



**ORIGINAL RESEARCH PAPER**

**Pathology**

**UTILITY OF PAPANICOLAOU TEST IN DIAGNOSIS OF CERVICOVAGINAL INFLAMMATION AT TERTIARY CARE CENTRE OF WESTERN INDIA.**

**KEY WORDS:** Pap smear, inflammation, bacterial vaginosis (BV), Trichomonas vaginalis (TV), and vaginal candidiasis (VC)

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**ABSTRACT**

**BACKGROUND:** Inflammation in cervical cytopathology smears (Pap smears) is very common and cause clinical dilemma in management of patients. Papanicolaou (Pap) test is simple, painless, quick and inexpensive cytology procedure for cervical screening based on cytological criteria. Although not the primary use and an imperfect test, it can be extremely useful in the diagnosis of some microorganisms like cocco-bacilli, candida and trichomonas vaginalis. Reproductive age group women is mainly affected with symptom of vaginal discharge in developing countries. Non-specific cervicitis or inflammatory changes in pap smear report are common which are usually unclear for clinical approaches.

**OBJECTIVES:** To investigate the frequency and severity of cervical inflammation and prevalence of pathogenic vaginal microorganisms in cervical smears among received sample.

**MATERIAL AND METHODS:** This cross-sectional study was carried out on received Pap smear samples of women referred from gynecological opd for 12 months. This study was conducted on 800 conventional Papanicolaou cervical smears. The frequency, severity of inflammation of cervix and prevalence of bacterial vaginosis (BV), Trichomonas vaginalis (TV), and vaginal candidiasis (VC) was determined in the samples. Also co-infection of vaginal microorganisms in Pap samples was evaluated. Percentage of the outcome parameters were calculated.

**RESULT:** The mean age of patients was 45 (ranged 20-80) years. Inflammation was reported in 500 (62.5%) samples out of 800. Mild inflammation was noted in 170 (21.25%), moderate in 208 (26%), severe in 122 (15.25%). The overall prevalence of BV, VC, and TV in Pap samples was (n=132, 16.5%), (27, 3.4%), and (n= 11, 1.4%) respectively. Overall, frequency of inflammation and prevalence of these vaginal microorganisms in women of reproductive age group females.

**CONCLUSION:** It is concluded from the present study, that women from reproductive age group are most commonly affected with moderate inflammation. Our study found that pap smear is simple, painless and quick investigation for screening of cervical inflammatory lesion and co-infection with vaginal micro-organisms also, although it is primarily for screening of malignancy. Prevalence of bacterial vaginosis is highest among all three micro-organisms followed by VC and TV in our study. It is necessary to increase awareness among women regarding symptoms like vaginal discharge and consequences of uterine cervix malignancy.

**INTRODUCTION**

The Pap smear has been in use for more than half a century as the primary screening test for pre invasive and invasive lesions of the uterine cervix. Although not the primary use and an imperfect test, it can be extremely useful in the diagnosis of some microorganisms. The use of the Pap smear in the diagnosis of several microorganisms including Candida, Trichomonas vaginalis, Leptothrix vaginalis, Herpes Simplex Virus, the causative agents of bacterial vaginosis, Actinomyces, Chlamydia trachomatis and other rarer organisms. The accuracy of diagnosis using the smear varies among the different organisms in question. [1]

The infections of the genital tract are common in reproductive age women and many women remain asymptomatic in presence of vaginitis or cervicitis. [2,3] The Papanicolaou test also known as Pap smear is a screening method used to detect potentially precancerous and cancerous processes in the cervix. Greek doctor Georgios Papanicolaou invented this test and it was named after him [4,5]. The Papanicolaou (Pap) test is a simple, quick, and painless procedure performed on cells from the uterine cervix and used as a screening test for the prevention of the cancer of uterine cervix. [6,7] On the basis of cytological criteria, possible presence of infection is reported in the result of cervical pap smear test. [8]

Non specific cervicitis or inflammatory changes in a smear report are common. These findings are usually unclear for clinical approaches and there is not an appropriate guideline

for management of such patients. The possible presence of infection in cervical smear tests is usually reported by the cytologists based on cytological criteria. Vaginal discharge is a common problem among women.

