

Original Article

“COMPARATIVE STUDY TO CHECK THE EFFICACY OF pH STRIP & pH GLOVES FOR DETECTION OF BACTERIAL VAGINOSIS IN A TERTIARY CARE HOSPITAL”

MOHAMMAD NIYAZ ALAM<sup>a\*</sup>, ALOK BHARDWAJ<sup>a</sup>, MOHAMMAD QUAISUL HODA<sup>a</sup>, CHITRA GUPTA<sup>a</sup>

Faculty of Pharmacy, Ram-Eesh Institute of Vocational and Technical Education 3, Knowledge Park-1, Kasna Road, Greater Noida, Gautam Budh Nagar, U. P, India

Email: niyazpharma@yahoo.co.in

Received: 11 Mar 2015 Revised and Accepted: 21 May 2015

ABSTRACT

**Objectives:** Comparative study to check the efficacy of pH Strip & pH Gloves for Detection of Bacterial Vaginosis in a Tertiary Care Hospital.

**Methods:** It was a duly approved, cross-sectional study in which 50 subjects were enrolled from Tertiary care hospital, New Delhi, India. Written informed consent was obtained from all women. Vaginal swabs were collected for vaginal pH measurement. Vaginal pH was evaluated immediately with the pH strips and vaginal pH glove simultaneously.

**Results:** The study was done on to check the efficacy of pH strip & pH gloves in pregnant as well as non-pregnant women by regularly visiting the tertiary care hospital. Among 50 subjects 8(16%) were Pregnant and 42(84%) were Non-pregnant. The mean age of the patients included in the study was found to be 26.1±19.5 years. After a Gram stain and microscopic examination of samples obtained from 50 women, those with intermediate flora and *Candida* infection were excluded and the final analysis was done on 40 samples. Among 40, 15 were diagnosed with BV and 25 had normal vaginal flora based on Nugents' score.

**Conclusions:** Our findings show that vaginal pH determination is relatively sensitive, but less specific in detecting women with BV. Both pH glove and pH strip are equally suitable for screening women with BV on an outpatient basis. Further studies are required to explore the possibility of self-evaluation of vaginal pH with pH glove at community level.

**Keywords:** Bacterial Vaginosis, Cross-sectional study, pH Strip, pH Gloves, Nugents' score.

INTRODUCTION

Vagina is a fibro muscular elastic tubular tract which is a sex organ and has two main functions: sexual intercourse and childbirth. In humans, this passage leads from the opening of the vulva to the uterus (womb), but the vaginal tract ends at the cervix. Unlike males, who have only one genital orifice, females have two, the urethra and the vagina. The vaginal opening is much larger than the urethral opening, and both openings are protected by the labia [1]. The vagina of a newborn is affected by the residual maternal estrogen still present. At birth, the vaginal mucosa is rich in glycogen and the vagina becomes colonized by lactic-acid producing bacteria, such as *Lactobacillus* spp., within the first day after birth [2]. These estrogen effects will slowly disappear by the fourth week after birth and the glycogen content will diminish. The vaginal pH becomes neutral or alkaline, likely due to the almost absence of lactic-acid producing microorganisms [3]. The healthy vagina of a woman of child-bearing age is acidic, with a pH normally ranging between 3.8 and 4.5 [4]. This is due to the degradation of glycogen to the lactic acid by enzymes secreted by the Döderlein's bacillus. The acidity retards the growth of many strains of pathogenic microbes [5]. An increased pH of the vagina (with a commonly used cut-off of pH 4.5 or higher), can be caused by bacterial overgrowth, as occurs in bacterial vaginosis and trichomoniasis, or rupture of membranes in pregnancy [4].

The pH of the upper vagina is normally acidic (pH 3.8-4.5). Leakage of amniotic fluid (normal pH 7.0-7.5) raises the pH in vaginal fluid to >4.5. Similarly, bacterial overgrowth, as occurs in bacterial vaginosis and trichomonas, may increase vaginal pH to >4.5. Vaginal yeast infections do not change the pH of the vagina [6].

