Comparative Study of PAP Smear and Microbiological Pattern in Bacterial Vaginosis in a Tertiary Care Hospital, South Bihar (India)

Imtiaz Ahmad¹, Rakesh Kumar Nirala², Chandan Kumar Poddar³, Pawan Kumar Chaudhary⁴

ABSTRACT

Introduction: Pap smears are routinely used in cervical cancer screening. The purpose of our study was to determine whether the Pap smear is of diagnostic value for the detection of bacterial vaginosis using vaginal gram stain as the diagnostic standard. The pap smear was a screening test to diagnose cervical cancer and also been used as a diagnostic test in cervical infection caused by different pathogenic organisms.

Material and Methods: A retrospective analysis of cervical pap smear study was done over a period of one years from September 2017 to September 2018 in a Tertiary Care Unit, Department of Pathology, Vardhman Institute of Medical Sciences, Pawapuri, Nalanda, Bihar and Associated Hospital of Bihar. This study was undertaken to determine the prevalence, clinical significance and seasonal variation in cervicovaginal infections. The Chi-square test was used for statistical analysis. A p-value of <0.05 was considered significant for statistical evaluation.

Results: One hundred and eight cases were determined as BV-positive by Pap smear, compared with 112 BV-positive cases determined by Gram stain. Sensitivity and specifity were 93% and 94% respectively with Pap smear and 97% and 94%, respectively with Gram stain. Positive predictive values were determined as 86% for the Pap-smear method and 88% for the Gram stain method. The comparison of results obtained from both groups with Amsel's criteria did yield significant difference. The prevalence of organisms among total infectious organisms is 48.4% (BV), 32% (Can), 13.4% (TV), and mixed infections 3.5% and 2.7% (BV + TV and BV + Can).

Conclusion: We concluded that cervical cytology is important for diagnosis of cervical infections. The most important cause of cervico vaginitis are bacterial vaginosis, next important cause is candidiasis. It is important to mobilize all reproductive age women to undergo pap smear examination and to prevent complications caused by infectious agents.

Keywords: Bacterial Vaginosis; Pap Smear; Gram Stain; Vaginal Culture

INTRODUCTION

Bacterial vaginosis was first described by Gardner and Dukes.¹ They regarded Gardnerella vaginalis as the etiologic agent of bacterial vaginosis, but this concept has been changed. Bacterial vaginosis is characterized by a shift in the vaginal flora from the dominant flora of Lactobacillus spp. to a mixed vaginal flora that includes G. vaginalis, Bacterioides spp., Mobiluncus spp. and Mycoplasma hominis.^{2,3}

The problem of vaginal discharge is probably the most frequently narrated complaint of woman of reproductive age group. 4,5 vaginal discharge constitute a considerable problem for many women causing discomfort, anxiety affecting women's quality of life and consuming considerable resources. Some vaginal discharges are normal and can vary with age, use of contraceptives, menstrual cycle and with the oestrogen level. 6,7

The vaginal flora is a dynamic ecosystem that can be easily altered. The most frequently encountered causes of vaginal discharge. Although there are four causes of vaginal discharges which cover almost 95% of cases. These are bacterial vaginosis, candidal vulvovaginitis, Trichomoniasis and normal physiological discharge.⁸

A common belief is that BV is the most common type of vaginal infection among women of reproductive age and accounts for at least one third of all vulvovaginal infections. BV is not caused by a single pathogen but rather it is a polymicrobial clinical syndrome. Common agents of BV include Gardnerella vaginalis, Mobiluncus, Bacteroides saprophytes and Mycobacterium Hominus.⁹

Candidiasis is mostly due to candida albicans¹⁰ and may be associated with diabetes, pregnancy and prolong use of antibiotics. Patient presents with vaginal discharge and pruritis. Discharge appears to be like curdled milk and deep erythema of vulva and vagina is often seen.

Trichomoniasis is a sexually transmitted disease (STD) that results from infection with flagellated protozoa named as Trichomonas Vaginalis. The prevalence of Trichomoniasis in American women is 3–5 million WHO estimates the worldwide prevalence of Trichomoniasis to be 170 million. The discharge is thin copious and pools in the vaginal vault. On examination vaginal and vulvar erythema is noted. The strawberry cervix in trichomoniasis resulting from punctuate

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haemorrhage is usually observed with colposcopy.8

Most of the patients are asymptomatic. Some patients show symptoms like increase in white discharge which appears homogeneous, is low in viscosity and evenly coats the vaginal mucosa and there is characteristic vaginal odor which smells like a fish. The patients may also experience itching and burning in the vaginal area.

Pap smear is a routine procedure done for cytological screening of cervical cancer.¹¹ It can also be used as a diagnostic test for bacterial vaginosis.

