence of more than 30 lactobacillus morphotypes yields a score of 0. Small Gram-variable rods (*Gardnerella vaginalis* morphotypes), small Gram-negative rods (*Bacteroides* species), and curved Gram-variable rods (*Mobiluncus*) were quantified from 1 to 4+ with regard to the number of morphotypes per oil immersion field (0, no morphotypes; 1+, less than 1 morphotype; 2+, 1–4 morphotypes; 3+, 5–30 morphotypes; 4+, 30 or more morphotypes). The scoring criteria summed the weighted quantitation to yield a score of 0–10 for each person. The criterion for BV was a score of 7 or higher; a score of 4–6 was considered as intermediate, and a score of 0–3 was considered as normal. In Gram stain scoring curved rod morphotype was given less weight because of its lower prevalence and the sense that it was seen as part of an end-stage process in patients with BV. The vaginal pH was measured in each of the women irrespective of their clinical presentation using the phenolphthalein paper (Qualigen, Glaxo India Limited; 3.5–6.0). This pH paper had been recalibrated in the laboratory for its specificity using three different buffers, i.e., 3.5, 4, and 5.0. The pH paper was placed on the surface of the speculum after it had been removed from the vagina. Approximately after 1 min the color of the strip was observed and compared with the color scale provided. A pH of 4.5 or less was classified as no vaginitis, whereas a pH of more than 4.5 was classified as vaginitis. This was an established technique for the measurement of vaginal fluid pH (1,19). The gynecologist read the vaginal pH after removing the speculum from the vagina to avoid interpersonal variation. Swab collected from the endocervix was used for *C. trachomatis* detection by polymerase chain reaction following the indigenously standardized technique (20). Statistical analysis was done using Epi Info version 6 package with Yates correction of χ^2 value.

RESULTS

Of the 358 women selected, aged 18–44 years, 185 were with clinical signs and symptoms of lower genital tract infections (LGTIs) such as vaginal discharge, cervicitis, and vaginitis with one or more children. Forty-five women had a history of two or more repeated spontaneous abortions (RSA), grouped as RSA. They did not have any child. Seventy-nine women who did not conceive within 2 years of their marriage but were trying to conceive were grouped as infertile. These three groups of women were considered as the study group,

whereas 49 women without any clinical signs and symptoms as well as with no proven infections were taken up as the control group. In this group, 93.9% of women were with normal vaginal pH (≤ 4.5), whereas in the study group 69.3% had normal vaginal pH (χ^2 : 11.69, $P < 0.0006$, Table 1).

Nugent's score of Gram stain revealed 77 (21.5%) women with BV infection. Among the women with symptoms of LGTIs, 63 had a high pH and 49 (77.8%) of them had BV, whereas 2 (3.2%) had *C. trachomatis*. Eighteen (14.8%) of 122 women with normal pH had *C. trachomatis* infection. Of the 45 women with RSA, 11 had high pH, 7 (63.6%) of them had BV infection, whereas 3 (8.8%) of the 34 women with normal pH had *C. trachomatis* infection. Three of these 7 BV-infected women did not have sign and symptoms of infection. Among the infertile women, 21 had high pH and all of them had BV infection, whereas in those with normal pH, 11 (18.97%) had *C. trachomatis* infection. Among these BV-infected women, 13 did not have sign and symptoms of infection. In total, 16 of the 77 (20.8%) BV-infected women were asymptomatic. *C. trachomatis* infection was observed in 12.3% of women with normal pH, whereas only 4.1% of women with high pH had this infection (Table 2). Among the 98 women with elevated vaginal pH, 77 (78.6%) had BV infection, whereas none of the subjects with normal pH had this infection. This indicated the positive and negative predictive values of vaginal pH to be 100 and 92.5%, respectively, when compared with Nugent's score of Gram stain for diagnosis of BV (Table 3).

TABLE 1. Women in Control and Study Groups and Their Vaginal pH

Groups	pH < 4.5	pH > 4.5	Total	χ^2	P value
Control	46	3	49	11.69	0.0006
Study	214	95	309		

TABLE 2. Sub-Groups of Subjects and Their Vaginal pH

Groups	pH<4.5					pH>4.5					Grand total
			BV +ve	BV -ve	Total			BV +ve	BV -ve	Total	
	BV +ve	Ct +ve	Ct +ve	Ct -ve		BV +ve	Ct +ve	Ct +ve	Ct -ve		
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)		
LGTI	0	18 (14.8)	0	104 (85.2)	122	49 (77.8)	2 (3.2)	0	12 (19.0)	63	185
RSA	0	3 (8.8)	0	31 (81.2)	34	7 (63.6)	0	0	4 (36.4)	11	45
Infertility	0	11 (18.9)	0	47 (81.0)	58	19 (90.5)	0	2 (9.5)	0	21	79
Normal	0	0	0	46 (100)	46	0	0	0	3 (100)	3	49
Total	0	32 (12.3)	0	228 (87.7)	260 (72.6)	75 (76.5)	2 (2.0)	2 (2.0)	19 (19.4)	98 (27.4)	358

BV, bacterial vaginosis; LGTI, lower genital tract infection; RSA, repeated spontaneous abortion.