The pH paper is impregnated with the indicator dye nitrazine (phenolphthalein). The color of the paper changes from bright yellow at pH 4.5 and lower to dark blue at pH 7.0 and higher. Changes in color shades occur with pH increments of 0.5 from pH 4.5 to 7.5. The pH of vaginal fluid is determined by comparing the color of pH paper that has come in contact with a fluid sample to a standard color chart provided with each roll of pH paper [7].

Bacterial Vaginosis (BV) is the most common vaginal infection among women in reproductive age. It is a condition of vaginal flora imbalance, in which the typically plentiful H<sub>2</sub>O<sub>2</sub> producing lactobacilli are scarce and other bacteria such as *Gardnerella vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum* and anaerobes (*e. g. Prevotella*, *Mobiluncus*, *Bacteroides*) are overly abundant. [8, 9]. Bacterial vaginosis (BV) is a vaginal infection characterized by loss of the normal protective lactobacilli and overgrowth of diverse anaerobes [15]. This infection is one of the leading causes of vaginal discharge and is more prevalent in HIV-1-infected women compared to uninfected women.

Women with BV are at higher risk of infection with human papilloma virus (HPV), Herpes simplex virus type 2 (HSV-2), *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and HIV [10, 11]. Given the high prevalence and gravity of associated morbidity, it is critical to diagnose and treat women, particularly pregnant women affected by BV appropriately. Conventional diagnostic methods for BV include the methods of Amsel criteria and Nugent. [12, 13]. An easy, rapid and inexpensive self-diagnostic test for BV may help to minimize the tendency to self-treat symptomatic BV blindly with antibiotics or treating inappropriately. Assessment of intra vaginal pH is a helpful, but frequently neglected procedure that can be used to evaluate vaginal health [14]. Bacterial vaginosis (BV) is highly prevalent among women in the reproductive age group.

Vaginal pH is a useful indicator for bacterial vaginosis. pH more than 4.5 of vaginal fluids is found to be indicative of bacterial vaginosis. In this regards pH determination if made a part of routine gynaecological examination can serve as a useful tool for preliminary diagnosis of bacterial vaginosis, moreover when the gloves, being used for routine examination are themselves pH sensitive and no additional procedures need to be carried out. Further these gloves can prove handy in self examination of vaginal pH and early detection of infections. The results of the study may be useful in developing a self diagnostic kit for bacterial vaginosis. Thus, pH determination during gynaecological examination which

has long been ignored can be made an integral part of routine gynaecological examination with availability of such gloves.

## MATERIALS AND METHODS

### Study population

In this cross-sectional study 50 subjects were enrolled from Gynaecological outpatient Clinics of Tertiary care Hospital, New Delhi, India, between 8<sup>th</sup> December 2013 to 8<sup>th</sup> March 2014 these women visited the hospital for complaints such as white discharge, abdominal pain and back ache. Informed written consent was obtained from all women. The study protocol was approved by the Institutional Ethics Committee of Ram-Eesh Institute of Vocational and Technical Education, Greater Noida. Women with history of gynaecological cancer/who had bleeding during the examination/used antibiotics/vaginal medication during the previous three weeks or those who had sexual intercourse in the last two days were excluded from the study.

### Sample collection

Vaginal swabs were collected for vaginal pH measurement, Gram stain, wet mount, and whiff test. Vaginal pH was evaluated immediately with the pH strips and vaginal pH glove simultaneously. Samples for Gram stain were collected and a smear was performed. Gram staining and Nugent scoring were done after transporting the smears to the laboratory with microscope facility (within 2 to 3 h of sample collection). The pH of secretions collected from the lateral vaginal wall was measured using a colour indicator ranging from 3.5-5.2. Secretions collected with other cotton tip applicator from the lateral wall were smeared on to a glass slide for Nugent Gram stain evaluation.

### Quality control

Quality control assessment was done for gloves as well as pH paper

### Statistical analysis

Descriptive statistics were calculated for all demographic and clinical variables. Patient characteristics were compared between women with and those without Bacterial Vaginosis by using the Student *t* test and Wilcoxon rank-sum test for continuous data and Chi-square test for non parametric categorical data.