Changes in balance of normal vaginal flora can cause an overgrowth of pathogens that lead to discharge. Although it is a common complain among sexually active women, there are still gaps in our knowledge about it. [9] Bacterial vaginosis (BV), Vaginal candidiasis (VC) and Trichomoniasis are the three infections most commonly associated with vaginal discharge [10] Bacterial vaginosis (BV), the most common form of vaginitis, is a complex condition where the ecosystem that guarantees vaginal antibiosis is completely altered. [11] BV may create a predisposition for serious sequelae such as postoperative infections in both obstetrics and gynecology, pre-term delivery, chorioamnionitis, urinary tract infections, endometritis, and pelvic inflammatory disease. [12,13] Trichomonas vaginalis (TV) is another common cause but some studies have not found its association with pregnancy complications [14] some studies indicated that TV during pregnancy was a predisposing factor for preterm delivery and delivering low-birth weight infants [15] Sensitivity, Specificity and Positive predictive value of Pap smear test for detection of BV has been reported 88.2%, 98.6% and 96.8% respectively. [16] The Pap smear test for detection of TV has showed sensitivity 98%, specificity 96% and PPV 88% [17] some recent studies have demonstrated the co-infection of vaginal pathogenic organisms in Pap tests [13, 18, 19] Our aim

was to investigate the frequency and severity of inflammation and prevalence of pathogenic vaginal microorganisms in cervical smears among received sample.

**MATERIAL AND METHOD**

This cross-sectional study was carried out on received Pap smear samples of women referred from gynecological opd over a period of 12 months at tertiary care centre of western India. This study was conducted on 800 conventional Papanicolaou cervical smears. These conventional smears were prepared by Gynaecologist at gynecological opd. After receiving, slides were fixed with 95% ethyl alcohol and were stained with the Papanicolaou stain by cyto technician. Slides were then mounted with DPX (Distrene Dibutyl phthalate Xylene) and Cytology smears were reviewed twice by two independent cytologists. All the data were manually collected and subsequently analysed. All females of age range 20 years to 80 years were included in our study. Women with Pregnancy, smoking, use of oral contraception and/or corticosteroids, regular use of vaginal douche, and women with chronic systemic diseases or systemic immunosuppression were excluded in our study. The frequency, severity of inflammation of cervix and prevalence of bacterial vaginosis (BV), Trichomonas vaginalis (TV), and vaginal candidiasis (VC) was determined in the samples. Also co-infection of vaginal microorganisms in Pap samples was evaluated.

Bacterial vaginosis is diagnosed by identifying *coccobacilli* or clue cells in Pap smears.

Trichomonas vaginalis (TV) is diagnosed by identifying trophozoites in Pap smears. Vaginal candidiasis (VC) is diagnosed by identifying fungal hyphae or budding yeasts were present in Pap smears. The presence of inflammation in the smears was divided into mild, moderate, and severe categories. Less than 30 inflammatory cells/high-power field was consider as mild inflammation. Moderate inflammation was diagnosed by 30 to 100 inflammatory cells/high-power field. If more than 100 inflammatory cells/high-power field than it was considered as severe inflammation.

**OBSERVATION AND RESULT**

The present study of 800 cases included patients from 20 to 80 years of age. Totally, 800 Pap smear samples were evaluated in which reproductive age group female were mainly affected with inflammation.

**Table 1 : The frequency of inflammation among studied population**

Presence of Inflammation	No. of patients
Yes	500(62.5%)
No	300(37.5%)

**Table 2: Distribution of inflammation severity among studied Pap smear samples**

Severity of inflammation	No. of patients
Mild	170(21.25%)
Moderate	208(26%)
Severe	122(15.25%)
Total	500(62.5%)

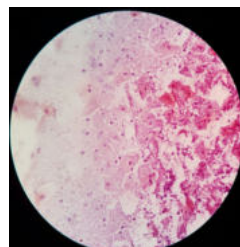
Out of 800 pap smear samples, Inflammation was reported in 500 (62.5%). Mild inflammation was seen in 170 smears (21.25%). Moderate inflammation was seen in 208 smears (26.0%) and severe inflammation was noted in 122 smears (15.25%). [Table 1,2]

