



Original Research Article

Cytomorphological spectrum of cervicovaginal infections and their prevalence with clinical correlation on pap smears: A three-year retrospective study

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ABSTRACT

Introduction: Cervical-vaginal infections are one of the most common infections seen in women of the reproductive age group. The important infectious agents in Gardnerella vaginalis, Candida, and Trichomonas vaginalis. Pap smear cytology is used as an important screening test for various epithelial cell abnormalities and cervical cancer. It is also useful in the diagnosis of various pathogenic microorganisms causing cervicovaginal infections.

Aim: To determine the prevalence of cervicovaginal infections on Pap smears, and study their cytomorphology and clinical correlation.

Material and Methods: This is a three-year retrospective study from June 2018 to May 2021 performed on conventional Pap smears in the Pathology laboratory of a Government hospital in North India. The prevalence of various cervicovaginal infections was determined and their cytomorphological spectrum was studied. The Bethesda System of classification was followed for reporting Pap smears. All the females attending Gynaecology OPD of the hospital with various complaints were included. Females with a history of cervical cancer and unsatisfactory samples were excluded from the study.

Results: Out of the total number 1344 pap smears examined during the three years, 1291 (96.058%) were reported as NILM, remaining 53(3.044%) were diagnosed as epithelial cell abnormalities and cervical cancer. Among the 1291 cases of NILM, 421(32.61%) smears were positive for specific pathogenic microorganisms. Out of, 421 smears, examined, 254(60.33%) were BV followed by 136 (32.30%) of candida infection. Trichomonas contributed to 22(5.22%) cases, whereas in one case (0.23%) each of Actinomyces and HSV was seen. Mixed infection by Trichomonas and leptothrix was seen in 4(0.950%) cases and Candida and leptothrix contributed to 3(0.712%) cases.

Conclusion: Cervico-vaginal infections are an important cause of morbidity in the reproductive age group. Cervical cytology is an invaluable method to diagnose these infections. About 90% of infections are caused by Bacterial Vaginosis and Candida. All women of the reproductive age group should undergo a Pap smear examination for timely diagnosis and proper management of cervicovaginal infections.

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1. Introduction

The introduction of the Pap smear method by George Papanicolaou in 1943 opened the flood gates for cervical

cytology. A Pap smear is considered to be the gold standard for screening various premalignant and malignant lesions of the cervix. It is useful in the diagnosis of species specific pathogenic microorganisms also.^{1,2} Majority of the patients present with vaginal discharge, postcoital bleeding, pain abdomen, dyspareunia, low back ache and

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other symptoms. 90% of infections are due to bacterial vaginosis and candidiasis. Actinomyces are common in women using IUDs.³ Viral infection by HSV and infection by Chlamydia trachomatous are rare. Mixed infections of Candida with Trichomonas and Leptothrix are seen occasionally. The accompanying cytopathic and inflammatory changes observed in Pap smears often give a diagnostic clue to the type of infectious agent. Hence this study was undertaken to not only determine the prevalence of cervicovaginal infections but also to study the accompanying cytomorphological changes.⁴

2. Materials and Methods

This is a three-year retrospective study done in the Government Hospital of North India. Due approval from the Ethical Committee was obtained. The patient's age ranged from 18 to 50 years. Pap smears were taken by a gynecologist in the OPD using a modified Ayres spatula. It was rotated full 360 degrees around the cervix. Both endo and ectocervix were sampled. The material obtained was gently smeared onto the clean glass slides, labeled, and put in the Coplin jar containing 95% alcohol as the fixative. The patient's requisition form duly filled and signed with short history along with the fixed slides was received in the pathology department. The slides were stained by Papanicolaou stain by the laboratory technician. The slides were mounted with DPX and appropriately labeled. They were screened by a pathologist. The Bethesda system 2014 was followed for reporting the smears⁴.

3. Results

The total number of pap smears examined was 1344. The age group ranged from 18 to 50 years. The mean age group affected was 30 to 40 years. (Table 1) Out of 1344 smears, 421(29.58%) were positive for specific microorganisms. The prevalence of bacterial vaginosis (BV) was 254 (60.33%), followed by Candidiasis 136 (32.30%) and Trichomonas seen in 22 (5.22%) cases. Herpes simplex virus (HSV) and Actinomyces infection noted in only in

.023%% cases. Mixed infection of candida and leptothrix (Lx) was seen in 3 (0.071%) cases whereas 4 (0.095%) cases of trichomonas with leptothrix were seen. (Fig- 1,2A-1E). The commonest age group affected by BV, TV, and Candida was 31-40 years. BV infected women presented with white discharge per vaginum 172(67.7%) followed by PID 52 (32.3%). The most common clinical presentation was vaginal discharge seen in 60 women(44%) followed by vaginal itching and burning sensation in the remaining 36 and 20 cases of Candidiasis. The patients with TV presented most commonly with foul-smelling yellow-green vaginal discharge 18(81%). The other complaints included PID and low backache