## RESULTS

The study was done on to check the efficacy of pH strip & pH gloves in pregnant as well as non-pregnant women by regularly visiting the tertiary care hospital. Among 50 subject 8 (16%) were Pregnant and 42(84%) were Non-pregnant (table 1). The mean age of the patients included in the study was found to be 26.1±19.5 years. After a Gram stain and microscopic examination of samples obtained from 50 women, those with intermediate flora and *Candida* infection were excluded and the final analysis was done on 40 samples. Of the 40 women, 15 were diagnosed with BV and 25 had normal vaginal flora based on Nugent's score. The mean age (in years) and body mass index (BMI in kg/m<sup>2</sup>) of the subjects were 26.1 and 22.2, respectively. A proportion of 33.3 per cent women were illiterate and 17.8 per cent had primary education. Illiterate women and women with only primary education had parity more than two compared to women with higher education ( $P<0.05$ ). Majority (79.6%) of the women had adopted tubectomy as a sterilization method.

Vaginal discharge was the symptom in 84.8 per cent of the subjects with BV and 85.3 per cent with no BV. Cervical erosion incidence was similar in women with and without BV. However, women with cervical erosion had more frequent abnormal vaginal discharge or thin homogenous discharge on examination ( $P<0.0001$ ) compared to women with healthy cervix. Similarly, laboratory diagnosed vaginal white blood cells by microscopy was more frequent ( $P<0.05$ ) in women with cervical erosion compared to women with healthy cervix.

Overall, women had vaginal pH>4.5, when measured with pH strips and pH glove respectively. The mean vaginal pH in women with BV measured by pH strips and pH glove was 5 and 4.9, respectively, and the difference in vaginal pH between BV and normal women was

significant ( $P<0.001$ ). Presence of clue cells and positive whiff test were significant ( $P<0.001$ ) for BV. Women with vaginal WBC>5 had vaginal pH>4.5 ( $P=0.002$ ). In the present study 3 (1.1%) women were infected with *Trichomonas*, but there was no significant difference between the groups.

Vaginal pH>4.5 detected by pH strips and pH glove had a sensitivity of 72 and 79 per cent, and a specificity of 60 and 53 per cent, respectively (table 2). Amsel's criteria had 76.6 and 95 per cent sensitivity and specificity, respectively. Among the combination criteria, clue cells and glove pH>4.5 had highest sensitivity and specificity. pH test when combined with positive amine (whiff) test had 46 per cent and about 75 per cent sensitivity and specificity, respectively. Thin homogenous discharge had the lowest specificity (27%). The positive predictive values (PPV) of pH strip (pH>4.5) was 71 per cent (confidence interval: 0.63-0.78) and the negative predictive values (NPV) was 62 per cent (confidence interval: 0.52-0.71) when compared with Nugent score.

## DISCUSSION

Vaginal pH of more than 4.5 was less than 80 per cent sensitive in diagnosing BV, that may be accurate only 60 per cent of the time. Inclusion of whiff test along with pH test further reduced the sensitivity, but improved specificity. The difference in mean pH measurement between the two methods (pH glove and pH strip) was not significant.

Reproductive tract infections continue to cause considerable morbidity among women. Our results confirmed the findings that women with bacterial vaginosis were more likely to have vaginal symptoms, specifically foul odour in comparison to healthy women. In resource-poor settings, the World Health Organization (WHO) syndromes management protocol for vaginal discharge is most commonly used to diagnose vaginal infections. It is based on clinical assessment with speculum examination only [16]. Though, this protocol is found to be effective in management of abnormal vaginal discharge [17], it is well known that cervical erosion can be associated with excessive non-purulent vaginal discharge due to the increased surface area of columnar epithelium containing mucus-secreting glands. The results of the present study confirmed that women with cervical erosion had vaginal discharge as the symptom in comparison to women with healthy cervix.

A previous study showed that the evaluation of pH plus amine (whiff) test was better than syndromes management protocols and easiest to implement in resource-poor settings [18]. Our results also confirm the same. In the present study 84.8 per cent women presented with abnormal vaginal discharge, but only 56.8 per cent of them were positive for BV. The sensitivity and specificity of Amsel criteria in our study were better than previous study [18, 19]. Our results demonstrated that clue cells were the most reliable single indicator for BV as reported previously [20]. However, identification of clue cells requires on-site microscopy facility, trained personnel and time.

