

# Inflammatory PAP smears and its microbiological association: A prospective study

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## Abstract

**Background:** Non specific inflammatory PAP smears are common and cause clinical dilemma in management of patient. Microbiological methods are more accurate method of diagnosis of pathogens of female genital tract. **Materials and Methods:** A total of 252 PAP smears and vaginal swabs of nonpregnant women referred to the laboratory, constituted the material of the study. **Results:** Pap smears showed high predictive values, comparable to microbiological testing in detection of pathogens. Apart from inflammatory pattern, a spectrum of diagnosis was given, based on cytological criteria. Majority of the PAP smears in which pathogens were detected, showed significant inflammation. Bacterial vaginosis constitutes the most common cause of vaginal discharge, followed by Trichomoniasis and then Candidiasis in our set up. Pap smears also revealed that 7 out of 252 cases were having various grades of cervical dysplasia. **Conclusions:** A report of inflammatory changes on the cervical Pap smear cannot be used to reliably predict the presence of a genital tract infection. Combination of PAP smear and simple, cost effective microbiological tests, coupled with primary clinical diagnosis lead to significant improvement in patient management.

**Keywords:** Vaginal discharge, Bacterial vaginosis, Candidiasis, Trichomoniasis.

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## Introduction

The infections of the genital tract are common in reproductive-age women. The Papanicolaou (Pap) test is a simple, quick, and painless cytology procedure, based on picking up of cells from the uterine cervix and used as a screening test for the prevention of the cancer of uterine cervix and the possible presence of infection based on cytological criteria [1]. The diagnosis of infections in cervical smear tests is based on cytological criteria. Non-specific cervicitis or inflammatory changes in a smear report are most common. The management protocol of such patients is unclear and need further testing by conventional and ancillary microbiological investigations or inflammatory change should be considered as minor.

The most common complaint in the reproductive age group is vaginal discharge. Symptomatic vaginal discharge is caused by inflammation due to infection of the vaginal mucosa. It occurs in 1-14% of all women in

the reproductive age group and the prevalence of vaginal discharge in India is estimated to be 30% [2].

The complications of untreated vaginal infections can lead to vaginitis, pelvic inflammatory diseases, infertility, endometriosis, urethral syndrome, pregnancy loss, preterm labour. Among the cases of vaginal discharge, Bacterial vaginosis is commonest cause followed by Candidiasis and Trichomoniasis.

The addition of a simple microscopic evaluation by Gram stain of the vaginal smear and wet mount visualization of motile *Trichomonas vaginalis* has evolved as a sensitive noncultural diagnostic technique for Bacterial vaginosis, Candidiasis and Trichomoniasis, respectively.

This study aimed to investigate the prevalence of pathogenic vaginal microorganisms and the presence of inflammation in Pap smear and to evaluate the possible co-infection of these organisms and association of dysplasia/ malignancy in them.

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## Materials and Methods

**Study Design and Setting:** The present Study, a prospective one, was conducted in a referral diagnostic centre in Western Odisha, India in association with referring hospitals and practicing gynaecologists in the area. PAP smears and vaginal swab of nonpregnant women of reproductive age, between December 2014 and December 2016 were included in this study. The study was conducted in collaboration with the management of the diagnostic centre.

**Inclusion Criteria:** Pap (papinaculaou) smears were taken in all the cases of the patients in reproductive age group.

**Exclusion Criteria:** Pregnant females were excluded from this study to avoid pregnancy associated changes in smears. Inadequate smears were also excluded from the study

**Study details and Variables:** Cytological diagnosis was accorded and provided for the correlation between cytological changes, and vaginal infection. Various inflammatory changes were recorded in Pap stained smears that included- presence of cells with enlarged nuclei, pyknosis or karyorrhexis, perinuclear halos, and vacuoles. Increased numbers of polymorphonuclear leukocytes or neutrophils were graded as mild, moderate and severe acute inflammatory smears.

## Results

A total of 252 women had smear tests and vaginal material examination for microbiological diagnosis of infections. All the predictive values were comparable in diagnosing infections- BV, Trichomoniasis and Candidiasis, with highest predictive values and Youden's Index (YI) for diagnosing Candidiasis (**Table 1 and 2**). Using PAP smear evaluation, 104(41.2%) out of 252 were rendered as normal PAP smears, with insignificant inflammation and no other etiological diagnosis. 148(58.8%) out of 252 were inflammatory smears with varying degree of inflammation and diagnosis ranging from, non specific inflammatory smears to other specific diagnosis.

Using the cytological criteria, various types of intraepithelial neoplasia and invasive carcinoma diagnosis was accorded (**Table 3**). In the intraepithelial neoplasia and invasive carcinoma category of diagnosis, there was concordant infection of Trichomonas in one case each of, Low grade squamous intraepithelial lesion (LSIL) and Atypical squamous cells of undetermined significance (ASCUS).

**Table-1: Infections detected by diagnostic tests.**

Type of diagnostic test	Bacterial vaginosis	Trichomoniasis	Candidiasis
Microscopic(Pap Smear)	22(8.7%)	12(4.7%)	09(3.5%)
Microbiological	27(10.7%)	15(5.9%)	11(4.3%)

Presence of other inflammatory cells- lymphocytes, plasma cells, histiocytes and epithelioid cells were also noted. Epithelial cells covered with blue-stained coccoid bacteria on the stained cervical smear together with a decreased number or a lack of lactobacilli was suggestive of bacterial vaginosis.