DISCUSSION

The flora of the female genital tract varies with the pH and estrogen concentration, which is dependent on the age of the host. In this study all women were within the reproductive age group, no one had diabetes, were not on any medication, and did not have sexual act on the previous night. These inclusion criteria were taken into consideration, as these factors are known to elevate the pH of vaginal secretion (1,4). Further, though there is no report on the influence of diabetes and hormonal medication on vaginal pH, they were not included in this study to eliminate the possible role of any such unknown factors that might or might not influence the pH of vaginal secretion. Once established as a marker of BV infection, further study may be done on individuals with these types of manifestation to evaluate their influence on vaginal pH. For women in the control group, the vaginal pH remained within the normal range and all of them were without BV and *C. trachomatis* infection. A sizeable percentage (69.3%) of women with different manifestations had normal pH and 15% of them had *C. trachomatis* infection, which might be the cause of their present clinical manifestations. This correlates well with other observations (16,17). They have established that chlamydial infections of the lower genital tract in women results in minimal inflammatory reaction and can progress into the upper genital tract to produce serious complications, such as

TABLE 3. Positive and Negative Predictive Value of Vaginal pH as a Diagnostic Tool

Vaginal pH	Nugent's score		Positive predictive value (%)	Negative predictive value (%)
	BV +ve	BV -ve		
pH > 4.5	77	21	100	92.5
pH < 4.5	0	260		

BV, bacterial vaginosis.

pelvic inflammatory diseases (PID), tubal factor infertility, and ectopic pregnancy. But neither the infection nor the present reproductive manifestations had changed the pH.

The average BV infection rate was 21.5%. All 77 BV-infected women were with high vaginal pH indicating 100% positive predictive value of vaginal pH for use as a tool for diagnosis of BV. But 21 women with high vaginal pH were negative for BV except 2 with *C. trachomatis* infection, indicating a negative predictive value of 92.5%. Another factor responsible for elevated vaginal pH is estrogen deficiency. However, estrogen level has not been estimated in this study.

BV has emerged as another risk factor for upper genital tract infection. Sixty-three women with LGTIs had high pH and 49 (77.8%) of them had BV, whereas 2 (3.2%) had *C. trachomatis*. This type of association has also been reported between the clinical diagnosis of BV and upper genital tract infections, where all participants underwent either endometrial biopsy or laparoscopy to obtain objective evidence of acute upper genital tract infection (14). Another report suggests, as observed in this study among the women with RSA, complications such as late miscarriage and preterm delivery to be associated with the presence of BV (15). A link has been demonstrated between LGTIs and subclinical PID in more than 25% of women with either *Neisseria gonorrhoea* (*N. gonorrhoea*) or chlamydia infection, whereas 15% of women with BV have subclinical PID, probably leading to fallopian tube damage and infertility (21). These infections might have played a similar role in the manifestation of the present symptom such as infertility. Similarly, infection with *N. gonorrhoea*, human papilloma virus, herpes simplex virus, and *C. trachomatis* in the carrier stage presents no inflammation and remains undiagnosed but the change in vaginal pH is only related to BV infection and not related to the present clinical manifestations. Patients with elevated vaginal pH were only treated if Nugent's score was 7 or higher or those with clear signs of vaginitis on per speculum examination to eliminate overtreatment. Treated subjects were followed up to establish complete cure of the infection but long-term follow-up of these cases was not done to establish whether they develop upper genital tract infection or had preterm delivery if became pregnant.

World Health Organization recommends the use of syndromic approach in management of RTIs/STIs in symptomatic women (22). If the syndromic approach had been followed in the current group of women, 16 (20.8%) women who had BV but were asymptomatic would have missed being treated. Utilizing a simple technique such as evaluating vaginal pH would help to detect and treat these asymptomatic women.

In conclusion, our observations suggest checking of chlamydia infection in women with manifestations such as LGTI, RSA, and infertility, though they are with normal vaginal pH. On the other hand, high vaginal pH correlated well with the occurrence of BV. In developing countries microscopes are not readily available in all clinical settings and hence an easily performed pH evaluation in the OPD setting could be safely used to diagnose BV.

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