This study is done to compare the characteristics of Pap smear and microbiological pattern among patients with abnormal vaginal discharge. The objective of this study was to compare the characteristics of Pap smear and microbiological pattern in bacterial vaginosis.

MATERIAL AND METHODS

A retrospective analysis of cervical pap smear study was done over a period of one years from September 2017 to September 2018 in a Tertiary Care Unit, Department of Pathology, Vardhman Institute of Medical Sciences, Pawapuri, Nalanda, Bihar and Associated Hospital of Bihar. Patients were included in the study if a pap smear was performed for routine screening and medical necessity. The study was approved by scientific review board and ethical clearance was obtained from the Ethical Committee of Vardhman Institute of Medical Sciences, Pawapuri, Nalanda, Bihar.

Inclusion criteria

All the patients with vaginal discharge between the age of 18-52 years were included.

Exclusion criteria

Pregnancy and vaginal bleeding.

Collection of sample: - After a written informed consent was obtained; brief history, general physical examination and per speculum examination was done by an experienced clinician.

A clean dry sterile Cusco's speculum was inserted, and the characteristics of the vaginal discharge were noted. A sterile cotton swab was used to obtain a cerivcovaginal sample and was sent to Department of Microbiology.

Samples for PAP smear were collected using a endocervical brush and wooden spatula, the samples were smeared on a clean glass slide cytofixed and sent to Pathology department.

Gram staining and Nugent's scoring system9

Gram-stained slides are examined under oil immersion (x1000). According to Nugent Scoring System, the smears are observed and quantified for the presence of the following morphotypes:

- Large gram-positive bacilli (Lactobacillus morphotypes)
- Small gram variable bacilli (Gardnerella morphotypes)
- Curved gram negative or gram variable bacilli (Mobiluncus morphotypes)

The number of organisms seen is quantified according to the following scale

• 0 = No morphotype

- 1+ = <1 organism per field
- 2+=1-4 organisms per field
- 3+=5-30 organisms per field
- $4+ = \ge 30$ organisms per field

A total numerical score (N score) is calculated by summing the scores for the three components as indicated in [Table 1].

Culture

A cotton-tipped applicator was used to transfer vaginal fluid onto a human blood bilayer medium (HB medium). HB plates were examined for G. vaginalis after 48 and 72 h of incubation at 37°C in 5% CO2 in air. G. vaginalis colonies appeared as small beta-hemolytic colonies on HB agar. The identification was confirmed by their characteristic Gram stain morphology showing small pleomorphic gram-variable rods, fermentation of starch and glucose but not mannitol, the inability to produce green discoloration of chocolate agar, and the inability to produce catalase and oxidase.

The fermentation medium used for identification of G. vaginalis consisted of 1% Proteose peptone no. 3 (Difco Laboratories), 0.3% meat extract (BBL Microbiology Systems), 0.5% NaCl, and 1% Andrade indicator. The pH was adjusted to 7.1 before the medium was autoclaved. To this base the appropriate sugar (1%) and fetal calf serum (1%) were added. After all the gram stain smears had been evaluated and the Gram stain diagnoses were made, the results were compared with those of the pap smear.

Pap smear examination

Pap smears, including squamocolumnar junction, were performed with endocervical brush, fixed in 95% ethanol and stained by the Papanicolaou method. They were evaluated under the Bethesda system guidelines for specimen adequacy and general categorization. Adequate transformation zone sampling was defined as the presence of at least 10 wellpreserved endocervical or squamous metaplastic cells singly or in groups.7 If there was a filmy background of small coccobacilli, individual squamous cells with a layer of coccobacilli along the margins of the cell membranes, and conspicuious absence of lactobacilli, the smear was evaluated as positive for bacterial vaginosis. Smears displaying the pattern characteristic of bacterial vaginosis were reported as 'shift in flora suggestive of bacterial vaginosis'. The smears were later re-examined for the presence of bacterial vaginosis by one pathologist who was blinded to the microbiological test results.

STATISTICAL ANALYSIS

The results of the Pap smears and vaginal cultures were analyzed and compared statistically. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic value of Pap smear and vaginal culture were determined with Gram stain used as the gold standard for diagnosis of bacterial vaginosis.

RESULTS

In the present study, a total of 220 cervical smears were studied. The age of participants ranged from 18 years to 52 years with the mean age being 35.00 years. All of the women

Lactobacilli	Score	Gardnerella, Bacteroides	Score	Curved gram negative bacilli	Score	Sum = n score
4+	0	0	0	0	0	0
3+	1	1	1	1+	1	3
2+	2	2	2	2+	1	5
1+	3	3	3	3+	2	8
0	4	4	4	4+	2	10

Table-1: Nugent's Scoring System.