Vaginal material was obtained from the posterior fornix using a sterile swab stick for 1) Wet mount-with normal saline and 10% of KOH 2) Gram stain-Gram stain slide is interpreted by using Nugent score. The diagnostic criteria used for microbiological diagnosis are: 1) Bacterial vaginosis – A Gram stain slide using both Amsel and Nugent criteria. [3,4]. 2) Candidiasis – If gram positive budding yeasts and pseudohyphae are seen on Gram stain. 3) Trichomoniasis – If wet smear microscopy is positive for motile Trichomonas vaginalis.

**Statistical Methods:** The various parameters were computed, based on the following formulae, Sensitivity = True positive/ (True positive + False negative); Specificity = True negative/ (True negative + False positive); Positive Predictive Value (PPV) = True positive/ (True positive + False positive); Negative Predictive Value (NPV) = True negative/ (True negative + False negative). Youden's index = Sensitivity + Specificity – 100.

**Table-2: Predictive value of Pap smear for diagnosis of infections.**

Infections	Sensitivity	Specificity	PPV	NPV	YI
BV	84.3	99.1( 2 FP)	93.1	97.8	83.4
Trichomoniasis	83.3	99.5(1 FP)	93.7	98.7	82.8
Candida	84.6	99.5( 1 FP)	91.6	99.1	84.1

BV-Bacterial Vaginosis, FP-False positive, PPV-Positive Predictive value, NPV-Negative predictive value, YI-Youden's index

**Table-3: Spectrum of Microscopic Diagnosis.**

Diagnosis	Numbers	No. of cases-Pathogens detected
Non Specific Inflammatory	(80/252) 31.7%	0
ASCUS	(06/252) 2.38 %	1-Trichomonas
ASC-H	(02/252) 0.79%	0
LSIL	( 03/252) 1.19%	1-Trichomonas
HSIL	(02/252) 0.79%	0
SCC	( 01/252) 0.39 %	0
AGUS	(01/252) 0.39%	0
AIS	( 00/252) 00	0
Adenocarcinoma	( 01/252) 0.39%	0
BV	(25/252) 9.92 %	25-BV
Trichomoniasis	( 10/252) 3.96%	12-Trichomoniasis
Candidiasis	(15/252) 5.95 %	15- Candidiasis
Concordant infections	( 02/252) 0.79%	2-Trichomoniasis and BV together
Normal PAP Smear	(104/252) 41.2%	0

## Discussion

The flora in vagina consists of different bacterial species in variable quantities and proportions. Bacterial vaginosis denotes a condition of alteration of vaginal flora, characterized by decreased *Lactobacillus* spp. and overgrowth of *Gardnerella vaginalis* together with anaerobes and potentially pathogenic bacteria, including *Ureaplasma urealyticum*, and *Mycoplasma hominis*. In our study, we carried out vaginal microbiological tests on vaginal material for a wide range of microorganisms in women with and without inflammation on Pap test.

Kelly et al [5], Wilson et al [6] reported that that inflammation on cytology is often associated with a genital tract infection. Inflammation on Pap smear has been associated with up to high 30–50% incidence of bacterial vaginosis [7], while in our study, it showed 18.7% incidence in inflammatory smears and 10.7%

(27/148) of overall smears examined. The *Candida* incidence was 7.4 (11/148) % in inflammatory smears and 4.3% in overall smears, which is not consistent with a recent report where, in a different population, the rate of *Candida* infection in patients with inflammatory changes was very high, 73.8% [8].

*Trichomonas vaginalis* is a parasite that causes symptomatic and asymptomatic infection of the female urogenital system. *T. vaginalis* is found more frequently in women with inflammation on Pap smear than in women without inflammation. [9] In our study, incidence was 10.1% (15/148) in inflammatory smears which is consistent with others reporting low prevalence [10]. This cross sectional study of 252 patients, whose pap smears were taken and simultaneous vaginal and cervical material was taken

for microbiological examination. The etiological diagnosis was reached in 172 (68.3%) of the patients included in PAP smear. In the remaining 31.7% of the patients, diagnosis could not be made with both PAP smear and the microbiological diagnostic approach.

Similarly, other studies showed that- in 10 to 58% of the patients complaining of vaginal discharge, diagnosis could not be reached using any of the diagnostic approaches under consideration [10].

This group of patients probably may have normal physiological discharge or non detectable conditions like viral vaginitis, aerobic vaginitis or vaginal lactobacillosis.

Pap smear results displayed that 7 (2.77%) patients out of 252 were having various grades of cervical dysplasia and 9 (3.5%) out of 252 were placed in indeterminate categories of - Atypical squamous cells of undetermined significance (ASCUS), Atypical squamous cells, can not exclude high grade lesion (ASC-H) and Atypical glandular cells of undetermined significance (AGUS), as compared to another study in which (2.67%) were having cervical intraepithelial neoplasia [11]. Only one concordant case of T vaginalis infection were seen in Low grade squamous Intraepithelial lesion (LSIL) and ASCUS, which was not significant enough to prove their causation in intraepithelial carcinoma or carcinoma cervix.

It was concluded that cervical intra epithelial neoplasia is common in our set up and can be diagnosed at early stage by routine Pap smear screening. When the microscopic diagnosis- PAP smear were compared with the microbiological diagnosis, PAP smear was found to have fair sensitivity, specificity, PPV and NPV in diagnosing infections of female genital tract.

Also, some recent studies have demonstrated the co-infection of vaginal pathogenic organisms in Pap tests [12], as was demonstrated in our study with detection of two cases of concordant BV and Trichomoniasis by both PAP smear and microbiological diagnostic tests.

## Conclusions

The results of our study suggest that inflammatory changes in PAP smears cannot be relied on as a harbinger of female genital tract infection. Further investigations including simple microbiological investigations, as conducted in the present study, to

other investigations like culture and other ancillary investigations are needed for detection of the possible presence of infection.

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