

Reliability of conventional Papanicolaou smear in diagnosing bacterial vaginosis among women with clinical genital infection

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Abstract

Objective: Bacterial vaginosis (BV) is a common reproductive tract infection (RTI) reported among Indian women. BV can influence the persistence of high-risk oncogenic human papillomavirus, a causative factor for cervical cancer. BV and cervical cancer are major public health issues in a developing country like India. It becomes important for a resource-constrained country like India with poor healthcare access to implement control measures to screen and treat RTI in an attempt to prevent the risk for cervical cancer. Papanicolaou (Pap) smear is an established screening tool for cervical cancer and the diagnosis of RTIs, forms a part of its evaluation. The present study explores the validity of conventional Pap smear in diagnosing BV. **Methodology:** Pap smear and Gram stain smear were collected for 254 women with clinically evident cervicitis/cervicovaginitis (genital infection). Using the Nugent score on Gram stain as a gold standard, we determined the sensitivity and specificity of Pap smear to diagnose BV. **Results:** The overall prevalence of BV in the study population was 44% using the Nugent score. Pap smear showed sensitivity and specificity of 70.9% (CI – 61.5% – 79.2%) and 56.8% (CI – 48.2%–65.2%), respectively. The positive predictive value of Pap smear to diagnose BV was 56.5% (CI – 47.8%–64.9%), and the negative predictive value was 71.2% (CI – 61.8%–79.4%). **Conclusion:** In the present study, conventional Pap smear demonstrates good accuracy to detect BV. Pap testing for cervical cancer screening can additionally serve as an effective screening tool for diagnosing BV among women with genital infection in healthcare settings.

Key words: Bacterial vaginosis, cervicitis, human papillomavirus, Nugent score, Papanicolaou smear

Introduction

The midterm report of the National AIDS Control Organization (NACO), India, which was based on a review of published and unpublished population-based studies, reported bacterial vaginosis (BV) to be the most common reproductive tract infections (RTIs) among Indian women.^[1] A huge burden of symptomatic/asymptomatic BV is reported worldwide.^[2,3] There is emerging evidence of a strong association between cervicitis and BV.^[4,5]

BV infection needs to be addressed as it has the potential to cause maternal morbidity due to its association with common conditions such as pelvic inflammatory disease, chorioamnionitis, and preterm labor in women.^[6] Due to the change in vaginal microbiological flora and inflammation associated with BV, it may help in acquiring and transmitting human papillomavirus (HPV) infection which is the main cause for cervical cancer. The inflammation of the cervix causes break in the cervical epithelium helping the HPV to gain entry in the actively proliferating basal cells of the cervical epithelium.^[7] Inflammation is known to cause DNA damage of the host cell leading to the integration of viral DNA, which leads to gradual progression of HPV infection to microinvasion and invasive cervical cancer. BV may serve as a cofactor for the persistence of high-risk HPV, thus diagnosing and treating BV infection may help to reduce the risk of cervical cancer in women.^[8-12]

Papanicolaou (Pap) smear beside an established screening test for the detection of cervical precancerous lesions is also posed to diagnose sexually transmitted infections (STI)/reproductive transmitted infections (RTIs).^[13] Pap smear has the potential

to serve as a cost-effective tool for dual screening of BV and cervical cancer which are the most common cause of morbidity and mortality reported among Indian women.^[1,14] Among the RTIs, the role of Pap smear to diagnose BV has conflicting results reported from several developed and developing countries.^[3,15-20] There is limited evidence from India about the accuracy of Pap smear in detecting BV. To the best of our knowledge, the only community-based study conducted from India demonstrated encouraging results of Pap smear to diagnose BV infection with sensitivity of 78%.^[3] The present study evaluates the accuracy of Pap smear to diagnose BV infection in women with clinically evident genital infection using the Nugent score on Gram-stained smear as the gold standard.^[21-23] The microbiology evaluation to diagnose BV infection by the Nugent score done for the women enrolled in the present study is a part of the main study which looks into “Performance of HPV DNA test in the presence of coinfection with common RTIs”.

Methodology

The study design is a prospective blinded cross-sectional study of women in the reproductive age group who presented for routine cervical cancer screening in a tertiary care institute between August 2016 and August 2018.

