

PAP SMEAR FOR SCREENING T. VAGINALIS

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ABSTRACT: BACKGROUND: Trichomonas vaginalis is sometimes seen in Papanicolaou stained smears, but because emphasis is placed on malignant cells in Papanicolaou stained smears, not much is done to search for this parasite in smears. In this study, cervical and vaginal specimens were examined by conventional Papanicolaou method for the presence of Trichomonas vaginalis microscopically. **MATERIALS AND METHODS:** Five hundred high vaginal swabs collected from gynaecology OPD were stained with Papanicolaou stain. **RESULTS:** One hundred and fifty (30%) out of 500 Papanicolaou stained smears screened, were positive for Trichomonas vaginalis. Out of them 76.67% of positive smears were from asymptomatic females. Presumptive diagnosis based on perinuclear halo and complete T. vaginalis had the highest sensitivity of 69.33%, while diagnosis based on perinuclear halo alone was 50.66% and 41.33% for diagnosis based on identification of complete organisms in Pap smear.

KEY-WORDS: Papanicolaou smears, Trichomonas vaginalis, perinuclear halo,

INTRODUCTION: Trichomonas vaginalis is a protozoan pathogen of the human urogenital tract. The prevalence of Trichomonas vaginalis has been reported to be as high as 26% among female STD clinic patients and 22% among HIV-positive women.¹ In infected female patients, symptoms include: vaginal discharge, vulvar pruritis, dysuria, and dyspareunia. Classical green, frothy, foul-smelling discharge occurs in 10% of the women. However, up to 50% of the infected female patients are asymptomatic.² In women, trichomoniasis may play a role in development of cervical neoplasia, postoperative infections, and adverse pregnancy outcomes and as a factor in atypical pelvic inflammatory disease and infertility.³ The presence of T. Vaginalis in the vagina increases predisposition to HIV seroconversion. Having trichomoniasis may increase the chance that an HIV infected woman passes HIV to her sex partner(s).^{4,5}

Diagnosis of T. vaginalis is an important public health issue, as asymptomatic patients may act as a reservoir for its transmission to their sexual partners. Wet mount preparations had a sensitivity of 40 to 75% while T. vaginalis culture had a sensitivity of 86 to 97%.^{6,7} However, expert personnel and laboratory support are required and they are not readily available in a primary care setting.² For direct microscopy only fresh specimens are of value.⁸ Diagnosis by

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wet-mount requires visualisation of viable, motile protozoa; therefore, specimens must be examined immediately. The sensitivity of wet-mount microscopy can be further reduced as a result of delays between specimen collection and examination,⁷ and with the large numbers of patients attending gynaecological clinics, an 'on the spot' examination of a vaginal swab is virtually impossible.⁹

Stained smears have the advantage that there can be considerable delay between preparation and staining and examination of a smear without loss of reliability in diagnosis. In primary care setting, the Papanicolaou (PAP) smear is a commonly performed screening test for cervical cancer. Thus, detection of *T. vaginalis* in PAP smear would mean an additional advantage provided the result is accurate.² *Trichomonas vaginalis* is sometimes seen in PAP smears where it is reported, but because emphasis is placed on malignant cells in PAP smears, not much is done to search for this parasite in smears.³ Moreover, studies have shown that PAP smear may detect *Trichomonas vaginalis* in a considerable number of culture-negative women.¹⁰ The aim of this study was to determine the suitability of PAP smear in the detection of *Trichomonas vaginalis* in cervical and vaginal specimens.²

MATERIALS AND METHODS: A total of 500 high vaginal swabs were collected from females in reproductive age group attending at gynaecology OPD. These smears were stained by conventional Papanicolaou method,¹¹ and screened microscopically for *Trichomonas vaginalis*. The study was carried in department of microbiology, during the period of August 2010 - August 2011.

PAPANICOLAOU METHOD¹¹: Each specimen was smeared on a clean grease free slide and fixed in ether-alcohol for 30 minutes. The specimens were then stained by the Papanicolaou method as follows: Harris's haematoxylin without acetic acid for 5 minutes, rinsed in tap water and differentiated in 1% acid alcohol for 30 seconds and blued in Scott's water for 2 minutes. Smears were taken to 95% alcohol and stained in OG6 for 2 minutes, rinsed in 95% alcohol and stained in EA 35 for 2 minutes. Smears were then taken to two changes of absolute alcohol, xylene and mounted in DPX. The stained smears were examined under the light microscope at low and high power objectives for the presence of *Trichomonas vaginalis* and perinuclear halo.