**Table 3 : The prevalence of pathogenic vaginal microorganisms among studied Pap smear samples**

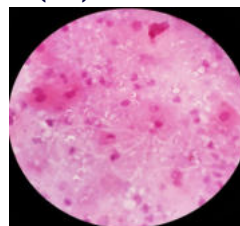
Microorganism	No. of affected people (%)
Bacterial vaginosis	132( 16.5%)
Vaginal Candidiasis	27(3.4%)
Trichomonas vaginalis	11 (1.4%)
Total	21.25%

The prevalence of vaginal microorganism was 21.3%

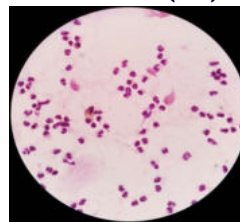
Out of 800 samples, Bacterial vaginosis was seen in 132 smears (16.5%), while Vaginal Candidiasis was noted in 27 smears (3.4%) and Trichomonas vaginalis was reported in 11 smears (1.4%) [Table 3] Overall, the presence of inflammation was significantly associated with candidiasis, but its severity was not correlated to the infection. Although, there was no significant relation between the presence of inflammation and BV, the severity of inflammation in specimen was significantly increased in association with BV. There was no significant association between BV and TV or VC.



**Figure 1: Clue cells and filmy background due to large number of coccobacilli. Shift in flora suggestive of Bacterial vaginosis (40x)**



**Figure 2: Clear halo surrounding the yeast form suggestive of Candida Glabrata (40x)**



**Figure 3: Pear shaped trophozoites of Trichomonas vaginalis (40x)**

**DISCUSSION**

In our study, total 800 pap smears were examined to find out the frequency and severity of cervical inflammation with prevalence of various vaginal micro-organisms also. The achieved results of our study were compared with different national and international studies. In present study, Inflammation was noted in 500 out of total 800 cervical pap smears. (62.5%). These findings were nearly similar to the study by Barouti et al, who found inflammatory smears 77.2% in their study.[9] while Stavroula baka et al, noted 55.7% in their study. [20] Other studies Fatma et al, Burke et al and Younis et al were noted frequency of inflammation 53%, 48% and 52% respectively in their study which was nearly similar with our study while Patel et al was noted 14.5% which was very low in compare with present study. [21,22,23,24]

**Table: 4 Comparison between various studies regarding frequency of Inflammation**

Fatma et al	53%
Burke et al	48%
Patel et al	14.5%
Younis et al	52%
Barouti et al	77.2%
Stavroula et al	55.7%
Present study	62.5%

Mild inflammation was seen in 170 smears.(21.25%), moderate inflammation in 208 smears (26%) and severe inflammation in 122 smears (15.25%) in present study, which is consistent with study by Barouti et al. Their result is similar to our study in which mild inflammation ( 26.1%), moderate (29.4%) and severe inflammation(22.2%) were noted. [9]

Among all three micro organisms, prevalence of BV was highest.( n=132, 16.5%). Wilson et al. also reported that inflammation on cytology is often associated with a genital tract infection [25]. Burke and Hickey demonstrated that the prevalence of infection 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 [24].

**Table: 5 Comparison between various studies regarding Prevalence of Bacterial Vaginosis**

Barouti et al.	17.2%
Stavroula et al	36.70%
Fatma et al	19.5%
Macroni et al	30.1%
Caixeta et al	41.0%
Present study	16.5%

Barouti et al. and Fatma et al [9,21] found their study that 17.2% and 19.5% women had bacterial vaginosis respectively. Their findings are consistent with our findings. While Stavroula et al, Macroni et al and Caixeta et al were reported higher frequency of bacterial vaginosis in their study which were 36.70%, 30.1% and 41.0% respectively. [20,26,27] These differences could be due to the characteristics of these communities and study populations.

**Table: 6 Comparison between various studies regarding Prevalence of Vaginal Candidiasis**

Barouti et al.	10.6 %
Stavroula et al	4.9%
Fatma et al	33.5%
Kafi et al	10.1%
Guducu et al	43.3%
Present study	3.4%

In our study, Vaginal Candidiasis was reported in 3.4% smears, which is consistent with findings of the study by Stavroula et al (4.9%). Barouti et al., Kafi et al, Fatma et al, Guducu et al were reported higher frequency of vaginal candidiasis in their study which were 10.6 %, 10.1%, 33.5% and 43.3% respectively.[9,20,21,28,29]

In our country, people suffer from poverty. The immune status, hygiene, level of education, and level of infection among patients may also have a profound influence on occurrence of an infection in the cervix. This may account for the wide variation in the presence of the organisms in the cervix among women in this study compared to other countries.