The inclusion criteria for the study were nonpregnant women in the age group of 30–50 years and having clinically evident cervicitis/cervicovaginitis (genital infection) on per speculum examination. The case definition of cervicitis was unhealthy cervix with the presence of cervical erythema and inflammation that bleeds on touch with mucopurulent/purulent discharge. Among 3900 women visiting the department of cancer screening during the period from August 2016 to

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August 2018, 2407 nonpregnant women in the age group of 30–50 years were screened for the eligibility criteria of clinically evident genital infection on per speculum examination. Two hundred and fifty-four women who satisfied the eligibility criteria were consented for recruitment in the study.

The women were then interviewed for sociodemographic data, reproductive history, medical history, and symptoms pertaining to STIs/RTIs such as white discharge per vaginam, lower abdominal pain, burning micturition, dyspareunia, and postcoital bleeding. The information was captured on a prestructured, validated proforma.

Smears of cervicovaginal discharge for Gram stain followed by Pap smear were taken for all the women enrolled in the study. All the women received treatment for genital infection(s).

Conventional Pap smears were obtained using sterilized, moistened cotton-tipped swab sticks, by standardized procedure, as a part of the routine screening test for cervical cancer. The Pap smear was collected from the transformation zone, lateral vaginal wall, and endocervix. The cytopathology department evaluated the Pap smears by the Bethesda system 2014.^[13] The cytologist was blinded to the clinical per speculum examination findings and the Nugent score on Gram stain.

Pap smear criteria for diagnosing BV was: “individual squamous cells covered by a layer of coccobacilli that obscure cell membrane, forming the so-called clue cells. The presence of a large number of inflammatory cells representing vaginitis with the conspicuous absence of lactobacilli” [Figure 1].

Sterile soft cotton-tipped swab stick was used to collect the discharge for Gram staining. The cervicovaginal swab was collected by inserting the cotton-tipped swab stick in the endocervix and rotated to collect the endocervical discharge, and the vaginal discharge was collected from posterior fornices and lateral vaginal walls for all women enrolled in the study. The discharge was uniformly spread on a glass slide. The smears were heat fixed and transferred to the Microbiology Department of collaborating institute for Gram staining and evaluation. The microbiologist was blinded to Pap smear results and clinical per speculum examination findings.

The gram-stained slides were evaluated for BV using the Nugent scoring system. The slides were read at $\times 1000$ using oil immersion. The Nugent score uses a system of scoring points allotted to number of different bacteria present in the sample,

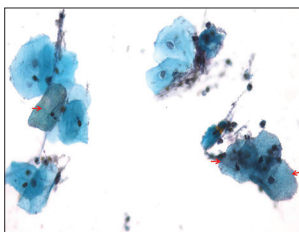


Figure 1: Papanicolaou smear showing superficial and intermediate squamous epithelial cells studded with coccobacilli giving the characteristic “Clue cells” appearance diagnostic of bacterial vaginosis (Papanicolaou, $\times 200$)

different bacterial morphology, and amount of lactobacillus present. The score ranges from 0 to 10. A total score of 0–3 is considered as normal vaginal flora, a score of 4–6 is classified as intermediate flora, and a score of 7–10 is considered to be consistent with a diagnosis of BV.

Ethical clearance

The main study was a collaborative study between the Department of Microbiology of Municipal General Hospital and cancer screening department of a tertiary cancer hospital. The study protocol was reviewed and approved by the Institutional Review and Ethics committees of participating centers.

Statistical analysis

The data were captured and analyzed using the Statistical Package for the Social Sciences (SPSS-v24) for frequency distribution. The test characteristics in terms of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Pap smear were calculated using Stata 13.0.

Results

A total of 254 women with clinically evident genital infection on per speculum examination were enrolled in the study. The mean age of women was 38 years. Among the women enrolled in the study, 204 (80.3%) women were symptomatic for STIs/RTIs complaints, whereas 50 (19.7%) were asymptomatic. One hundred and twelve (44.1%) women were diagnosed with BV by the Nugent score whereas Pap smear reported BV in 138 (55.4%) women [Table 1].

