Original Article

Prevalence of Bacterial Vaginosis and Comparison of the Efficacy of Gram Stain and Pap Smears in Its Detection

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Abstract
Background: *Bacterial vaginosis (BV), a disturbance of vaginal microflora, is a common cause of vaginal symptoms and is associated with an increased risk of acquisition of sexually transmitted infections, HIV, and with adverse pregnancy outcomes.*
Objectives: *We studied the overall prevalence of BV in symptomatic women, compared the efficacy of Gram stain and Papanicolaou (Pap) smear and determined the sensitivity and specificity of clinical criteria.*
Material and Methods: *The present prospective and analytical study was a part of ICMR funded project done over a period of 2 months. This study included 150 non pregnant women attending gynaecological outpatient department. High vaginal smears were Gram-stained and examined and characterized into 5 grades of vaginal flora and diagnose BV.Smears were PAP-stained and examined. The results of the 2 methods were compared.*
Results: *In our study, prevalence of BV was 23.3% using clinical criteria as gold standard. Considering the clinical criteria as gold standard, the sensitivity, specificity, positive predictive value and negative predictive value of Gram stain for diagnosis of bacterial vaginosis were 80.0%, 98.3%, 93.3% and 94.2%, respectively. Considering the clinical criteria as gold standard, the sensitivity, specificity, positive predictive value and negative predictive value of Pap stain for diagnosis of bacterial vaginosis were 60.0%, 94.8%, 77.8% and 88.6%, respectively.*
Conclusions: *Given the high prevalence, it is critical to diagnose and treat women, affected by BV appropriately. If Amsel'scriteria is accepted as the gold standard for diagnosis of BV, Gram stain has greater sensitivity and specificity.*
Keywords: *Bacterial vaginosis, Gram stain, Pap stain.*

Introduction

The most frequently encountered complaint in gynecological out-patient clinics is vaginal discharge. Bacterial vaginosis (BV) is the most common vaginal infections among women in reproductive age. Usually there is a biological balance in the microorganisms living in vaginal mucosa. The most important role in the continuity of this balance and in preventing the growth of pathogenic microorganisms is that of *Lactobacillus* species. Lactobacilli produce an acidic medium inthe vagina via hydrogen peroxide (H_2O_2), whichtransforms glycogen present in vaginal epitheliumto lactic acid[1]. The acidic medium produced bylactobacilli suppresses the growth of other microorganisms.If the balance of vaginal flora is alteredagainst lactobacilli, a clinical picture of itching, pain and vaginal discharge or smell will beobserved. In bacterial vaginosis (BV), micro organisms that are short, rod-like and mostly anaerobic,with variable Gram stain properties such as *Gardnerella vaginalis*, *Bacteriodes*, β- streptococci and the Mobilincus–Falcivibrio group replace the usual vaginal flora formed by lactobacilli[1,2]. BV has been linked to low birth weight infants, preterm delivery, chorioamnionitis, post hysterectomy cuff cellulitis, post surgical endometritis, endometritis following vaginal delivery and pelvic inflammatory disease.―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.

We undertook this study to know the overall prevalence of BV in symptomatic women, to compare the efficacy of Gram stain and Papanicolaou (Pap) smear in diagnosis of bacterial vaginosis (BV) and to determine the sensitivity and specificity of clinical criteria for the diagnosis of BV.

Material and Methods

This prospective and analytical study was a part of ICMR funded project done over a period of 2 months (August 2016- Sep 2016). This study included 150 non pregnant women attending gynaecological outpatient department. Clinical data was collected using a structured question-naire. Informed written consent was obtained from all women. High vaginal smears were Gram-stained and examined and characterized into 5 grades of vaginal flora and diagnose BV. Smears were PAP-stained and examined. The results of the 2 methods were compared.

Inclusion criteria: Women aged 18–50 years visiting our hospital for complaints such as white discharge, bad-smelling vaginal discharge, itching, pain abdomen and backache.

Exclusion criteria: Women with history of gynaecological cancer, using oral contraceptives and vaginal spermicides, who had bleeding during the examination and used antibiotics/vaginal medication during the previous three weeks.

Statistical analysis: The statistical analysis was done using SPS windows. Prevalence, specificity and sensitivity of the results obtained from Pap and Gram stains were calculated.

Sample collection: Vaginal swabs were collected for vaginal *p*H measurement, Gram stain, wet mount, and whiff test. The pH of the vaginal discharge was evaluated with litmus paper (Merck KGaAAcilit pH, Darmstad, Germany). An amine test was performed to the appropriately taken vaginal discharge sample with 10% KOH. Samples were taken from the vaginal lateral wall and posterior fornix with brushes, and spread out on three preparations. One of these preparations was examined immediately by light microscope; appropriate fixation, Gram stain and classic Papanicolaou (Pap) stain were applied to the other preparations.