Though the sensitivity of vaginal pH in detecting BV was considerably lesser, the specificity was much higher in the current study compared to an earlier study [21]. False elevations in pH can be encountered when semen and mucus were sampled, exclusion of women who had coitus in the previous two days might have contributed to improved specificity and an exclusion of women with *Candida* infection to improved negative predictive value in the present study. However, exclusion of women with intermediate flora, which is not practical, might have falsely contributed to better specificity. Contrary to our study, pH and whiff test together had a high sensitivity and specificity in previous study [21].

Whiff test seems less practical and requires a good sense of smell [22]. However, inclusion of whiff test along with pH test improves specificity. A study in a population with low prevalence of BV showed correlation of high vaginal pH with BV and suggested vaginal pH as a simple tool for the diagnosis of BV [23].

Self-sampling the vagina seems to be acceptable to women of multiple ethnic groups [24]. Self-sampling of vaginal pH seems suitable for implementation before using over the counter products for presumed vaginitis [24]. Moreover, a better informed self-

diagnosis would ultimately reduce delayed treatment and possible secondary complications. A major limitation of the study was exclusion of women with intermediate flora that might have contributed to better sensitivity and specificity of the pH test.

## CONCLUSION

Our findings show that vaginal pH determination is relatively sensitive, but less specific in detecting women with BV. Inclusion of whiff test along with pH test may reduce the sensitivity, but improves specificity. Both pH glove and pH strip are equally suitable for screening women with BV on an outpatient basis. Further studies are required to explore the possibility of self-evaluation of vaginal pH with pH glove at community level.

## ACKNOWLEDGEMENT

All authors acknowledge the full support of Ram-Eesh Institute of Vocational and Technical Education, 3, Knowledge Park-1, Kasna Road, Greater Noida, for providing the infrastructure to conduct the study.

**Table: 1 Distribution characteristics among study population**

Subjects	No of patients	% of patients
Pregnant Women	08	16
Non-Pregnant Women	42	84
Total	50	100

**Table 2: Sensitivity, specificity, and 95% confidence intervals of the clinical criteria for diagnosing bacterial Vaginosis**

Parameter to be assessed	Bacterial Vaginosis N=35	No Bacterial Vaginosis N=15	P value	Total N=50
Age (Yr) (mean±SD)	26.1±4.8	27.8±5.2	0.386	27.0±4.0
BMI (Kg/m <sup>2</sup> )	22.5±5.1	22.0±5.0	0.996	22.2±5.1
Foul smelling odor	15(43.5)	5(32.2)	0.035	20 (37.8)
Vaginal Discharge	30 (84)	13(85)	0.835	43 (84.8)
pH Gloves(mean±SD)	4.9±0.35	4.6±0.135	0.001	
pH Strip (mean±SD)	5.0±0.54	4.6±0.53	0.001	
Clue cells present	33(95.5)	2(10.3)	0.001	35 (58.9)
Positive whiff test	16(46.1)	3(21.6)	0.001	19 (35.6)
Amsels Criteria	26(76.6)	2(13.8)	0.001	28 (49.6)
<i>Trichomonas</i>	2 (1.3)	1(0.9)	0.735	3 (1.1)
Vaginal WBC>5	7(20.8)	3(18.1)	0.584	10 (19.6)
pH Paper>4.5	25(72.1)	6(39.7)	0.001	31 (58.1)