Identification of one or more of the following morphological characters was considered to be conclusive of *T. vaginalis*: a pear-shaped, oval to round, cyanophilic organism that ranges in size from 15-30 microns; pale nucleus, vesicular and centrally located; cytoplasmic eosinophilic granules or flagella. The presence of perinuclear halo in the epithelial cells was also used as a presumptive diagnosis for *T. vaginalis*. These criteria were also used to distinguish trichomonads from cytoplasmic fragments.

RESULTS: One hundred and fifty (30%) out of 500 PAP smears screened were positive for *Trichomonas vaginalis*. On retrospective analysis 76.67% of positive smears were from asymptomatic females. Only 23.33% had history of vaginal discharge and pruritis. 25(5%) of 150 positive PAP smears showed *Candida* infection and all were symptomatic women. See table1

Presumptive diagnosis based on perinuclear halo alone was 50.66% while diagnosis based on identification of organisms alone in PAP smear was 41.33%. When both the morphological characters of *Trichomonas vaginalis* were given equal consideration for

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identification, i.e slide was considered positive when any one or both the characters are present; diagnosis of *Trichomonas vaginalis* rose to the sensitivity of 100%. See table 2.

DISCUSSION: Papanicolaou is the best staining method in cytology, because it helps to effectively differentiate malignant cells from non-malignant cells. It also stains the cytoplasm and its contents. Its ability to differentiate acidophilic materials from basophilic materials as well as its ability to stain non-cellular substances such as fibrin, crystals and pigments, makes it an essential stain in. *T. vaginalis*, the causative organism for trichomoniasis is the most common curable sexually transmitted organism worldwide. It parasitizes both males and females where it is sometimes asymptomatic in the early stages of the infection. *T. vaginalis* infection is said to play a role in the development of cervical neoplasia, postoperative infections, and adverse pregnancy outcomes and as a factor in atypical pelvic inflammatory disease and infertility. In our study 76.67% of females in reproductive age group were asymptomatic carriers of *T. Vaginalis*, and are at risk of developing above said complications until treated in time. There is also epidemiological and experimental evidence that PAP smears are beneficial in detecting infections that are risk factors associated with cervical cancer. It can detect certain viral, bacterial, and fungal infections of the cervix and vagina. Culture is a very sensitive method of detecting *T. vaginalis* but it is expensive and time consuming.

In the present study very broad criteria were used for the identification of *Trichomonas vaginalis* in PAP smears, to increase the sensitivity. Twenty three percent of the women with *Trichomonas* - positive PAP smears had genital symptoms, while 77% of women were asymptomatic carriers of *Trichomonas vaginalis*. Thus our data suggest that PAP smear may detect *Trichomonas vaginalis* in a considerable number of asymptomatic women. Steven KF Loo. et al² reported that Pap smear could diagnose *T. vaginalis* infection in 42% of asymptomatic carriers.

The comparison of positive results showed that the highest sensitivity was found when diagnosis was based on perinuclear halo and/or *T. vaginalis* in PAP smear followed by sensitivity of 50.66% when diagnosis was based on perinuclear halo alone. Presumptive diagnosis based on identification of complete organisms alone in Pap smear was 41.33%. Avwioro O G³ also reported a similar sensitivity pattern of 65.77%, 52.63% and 42.11% for diagnosis based on both perinuclear halo and *T. vaginalis*, perinuclear halo alone and complete organisms in PAP smear respectively. In our study, sensitivity for the PAP smear was 100 % which is in comparison with previous studies in the diagnosis of *T. vaginalis*. The reported sensitivities ranged from 83 to 99%.^{12,13}

CONCLUSION: Papanicolaou smears used for routine screening of cervical cancers can also be used for screening *T. vaginalis* infection in females. Papanicolaou smears can detect trichomoniasis in asymptomatic patients and is suggested to be one of the best screening tools for asymptomatic carriers of *T.vaginalis* in females. This helps in early detection and treatment of infection.

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Table 1: Prevalence of T.vaginalis in PAP smears

Total number of PAP positive specimens	150	100 %
Presence of genital symptoms	35	23.33%
Absence of genital symptoms	115	76.67%
Concomitant sexually transmitted disease	25	16.66%

Table 2: Comparison of Morphological features for identification of T vaginalis in PAP smears

Investigation	No.	Sensitivity%
Total positive for perinuclear halo alone (suggestive of T. vaginalis)	76	59.66
Total positive for complete parasite alone seen in PAP smears	62	41.33
Total positive for both perinuclear halo and complete T. vaginalis	12	8
Total positive for perinuclear halo and complete T. vaginalis: alone or both	150	100