**Table 7: Comparison between various studies regarding Prevalence of Trichomonas Vaginalis**

Barouti et al.	0.4%
Stavroula et al	0.2%
Present study	1.4%

The prevalence of Trichomonas Vaginalis was 1.4% in our study which was higher than study by Barouti et al. and Stavroula et al., 0.4% and 0.2% respectively.[9,20] However, this rate was 18% in the study in Hamedan (30) and 19% in the African study (31). A probable reason for these variations could be differences in Pap sampling quality and cytologists' skill. Burke et al concluded that women with an inflammatory smear were more likely to mask the infection than women whose smear shows no evidence of inflammation (24).

**CONCLUSION**

It was concluded from the present study that, Papanicolaou test

( P&P smears ) in cytopathology has important role to diagnosis of inflammation of lower female genital tract, although it is primarily for screening of malignancy. Many times, inflammation in pap smears are associated with vaginal microorganisms and these patients should be considered for proper treatment for it. Finding BV,VC and TV in pap smears might be considered as an indication of treatment without performing other diagnostic test. Treatment of asymptomatic infection prevent further complications in selected patients. Candida is normal commensal bacteria in vagina, therefore asymptomatic patient may not require treatment. Pap smears are helpful for diagnosis of frequency and severity of cervical inflammation also. BV is major problem of this study group followed by VC and TV. It is necessary to increase awareness among women regarding symptoms like vaginal discharge and consequences of uterine cervix malignancy.

