

Original Research Article

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Comparison of Wet Mount Examination with Giemsa Staining and Fluorescent Staining for Detection of *Trichomonas vaginalis* in Clinically Suspected Cases of Vulvovaginitis

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ABSTRACT

Keywords

Trichomonas vaginalis, Wet mount examination, Giemsa staining, Acridine orange staining.

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Trichomonas vaginalis is an important cause of the premature rupture of membranes, premature delivery, pelvic inflammatory disease, urethritis, and chronic prostatitis. Wet mount is the most widely used method for the detection of *T. vaginalis* in clinically suspected cases of vulvovaginitis. The purpose of this Study was to compare various microscopic techniques for detection of *T. vaginalis* like wet mount examination, Giemsa and Acridine orange staining. The Cross-sectional Analytical study was conducted in the Department of Microbiology, attached to a Tertiary Care Hospital in Central India after obtaining ethical clearance over a time period of two months. Out of 30 cases, only two (6.66%) were detected positive for Trichomoniasis. All the 30 vaginal samples (cases) tested for detection of *T. vaginalis* were found to be negative by Wet mount examination. Whereas, Giemsa staining and Acridine orange staining were positive in 1 (3.33%) and 2 (6.66%) case respectively. Present study highlights that Acridine orange staining was found to be the best microscopic method when compared with wet mount examination and Giemsa staining. This will also provide rapid screening of vaginal smears in patients suffering from vaginal discharge and thus, help in early diagnosis and prompt treatment of patients.

Introduction

Trichomoniasis is the most common non-viral sexually transmitted disease (STD) worldwide. It was estimated that approx. 7.4 million new cases of *Trichomonas vaginalis* (*T. vaginalis*) infection occurred in 2000 in USA (Weinstock *et al.*, 2004).

Population-level data from the National Longitudinal Study of Adolescent Health have documented that *T. vaginalis* affects

2.8% of young adult women, a prevalence seven times higher than that of *Neisseria gonorrhoeae* in this age group (Miller *et al.*, 2005).

Vaginitis due to *Trichomonas vaginalis* clinically manifests with symptoms of vaginal itching, odor, purulent, foul-smelling and vaginal discharge, which is associated with pruritis, dysuria and dyspareunia (Schwebke

et al., 2004; Swygard *et al.*, 2004). The discharge usually exceeds pH 6.0, and a 'strawberry' cervix, which is characterized by punctate hemorrhagic lesions, is described. These classic symptoms are only seen 20% of the time, which often leads to women with these vaginal symptoms being empirically treated for yeast infections or bacterial vaginosis.

Recent studies also show that *T. vaginalis* is an important cause of the premature rupture of membranes, premature delivery, pelvic inflammatory disease, urethritis, and chronic prostatitis.

Trichomoniasis infection also enhances the transmission of human immunodeficiency virus infection (Nanda *et al.*, 2006). *T.vaginalis* is highly prevalent and is associated with adverse outcome, improved detection of *T. vaginalis* is needed.

In various clinical settings, wet mount is the most widely used method for the detection of *T. vaginalis* in clinically suspected cases of vulvovaginitis (Wiese *et al.*, 2000).

Although, wet mount examination is most cost effective diagnosis test, but it has low sensitivity resulting in under diagnosis of the diseases. Therefore, to overcome this problem, various staining techniques like Giemsa and Acridine orange can be used along with wet mount examination for diagnosis of *T.vaginalis* infection.

Therefore the plan was to compare various microscopic techniques for detection of *T. vaginalis* like wet mount examination, Giemsa and Acridine orange staining.

Materials and Methods

This Cross-sectional Analytical study was conducted in the department of Microbiology, attached to a Tertiary care hospital in central

India over a time period of two months from 1st August to 31st October 2016 after obtaining Ethics Clearance.

A total of 30 vaginal swabs were collected from patient of reproductive age group (15-45 years).

Collection of Samples

A total of 30 vaginal swabs were collected from patient of reproductive age group (15-45 years) by clinicians in suspected cases of vulvovaginitis after written informed consent.

The clinicians obtained three vaginal samples/swabs [Himedia Laboratories Pvt. Limited, Mumbai] (first swab for detection of motile trichomonas by wet-mount microscopy & second swab was used to prepare smear for Giemsa staining and third swab was used to prepared smear for Acridine orange staining for detection of *Trichomonas vaginalis*) from each patient by swabbing the vaginal vault with the help of vaginal speculum and then send to laboratory within 10 min of collection.