Among 254 women, five (2%) women had Pap smear reported inadequate for evaluation. Eighty-nine (35%) women had normal/inflammatory smear reported without infection. Overall in the study population ($n = 254$), Pap smear reported BV infection in 138 (54.3%) women and epithelial cell abnormality in 46 (18.1%) women. Thirty-one (12.2%) women among the study population had BV infection associated with epithelial cell abnormalities reported on Pap smear. Candida infection or trichomoniasis infection was reported in seven (2.8%) women [Table 2].

Using the Nugent score for diagnosing BV as the gold standard, Pap smear showed sensitivity and specificity of 70.9% (confidence interval [CI] – 61.5%–79.2%) and 56.8% (CI – 48.2%–65.2%), respectively.

Table 1: Prevalence of bacterial vaginosis among women with clinical evident genital infection

Clinical symptoms and diagnostic results	<i>n=254, n (%)</i>
Clinical symptoms of cervicitis	
Symptomatic	204 (80.3)
Asymptomatic	50 (19.7)
BV diagnosis by Nugent score on gram stain*	
BV positive (score 7-10)	112 (44.1)
BV negative (score 0-6)	142 (55.9)
BV diagnosis by conventional Pap smear as per the Bethesda classification	
BV positive (shift in flora suggestive of BV)	138 (55.4)
BV negative	116 (45.7)

*Nugent score: Score of 0-3=Normal vaginal flora, Score of 4-6=Intermediate flora, Score of 7-10=BV. BV=Bacterial vaginosis, Pap=Papanicolaou

Table 2: Distribution of Papanicolaou smear results among women with clinically evident genital infection

Category of Pap smear by the Bethesda classification	n=254, n (%)
Inadequate for evaluation	5 (2)
Normal/inflammatory smear with no infection	89 (35)
Total BV-positive smear reported	138 (54.3)
Total epithelial cell abnormalities reported	46 (18.1)
ASCUS	13 (5.1)
LSIL	26 (10.2)
HSIL	7 (2.8)
BV with epithelial cell abnormalities (ASCUS, LSIL, HSIL)	31 (12.2)
Other single RTIs (candida/trichomoniasis)	7 (2.8)

RTIs=Reproductive tract infection, ASCUS=Atypical squamous cells of undetermined significance, LSIL=Low-grade squamous intraepithelial lesion, HSIL=High-grade intraepithelial lesion, BV=Bacterial vaginosis, Pap=Papanicolaou

Table 3: Comparison of Papanicolaou smear and Nugent criteria for the diagnosis of bacterial vaginosis

Pap smear report	Nugent score* on gram stain		Total
	Score 7-10 (positive)	Score 0-6 (negative)	
BV positive	78	60	138
BV negative	32	79	111
Total	110	139	249

*Nugent score: Score of 0-3=Normal vaginal flora, Score of 4-6=Intermediate flora, Score of 7-10=BV. Using Nugent score on gram stain for diagnosing BV as the gold standard, Pap smear showed sensitivity 70.9% (CI - 61.5%-79.2%), specificity 56.8% (CI - 48.2%-65.2%), positive predictive value 56.5% (CI - 47.8%-64.9%), and negative predictive value 71.2% (CI - 61.8%-79.4%). BV=Bacterial vaginosis, CI=Confidence interval, Pap=Papanicolaou

PPV was 56.5% (CI - 47.8%–64.9%) and NPV was 71.2% (CI - 61.8%–79.4%) [Table 3].

Discussion

As per the NACO 2012 report, the prevalence of BV infection among Indian women varied between 17.8% and 63.7%.^[1] In the present study population, the prevalence of BV infection was 44.1% by the Nugent score. About 19.7% of women were asymptomatic, which indicates a possibility of large number of undiagnosed asymptomatic genital infections among Indian women [Table 1]. BV infection has the potential to serve as a cofactor for persistent high-risk HPV in the pathogenesis of cervical precancerous/cancerous lesions.^[9] Among the present study population, 18.1% ($n = 46/254$) women reported epithelial cell abnormalities on the Pap smear [Table 2]. Among 46 epithelial cell abnormalities (ASCUS and above lesions) reported on Pap smear, 67.3% ($n = 31/46$) of patients were reported to be associated with BV infection demonstrating the need to treat BV infection.

Pap smear is an established screening tool to detect epithelial cell abnormalities associated with cervical precancerous/cancerous lesions. The Amsel criteria and Nugent score are the two most commonly evaluated gold standard methods to diagnose BV infection.^[3,15,17-19,24-26] The aim of the present study was to evaluate the test characteristics of Pap smear as a screening tool for diagnosing BV infection using a Nugent score of 7 and above.