**REFERENCES**

- [1] Fitzhugh VA, Heller DS. Significance of a diagnosis of microorganisms on a pap smear, J Low Genit Tract Dis. 2008;12:40-51
- [2] J. M. Klomp, M. E. Boon, M. Van Haften, and A. P. M. Heintz, "Cytologically diagnosed Gardnerella vaginalis infection and cervical (pre)neoplasia as established in population-based cervical screening," The American Journal of Obstetrics and Gynecology, vol. 199, no. 5, pp. 480.e1-480.e5, 2008.
- [3] M. H. Bukhari, M. Majeed, S. Qamar et al., "Clinicopathological study of Papanicolaou (Pap) smears for diagnosing of cervical infections," Diagnostic Cytopathology, vol. 40, no. 1, pp. 35-41, 2012.
- [4] Vijaya Lakshmi P, Sree Gouri S.R. Study and Analysis of Two Hundred Cervical PAP Smears in Our Hospital. International Journal of Contemporary Medical Research. 2016;3(9):2787-89.
- [5] Pap smear. By Healthline medical review team. www.healthline.com/health/pap-smear# Overview/1/2015/11.
- [6] G. N. Papanicolaou, "A new procedure for staining vaginal smears," Science, vol. 95, no. 2469, pp. 438-439, 1942.
- [7] S. P. Michalas, "The Pap test: George N. Papanicolaou (1883- 1962). A screening test for the prevention of cancer of uterine cervix," European Journal of Obstetrics Gynecology and Repro- ductive Biology, vol. 90, no. 2, pp. 135-138, 2000.
- [8] J. G. Bertolino, J. E. Rangel, R. L. Blake Jr., D. Silverstein, and E. Ingram, "Inflammation on the cervical Papanicolaou smear: the predictive value for infection in asymptomatic women," Family Medicine, vol. 24, no. 6, pp. 447-452, 1992.
- [9] Barouti et al, "The pathogenic microorganisms in papanicolaou vaginal smears and correlation with inflammation", Journal of family and reproductive health, Vol 7, No:1, March 2013
- [10] Owen MK, Clenney TL. Management of vaginitis. Am Fam Physician 2004; 70:2125-32.
- [11] Mårdh P-A. The vaginal ecosystem. Am J Obstet Gynecol 1991;165:1163-116
- [12] Eschenbach DA, Hillier S, Critchlow C, et al. Diagnosis and clinical manifestations of bacterial vaginosis. Am J Obstet Gynecol 1988; 158:819-828.
- [13] Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. N Engl J Med 1995; 333:1737-1742.
- [14] Azarogoon A, Darvishzadeh S. Association of bacterial vaginosis, trichomonas vaginalis, and vaginal acidity with outcome of pregnancy. Arch Iran Med 2006; 9:213-17.
- [15] Cotch MF, Pastorek JG 2nd, Nugent RP, Hillier SL, Gibbs RS, Martin DH, et al. Trichomonas vaginalis associated with low birth weight and preterm delivery. The vaginal infections and prematurity study group. Sex Transm Dis 1997; 24:353-60.
- [16] Platz-Christensen JJ, Larsson PG, Sundstrom E, Wicqvist N. Detection of bacterial vaginosis in wet mount, papanicolaou stained vaginal smears and in gram stained smears. Acta Obstet Gynecol Scand 1995; 74:67-70.
- [17] Loo SK, Tang WY, Lo KK. Clinical significance of trichomonas vaginalis detected in papanicolaou smear: A survey in female social hygiene clinic. Hong Kong Med J 2009; 15:90-3
- [18] Demirezen S, Korkmaz E, Beksas MS. Association between trichomoniasis and bacterial vaginosis: Examination of 600 cervicovaginal smears. Cent Eur J Public Health 2005; 13:96-8.
- [19] Myer L, Denny L, Telerant R, Souza M, Wright TC Jr, Kuhn L. Bacterial vaginosis and susceptibility to hiv infection in south african women: A nested case-control study. J Infect Dis 2005; 192:1372-80
- [20] Stavroula baka et al, ' Inflammation on the Cervical papanicolaou smears: Evidence of infection in asymptomatic women?, Infectious Diseases in obstetrics and Gynecology, Volume 2013
- [21] Fatma et al, 'Cervical pathological changes among women with vaginal discharge attending teaching hospital.' J cyto/2017 Apr-Jun; V 34(2):90-94
- [22] Patel V, Pednekar S, Weiss H, Rodrigues M, Barros P, Nayak, et al. Why do women complain of vaginal discharge? A population survey of infectious and psychosocial risk factors in a South Asian community. Int J Epidemiol. 2005;34:853-62.
- [23] Younis N, Khattab H, Zurayk H, Elmouelhy M, Amin M. A community study of gynecological and related morbidities in rural Egypt. Stud Fam Plann. 1993;24:175-86.
- [24] Burke C, Hickey K. Inflammatory smears--is there a correlation between microbiology and cytology findings? Ir Med J 2004; 97:295-6.
- [25] J. D. Wilson, A. J. Robinson, S. A. Kinghorn, and D. A. Hicks, "Implications of inflammatory changes on cervical cytology," British Medical Journal, vol. 300, no. 6725, pp. 638-640, 1990.
- [26] Marconi C, Marii T, Daniela D, Silva C, Márcia G. Silva Prevalence of and risk factors for bacterial vaginosis among women of reproductive age attending

- cervical screening in Southeastern Brazil. *Int J Gynaecol Obstet.* 2015;38:85–93.
- [27] Caixeta R, Ribeiro A, Segatti K, Saddi V, Figueiredo A, Carneiro, et al. Association between the human papillomavirus, bacterial vaginosis and cervicitis and the detection of abnormalities in cervical smears from teenage girls and young women. *Diagn Cytopathol.* 2015;38:14–18.
- [28] Kafi S, Mohamed A, Musa H. Prevalence of sexually transmitted diseases (STD) among women in a suburban Sudanese community. *Ups J Med Sci.* 2000;105:249–53.
- [29] Güdücü N, Gönenç G, İnci H, Yılmaz A, Başsüllü N, Dunder I. Clinical importance of detection of bacterial vaginosis, trichomonas vaginalis, candida albicans and actinomyces in Papanicolaou smears. *Clin Exp Obstet Gynecol.* 2012;39:333–6.
- [30] Shobeiri F, Nazari M. A prospective study of genital infections in hamedan, iran. *Southeast Asian J Trop Med Public Health* 2006;37 Suppl 3:174-7.
- [31] Romoren M, Velauthapillai M, Rahman M, Sundby J, Klouman E, Hjortdahl P. Trichomoniasis and bacterial vaginosis in pregnancy: Inadequately managed with the syndromic approach. *Bull World Health Organ* 2007; 85:297-304.