Criteria for diagnosing BV: The clinical criteria reported by Amsel *et al* [2] for diagnosing bacterial vaginosis were also independently evaluated to determine their sensitivity and specificity: thin

homogeneous discharge, vaginal pH greater than 4.5, positive whiff test or release of amine odour after addition of 10 per cent KOH, and clue cells on microscopic evaluation. The presence of any three of the four Amsel's criteria confirms BV.

Pap stain- In evaluation of Pap stain under 400 magnification, according to the method of Davis andcolleagues[3], cases with the presence of microorganismsother than lactobacilli in a thin filmform, the presence of clue cells which are formedby the covering of the cytoplasmic membranesof squamous cells by non-*Lactobacillus* microorganisms and a predominance of non-*Lactobacillus* bacteria in most areas, were accepted as BV-positive cases

Gram stain- In evaluation of Gram stain, the grading system that used was as follows:-
Grading scheme for Gram- stained vaginal smears (Ison and Hay2002)

Grade of flora	Definition of flora	Description
0	Normal [a]	Epithelial cells only- no bacteria seen
I	Normal	Lactobacillus morphotype only
II	Intermediate	Reduced lactobacillus morphotype with mixed bacterial flora
III	Bacterial vaginosis	Mixed bacterial flora with few or absent lactobacillus morphotype
IV	Normal in asymptomatic women [b]	Epithelial cells covered with gram- positive cocci with few or absent lactobacillus morphotype

Normally found during or immediately post antibiotic therapy.
May be a cause of aerobic vaginitis or be of importance in pregnant women.

Ethical considerations: Informed consent was obtained from all the participants. Departmental Institutional Ethics Committee (IEC) approval was obtained Institutional Ethical committee Ref. No. IGIMS/Patho/111.

Results

In the prospective study of 150 non-pregnant women of child-bearing age were included, 35 were clinically diagnosed to have BV. Gram-stained vaginal smears and PAP-stained cervical smears were collected from all enrolled patients. Grading scheme for Gram- stained vaginal smears (Ison and Hay2002) was used and patients with intermediate scores were considered to be negative. Prevalence of BV in our study was 23.3% using clinical criteria as gold standard. Considering the clinical criteria as gold standard, the sensitivity, specificity, positive predictive value and negative predictive value of Gram stain for diagnosis of bacterial vaginosis were 80.0%, 98.3%, 93.3% and 94.2%, respectively.(Table 1) Considering the clinical criteria as gold standard, the sensitivity, specificity, positive predictive value and negative predictive value of Pap stain for diagnosis of bacterial vaginosis were 60.0%, 94.8%, 77.8% and 88.6%, respectively (Table 2)

Table 1: Sensitivity and specificity of clinical criteria for the diagnosis of BV-Gram stain

		Clinical criteria		Total
		Positive	Negative	
Gram stain	Positive	28 (TP)	2 (FP)	30
	Negative	7 (FN)	113 (TN)	120
Total		35	115	150

Table 2: Sensitivity and specificity of clinical criteria for the diagnosis of BV-Pap stain

		Clinical criteria		Total
		Positive	Negative	
Pap stain	Positive	21 (TP)	6 (FP)	27
	Negative	14 (FN)	109 (TN)	123
Total		35	115	150
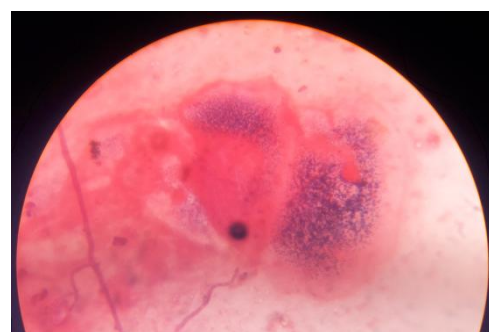
Gram- stained smear (Grade III)

Discussion

The prevalence of BV in this sample was 23.3%, which is in the range of previous findings for other populations in India.[4,5.] According to Enver Vardaretal.[6] the sensitivity of the Gram stain method was calculated as 97% and the sensitivity of the Pap smear method as 93% which is different from our findings.One of the most important complications of bacterial vaginosis is cervical dysplasia. It is hypothesized that bacterial vaginosis could be important in the development of neoplasia of the cervix, because the abnormal microflora in this condition produce carcinogenic nitrosamines. In our study, none of the patients had cervical intraepithelial neoplasia.

Conclusions

Given the high prevalence, it is critical to diagnose and treat women, affected by BV appropriately.

If Amsel's criteria is accepted as the gold standard for diagnosis of BV, Gram stain has greater sensitivity and specificity.

References

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