## CONFLICT OF INTERESTS

Declared None

## REFERENCES

- Gorodeski GI, U Hopfer, CC Liu, E Margles. Estrogen acidifies vaginal pH by up-regulation of proton secretion via the apical membrane of vaginal-ectocervical epithelial cells. *Endocrinol* 2005;146:816-24.
- Verstraelen H. Bacterial vaginosis: a sexually enhanced disease. *Int J STD AIDS* 2008;19:575-6.
- Cauci S, S Driussi, D De Santo, P Penacchioni, T Iannicelli, P Lanzafame, et al. Prevalence of bacterial vaginosis and vaginal flora changes in peri-and postmenopausal women" *J Clin Microbiol* 2002;40:2147-52.
- Manetta A, Pinto JL, Larson JE, Stevens CW, Pinto JS, Podczaski ES. Primary invasive carcinoma of the vagina. *Obstet Gynecol* 1988;72(1):77-81.
- Martin R, JE Suarez. Biosynthesis and degradation of H2O2 by vaginal lactobacilli. *Appl Environ Microbiol* 2010;76:400-5.
- Simoes JA, Discacciati MG, Brolazo EM, Portugal PM, Dini DV, Dantas MCM. Clinical diagnosis of bacterial vaginosis. *Int J Gynecol Obstet* 2006;94:28-32.
- Davidson KM. Detection of premature rupture of the membranes. *Clin Obstet Gynecol* 1991;34(4):715-22.
- World Health Organization, Geneva. Sexually transmitted and other reproductive tract infections; 2005. p. 11-21.
- Thorsen P, Jensen IP, Jeune B, Ebbesen N, Arpi M, Bremmelgaard A, et al. Few microorganisms associated with bacterial vaginosis may constitute the pathologic core: a population-based microbiologic study among 3596 pregnant women. *Am J Obstet Gynecol* 1998;178:580-7.
- Gillet E, Meys JFA, Verstraelen H, Bosine C, Sutter DP, Temmerman M, et al. Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. *BMC Infect Dis* 2011;11:10-6.
- Cherpes TL, Meyn LA, Krohn MA, Lurie JG, Hillier SL. Association between acquisition of Herpes simplex virustype 2 in women and bacterial vaginosis. *Clin Infect Dis* 2003;37:319-25.
- Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK. Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983;74:14-22.
- Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improving by a standardized method of Gram stain interpretation. *J Clin Microbiol* 1991;29:297-301.
- World Health Organization, Regional Office for the Western Pacific, Manila. Syndromic case management of STD; 1997.
- Eschenbach DA, Hillier S, Critchlow C, Stevens C, DeRouen T, Holmes KK. Diagnosis and clinical manifestations of bacterial vaginosis. *Am J Obstet Gynecol* 1988;158(4):819-28.
- Choncleying V, Skor S, Kemapunmans M, Law M, Creator A, Rowe P. Evaluation of two clinical protocols for the management of woman with vaginal discharge in Southern Thailand. *Sexually Transmitted Infections* 1998;74:194-201.
- Posner SF, Kerimova J, Aliyeva F, Duerr A. Strategies for diagnosis of bacterial vaginosis in a resource-poor setting. *Int J STD AIDS* 2005;16:52-5.
- Schwebke JR, Hillier SL, Sobel JD, McGregor JA, Sweet RL. Validity of the vaginal gram stain for the diagnosis of bacterial vaginosis. *Obstet Gynecol* 1996;88:573-6.
- Thomason JL, Gelbart SM, Anderson RJ, Walt AK, Osypowski PJ, Broekhuizen FF. Statistical evaluation of diagnostic criteria for bacterial vaginosis. *Am J Obstet Gynecol* 1990;162:155-60.
- Thulker J, Kriplani A, Agarwal N. Utility of pH test & Whiff test in syndromic approach of abnormal vaginal discharge. *Indian J Med Res* 2010;131:445-8.
- Pavletic AJ, Hawes SE, Geske JA, Bringe K, Polack SH. Experience with routine vaginal pH testing in a family practice setting. *Infect Dis Obstet Gynecol* 2004;12:63-8.
- Pramanik JM, Kerkar SC, Mehta PB, Potdar S, Salvi VS. Use of vaginal pH in diagnosis of infections and its association with reproductive manifestations. *J Clin Lab Anal* 2008;22:375-9.
- Forrest S, Mc Caffery K, Waller J, Desai M, Szarewski A, Cadman L, et al. Attitudes to self-sampling for HPV among Indian, Pakistani, African, African-Caribbean and white British women in Manchester, UK. *J Med Screening* 2004;11:85-8.
- Ferris DG, Francis SL, Dickman ED, Miler-Miles K, Waller JL, McClendon N. Variability of vaginal pH determination by patients and clinicians. *J Am Board Family Med* 2006;19:368-73.