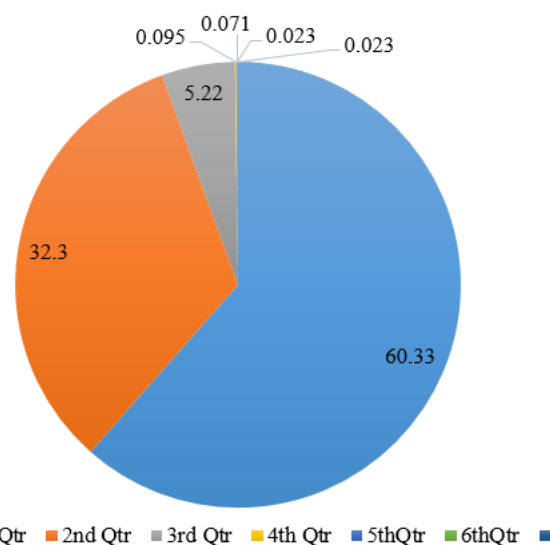


Fig. 1: Frequency of 1.Bacterial Vaginosis 2.Candida, 3.Trichomona vaginalis 4,5 Mixed infections 6 HSV 7Actinomyces

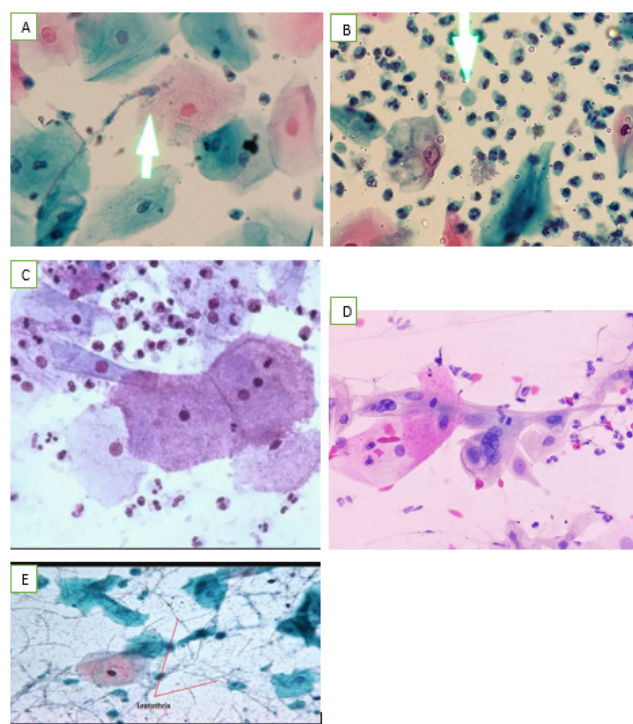


Fig. 2: **A:** Pap smear showing yeast-like forms and pseudohyphae of Candida species with variable surrounding inflammation. (Pap; 400X); **B:** Pap smear shows trophozoite forms of Trichomonas vaginalis species (arrow) with background altered flora. (Pap; 400X); **C:** Gardnerella vaginalis (clue cell) - the surface of squamous cell is obscured by a large accumulation of anaerobic short rod like bacilli (Pap,X400); **D:** Herpes Simplex Virus-Papsmear showing multinucleate squamous cell (Pap,X400); **E:** Leptothrix - long, curving, filamentous bacteria (Pap,400X).

Table 1: Frequency of Bacterial Vaginosis (BV), Candida, Trichomonas Vaginalis (TV) and mixed infections in different age groups.

Organism	18-30	31-40	41-50	Total
Bacterial Vaginosis (BV)	81	111	62	254
Candidiasis (Can)	43	72	21	136
Trichomonas Vaginalis (TV)	8	10	4	22
Candidiasis + Leptothrix (LX)	1	2	0	3
LX+TV	1	2	1	4
Actinomyces	0	1	0	1
Human Papilloma Virus (HPV)	0	1	0	1

Table 2: Chief complaints of bacterial vaginosis, candidiasis trichomonas vaginalis and mixed infections

Presenting complaints	BV	CAN	TV	ACT	CAN+LX	TV+LX	HSV
Vaginal Discharge	172	60	18	0	2	3	0
PID	52	8	2	1	0	0	0
Dyspareunia	14	4	0	0	0	0	0
Low backache	14	8	2	0	0	1	0
Vaginal Itching	2	36	0	0	1	0	0
Vaginal burning	1	20	0	0	0	0	1