N score	Report		
0-3	Gram stain indicates normal bacterial vaginal flora		
4-6	Gram stain reveals altered vaginal flora that is not consistent with bacterial vaginosis		
>7	Gram stain indicates the presence of bacterial vaginosis.		
Table-2: Interpretation of Nugent's Score.			

Characteristics (colour)	Number	Percentage		
Yellow –Gray-Discharge	163	74		
Gray-White	46	21		
Yellow-Green	11	5		
Total	220	100		
Table-3: Characteristics of vaginal discharge				

Education	Number	Percentage		
Married	213	97		
Nonmarried (divorced, widows)	07	3		
Illiterate	62	28		
University Education	24	11		
Low socioeconomic status	141	64		
	Table-4: Patient data showing married, and educa	tion.		

Organism	18 - 30 Years	31 - 40 Years	41 - 50 Years	> 50 Years	Total	p-value
Bacterial Vaginosis (BV)	34	44	24	4	106	0.003
	15.4%	20.1%	11.2%	1.7%	48.4%	
Candida species (Can)	28	29	11	2	70	0.005
	12.8%	13.2%	5.0%	1.0%	32.0%	
Trichomona vaginalis (TV)	7	14	6	3	30	0.001
	3.2%	6.3%	2.7%	1.2%	13.4%	
BV+TV	2	4	2	0	8	0.003
	1.0%	1.9%	.6%	.0%	3.5%	
BV+Can	2	2	1	1	6	0.004
	1.0%	.7%	.6%	.4%	2.7%	

Table-5: Frequency of BV, Candida, TV and mixed infections in different age groups. P-value of <0.05. Hence, the results are statistically significant.

were suffering from vaginal discharge, which was a yellowgray discharge in 74% of cases, white in 21% and yellowgreen in 5% [Table-3]. Itching was observed in 86% and bad smell was observed in 94%. Post-coital odor was observed in 96%. One of the most common signs and symptoms, mentioned by 147 women (67%), was smelly odor, Seventythree (33%) also complained of odorless vaginal discharge. Most patients (213 women, or 97%) were married; and 62 (28%) patients were illiterate. Only 24 (11%) had university education [Table-4]. One hundred and forty-one (64%) patients were of low socioeconomic status. Seventy-nine patients (36%) were from Nalanda, Bihar.

One hundred and eight cases were determined as BV-positive by Pap smear, compared with 112 BV-positive

cases determined by Gram stain. Sensitivity and specifity were 93% and 94% respectively with Pap smear and 97% and 94%, respectively with Gram stain. Positive predictive values were determined as 86% for the Pap-smear method and 88% for the Gram stain method. The comparison of results obtained from both groups with Amsel's criteria did yield significant difference.

The prevalence of organisms among total infectious organisms is 48.4% (BV), 32% (Can), 13.4% (TV), and mixed infections 3.5% and 2.7% (BV + TV and BV + Can) [Table -5/Fig-1, 2].

DISCUSSION

Bacterial Vaginosis cases diagnosed on routine pap smears

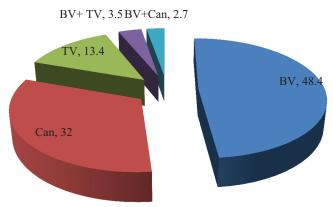


Figure-1: Frequency of Bacterial Vaginosis, Candida, Trichomona vaginalis and mixed infections.

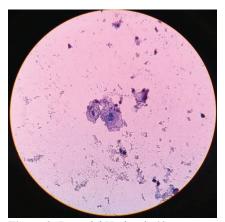


Figure-2: Bacterial Vaginosis 40 x.

were studied for Amsel's clinical criteria and Nugents scoring in gram staining. BV is the most frequent cause of vaginitis, and is characterized by increase in growth of anaerobic and aerobic microorganisms due to an unbalanced eco-systemin the vagina. 12 Gardner and Dukes 13 were the first to report Haemophilus vaginalis as a cause of non specific vaginitis in1955. Several studies have identified BV as the leading vaginal infection. 14-16 In our study we found BV as the main infectious agent in 48.4% among total infectious agents, which is very high and 7.12% among total cases of the patients, which is similar to Krabulut's result. The diagnosis of BV was made by the presence of clue cells i.e., mature squamous cells are covered by cocobacilli, typically extending beyond the cell margin and relative absence of lactobacilli on mixed flora. Half of the patients with BV are asymptomatic; the Pap smear study may be only means of diagnosis. ¹⁷ For the diagnosing BV most of the studies proved that the finding of clue cells in the Pap smear examination had 100% sensitivity and a 96% specificity. 18 The frequency of clue cells are decreased due to the less variable pH in older women and in younger women BV is more frequent with related pH alterations.19