The 1991 Bethesda system for reporting cervicovaginal cytology stated predominance of coccobacilli to be consistent with the shift of vaginal flora but was not

sufficient to diagnose BV infection.^[27] Later, Prey *et al.* demonstrated that 96% of women showing a predominance of coccobacilli on Pap smear also had reported BV infection on Gram stain.^[15] Tokyol *et al.* in their study reported the sensitivity and specificity of Pap smear in diagnosing BV to be 43.1% and 93.6%, respectively, using the Nugent score as the gold standard.^[18] The low sensitivity of Pap smear to diagnose BV infection may be attributed to evaluating cervical swab instead of the vaginal swab for diagnosing BV infection on Gram staining since BV is primarily a vaginal infection.^[19]

Compared to other studies,^[17-19] the present study reported an improved sensitivity of 70.9% and specificity of 56.8% of Pap smear to diagnose BV infection. The PPV and NPV of Pap smear to diagnose BV was 56.5% and 71.2%, respectively [Table 3].

The higher sensitivity of Pap for detecting BV infection in the present study may be attributed first to the standard procedure of performing Pap smear as per protocol of the institute to include the area of transformation zone, vagina, and endocervix, and hence that Pap smear has both vaginal and cervical components. Second, women enrolled in the study were with a clinically evident genital infection on per speculum examination, and thus, the positivity rates of BV were expected to be high among these women. Third, Pap smear slides were reported by trained cytotechnologist of the tertiary care cancer institute with the National Accreditation Board for Testing and Calibration Laboratories accredited laboratory. The reasons for low specificity of Pap smear to diagnose BV infection may be due to first, the vaginal microbiological flora consists of several types of obligate and facultative anaerobic bacteria that are commensal, including *Gardnerella vaginalis*, *Peptostreptococcus* species, and *Bacteroides* species.^[28] Overgrowth of the above-mentioned organisms is the main cause for BV infection among women. The Nugent score on Gram stain has a standard scoring system that takes into consideration different bacterial morphologies of the above-mentioned organisms and their number present in the given high-power field (HPF) along with lactobacilli to distinguish the normal/intermediate vaginal flora from BV infection. Pap smear unlike Nugent score lacks standardized scoring system to identify the number of bacteria and lactobacilli present per HPF, a needed prerequisite for diagnosing BV infection. The standard Pap stain used for Pap smear under good magnification power of objective lenses can identify the morphology of the bacteria (cocci, bacilli, and coccobacilli) but has limitations to comment on Gram-positive/Gram-negative nature of bacteria. Finally, criteria to diagnose BV infection by Pap smear which is consistent with “shift of vaginal flora suggestive of BV” and not “vaginitis” may be due to normal/intermediate vaginal flora due to other obligate and facultative anaerobic bacteria present in vaginal flora that clearly does not prove clinical infection.^[29,30]

Conclusion

BV infection is the most common female RTI reported from the Indian subcontinent. Unwillingness on the part of Indian women to attend health care facilities for STI/

RTI screening and treatment due to poor access, an inadequate number of laboratory diagnostic facilities in public health program settings makes them prone for lower genital tract infections. Persistent untreated genital tract infections can increase the susceptibility of acquiring HPV infection. Acquisition and persistence of high-risk HPV have significant implications for contributing to cervical carcinogenesis.^[10,31]

The Pap smear primarily is a screening test for cervical cancer however, the reporting of STIs/RTIs forms a part of evaluation as per the framework provided by the Bethesda system for evaluation of Pap smears.^[13] Since the accuracy of diagnosing clinical BV infection varies and the majority of women with BV infection can be asymptomatic, Pap smear may serve as a means of diagnosing BV infection in resource-constrained countries like India. In the present study, Pap smear was found to be a fairly reliable tool for diagnosing BV among women with genital infection without the requirement of additional diagnostic tests or cost of Gram stain, which itself is not routinely done in Indian health-care settings. Thus, facilities of Pap smear where available should be utilized to screen and treat women for BV promptly to reduce associated morbidity with BV infection.

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Conflicts of interest

There are no conflicts of interest.

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