4. Discussion

Cervicovaginal infections are common in women of reproductive age group. Despite being treatable, they still contribute to being a major health issue in many developing countries. Ignorance, illiteracy, poverty limited resources, poor personal hygiene and associated social stigma leading to the visits of gynecology clinics, are various factors that contribute to the increased prevalence of these infections among women.⁵ Bacterial vaginosis, Candida, and TV together constitutes about 90% of the cases. A Pap smear is a quick and cheap method for screening not only premalignant and malignant lesions in the cervix but also specific microbial infections by identification of the pathogen and/ or their characteristic cytological alterations.⁶ Bacterial Vaginosis is the leading cause of cervicovaginal infection seen in various studies.⁷⁻⁹ In our study also, BV was the most common infectious agent among all accounting for 60.3% of cases, and 1 of total smears examined in three year period. Simoes Barbosa A et al reported similar results in their study.¹⁰ Chief complaints in patients with bacterial vaginosis ranged from foul-smelling thin gray, white, or green vaginal discharge to PID and dyspareunia.¹¹ The diagnosis of Gardnerella vaginalis infections was made by the presence of 'clue cells' in pap smears; consisting of keratinocytes covered by dark bacillary forms in a background of mild to moderate inflammation along with relative absence of lactobacilli.¹¹ [Figure 2]. Enrique OE et therein his study showed that the presence of clue cells on Pap smear examination had sensitivity and specificity of 100% and 96% respectively.¹² The frequency of clue cells in younger women is more frequently related to alterations of pH.¹³ In the present study, middle-aged women had a significantly higher prevalence of BV. Detection of infection

(BV) is important as it leads to endometritis, urinary tract infections, preterm delivery, chorioamnionitis, and pelvic inflammatory disease.¹⁴ It also leads to an increased risk of HIV acquisition.¹⁵ Mixed infections are also seen with BV like TV and Candidiasis. Vaginal Candidiasis is the most common fungal disease seen in reproductive age women and affects the majority of women at some stages of their life. The patients present with curdy white discharge and pruritis. Candida infection was diagnosed by the variable distribution of yeast forms (spores) or pseudohyphal forms along with alteration in the normal flora in a background of moderate to heavy neutrophilic infiltrates. The pseudohyphal forms were more commonly seen than spores. In the present study, the prevalence rate of Candidiasis was found to be 32.30% of total infectious agents and 9.3% of total cases. This is similar to studies conducted by Adad et al., and Bukhari et al.^{8,16} In the present study younger age group were more affected.

Trichomonas vaginalis (TV) is the most common sexually transmitted disease in women of all age groups.¹⁷ Trichomonas vaginalis infections are characterized by the presence of trophozoite forms in a background of severe inflammation composed of neutrophils, lymphocytes, plasma cells, and histiocytes. In TV infected patients, the symptoms range widely from vaginal discharge and pruritis to dysuria and dyspareunia.¹⁸ The present study matches with this clinical profile. The classical green, frothy, foul-smelling discharge was documented in 10% of patients. It also acts as a contributory factor in cervical malignancy, postoperative infections and poor pregnancy outcomes, PID, and rarely infertility.¹⁹ In the present study, the prevalence of Trichomonas infection is 5.07% which is similar to other studies.^{18,19} Herpes simplex Virus (HSV) is one of the sexually transmitted infections and is a rare cause of abnormal Pap smears as observed in the

present study. The cytological diagnosis of genital herpes depends upon the recognition of multinucleated giant squamous cells in Papanicolaou-stained smears. Infection with the herpes simplex virus is characterized by cell fusion with the formation of large syncytia of epithelial cells. Moulding and ground glass appearance of nuclei is seen. Cytological diagnosis on Pap smears offers a rapid and convenient method of detecting infection which can, later on, be confirmed by the conventional virological method.²⁰ Actinomyces-like organisms rarely appear on routine PAP smears. Our study showed very few cases contributing to around 0.2%. Güdücü et al., reported only 33.5%, 30.4%, 43.3%, and 0% of patients with bacterial vaginosis, *Trichomonas vaginalis*, *Candida* and *Actinomyces*, respectively on Pap smear.²¹ A study conducted to compare PCR analysis, for the diagnosis of genital *Actinomyces*, reported that culture and Pap smear have low while PCR analysis has high sensitivity and specificity for the identification of *Actinomyces*.¹⁹

5. Conclusion

The study concludes that the Papanicolaou test for examining cervical smear is important for detecting cervicovaginal infections. Despite its limitations, it is a cost-effective, quick, and acceptable method for most women. The most important cause of cervicovaginitis found to be Bacterial Vaginosis followed by candidiasis and TV. HSV and *Actinomyces* species were seldom noted. Higher prevalence of BV and *Candida* infection is seen in younger to the middle-aged group while the TV in the middle-aged group. Hence, it is important to mobilize all reproductive-age women to undergo Pap smear examination to prevent gynecological and obstetrics complications caused by infectious agents and it may also uncover much rarer causative organisms that may or may not have a role in carcinogenesis.

6. Acknowledgments

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7. Conflict of Interest

None.

8. Source of Funding

None.

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