In a study by Lamont R. F.et al²⁰ pap-staining of vaginal smears is shown to be a useful instrument for diagnosing BV compared with the Amsel clinical criteria as well as with the mean Nugent score, in Gram stained smears. With regard to diagnostic accuracy, very little difference is found among the three staining methods when the same scoring

system is used to compare the different staining methods in many countries. Narasimha.A.et al²¹ reported that pap smears have a sensitivity and specificity of 90% and 97% respectively. Pap-stained vaginal smears can be used as a wholly adequate alternative to Gram-stained smears for BV diagnosis. It has been suggested that the presence of clue cells on the Pap smear agrees reasonably well with clinical criteria. So Pap smear test which is a simple, quick, painless procedure employed to screen cervical cancer can also be used for diagnosing cervicovaginal infections. Donder et al22 reported that Prevalence of BV was higher in the inflammatory smear group, thus supporting that women with an inflammatory smear are more likely to harbour genital tract infection than women whose smear shows no evidence of inflammation. Previous studies demonstrated that women with no inflammatory changes on cervical smears can also harbour genital tract pathogens. Inflammation on Pap smear had a relatively low predictive value for the presence of vaginal pathogens in asymptomatic women. A high rate of BV was found both in women with inflammatory changes and in those without inflammatory changes on pap smear, suggesting that inflammation on pap smear is a poor indicator of cervical infection. Pap smears are less specific because standardized criteria for evaluation of Pap smears has not been routinely applied. Amsel's criteria is the most common method of identifying BV.Patients were diagnosed as having Bacterial Vaginosis if they fulfilled any three of the following four criteria:

- 1. Thin, homogenous watery vaginal discharge.
- 2. Vaginal pH above 4.5.
- 3. A fishy smell on addition of 10% KOH to vaginal fluid (Whiff's test).
- 4. Presence of clue cells on saline wet mount.

In our study, younger and middle aged women had significantly higher prevalence of BV. The diagnosis of BV is important as it causes endometritis, urinary tract infections, preterm delivery, chorioamnionitis and pelvic inflammatory disease.²³ BV also increases the risk of HIV acquisition.²⁴ Mixed infections are also observed when BV with TV and BV with Candidiasis.

The most common fungal disease in women is vaginal cadidiasis and affects 75% of women at some stage in their lifetime. The patients are present with curdy white discharge and prurities. Candida species was diagnosed when yeast forms or pseudohyphae are present and mixed infections candida with BV are seen. Adad et al., reported an increase of Candida infection over the last decade (8.1% in 1978 to 22.5% in 1998).²⁵ Our study showed 32% of higher prevalence rate among total infectious agents and 4.61% of Candida infection from total cases similar to Adad et al., and Bukhari et al., 6.5%.^{25,26} In our study, younger and middle aged women had significantly higher prevalence of cadidiasis.

The most common sexually transmitted disease in sexually active women in all age groups is Trichomonas vaginalis (TV).²⁷ Approximately 57-10 million people are affected by TV globally, majority living developing countries.^{28,29} Our

study showed the occurrence of TV was 13.4% of higher prevalence rate among total infectious agents and 1.73% from total cases. Our study showed middle aged women had significantly higher prevalence of TV.

TV was diagnosed as a unicellular organism of ovoid or pear shaped with single nucleus present [Fig-2]. The pelvic inflammatory disease, infertility, genitourinary tract infection premature labor, ectopic, pregnancy, and there was an increased chance in the risk of both the transmission and acquisition of Human Immunodeficiency Virus (HIV) in TV patients. 15 Vikki M et al., studies showed that epidemiological association between TV infection and subsequent cervical neoplasia and carcinoma.³⁰ The cause for the development of cancer was chronic inflammation either specific or nonspecific has been associated with malignancy.³¹ In the previous literature mixed infections (BV+ Can, BV+TV) were not determined but were slowly started to find the mixed infections in pap smear. Our study showed mixed infections of 3.5% and 2.7% (BV+TV and BV+Can) of prevalence rate among total infectious agents and BV+ can infection was 0.37% and TV with BV was 0.48% of total cases.

CONCLUSION

Bacterial vaginosis (BV) is diagnosing by Pap smear is the most simple and a quick test which is beneficial in diagnosing cervical infections like Bacterial vaginosis. Control of these infections is possible through regular screening and treatment. Early diagnosis of BV can help prevent further complications, by commencing appropriate treatment. By using Amsel's clinical criteria and Nugent's scoring, BV can be diagnosed effectively in Pap smears.

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