The clinicians were asked to vary the order in which they obtained the vaginal swabs to prevent sampling bias.

Procedure for sampling

After the receiving of sample in the laboratory and procedure as followed-

1st swab - was placed in screw capped sterile plastic tube containing 0.5 ml of 0.9% saline and used immediately for detection of motile *Trichomonas* by wet-mount microscopy.

Wet Mount Examination

First swab was processed for wet-mount microscopy. After vigorous mixing of the swab in the saline, the swab was pressed

against the side of the tube to express as much fluid as possible. One drop of expressed fluid was placed on a clean glass slide with a cover slip and the slide was examined for detection of motile *Trichomonas* (exhibiting twisting motility) with a brightfield microscope at 10x and 40x by the clinician or laboratory technician within 10 min of collection.

The *Trichomonas* were identified by their size (10-20 µm), round or oval shape, and characteristic twitching motility (Cheesbrough, 2000).

IInd swab was used to prepare smear for Giemsa staining (Hi Media Laboratories, India) for detection of *Trichomonas vaginalis*. Procedure was as follows:

Giemsa Staining

The prepared smear was fixed by immersion in methanol for one minute and allowed to dry. It was then stained with Giemsa stain (Hi Media Laboratories, India), diluted 1 part to 19 parts of 1/15M phosphate buffer, pH 7.2 for 10 min and scanned for *Trichomonas vaginalis* at 10X magnification and then at a magnification of 40x. At least 30 fields were examined before a negative finding was recorded.¹⁸

Both the internal and external structures of the organism were clearly visualized. The former stained dark blue with a red nucleus and the latter was sharply outlined, showing clearly the flagella and the undulating membrane [Figure A]. IIIrd swab was used to prepare smear for Acridine orange staining using staining kit by HiMedia, India for detection of *Trichomonas vaginalis*. Procedure was as follows:

Acridine Orange Staining-

The prepared smear was fixed with methanol for 2-3 min and covered with phenolic

auramine dye [S042] which was allowed the stain to remain on the surface of the slide for 15 minutes without drying rinse with distilled water and draining off excess liquid. Slides were flooded with decolorizer [S043] for 2-3 mins. The slides were rinsed thoroughly with distilled water, and counterstained with [S044] for 3-4 mins by flooding. After being rinsed with distilled water & allowed to air dry, the slide was examined under a fluorescence microscope (with a 470-490 nm filter) at a magnification of 40X (Schee *et al.*, 1999).

Epithelial cells fluoresced light green with a bright green nucleus. The nuclei of leucocytes (Pus cells) fluoresced bright green and bacteria stained bright red colour. Trophozoites of *T. vaginalis* were seen as characteristic brick red colour with a yellowish green nucleus. The images from the fluorescent microscopes cannot be captured as we don't have the facility for the same.

All data was maintained in Microsoft office Excel. Statistical analysis was carried out using Excel and test of proportion were used for analysis.

Results and Discussion

A total of 30 vaginal swabs were collected from patient of reproductive age group (15-45 years) after Institutional Ethics committee clearance.

The age wise distribution of the patients from whom the samples were collected is as given in Table 1. The observation for wet mount, Giemsa stain and Acridine orange stain for the samples collected were as given in Table 2.

Of 30 cases only two (6.66%) were detected positive for Trichomoniasis by Direct microscopic examination.

Wet mount examination is the most frequently used method for diagnosis of Trichomoniasis in women. In study done by Razia Khatoon, Noor Jahan *et al.*, (Razia *et al.*, 2014) shows, out of the 615 cases, 25 (4.1%) cases were positive for *T. vaginalis* infection by wet mount examination with sensitivity of 67.6%. In our study, all the 30 cases were found to be negative by wet mount examination technique which may be because of chance as the sample size is small. The less number of organisms and inappropriate amount of sample collected due to variable

absorption rate of the cotton swab used may lead to negative results. Our tertiary hospital being in the rural area and population with low socioeconomic category, mostly resort to the quacks and the over the counter prescriptions are very common. Majority of the patients reports to have taken anti - protozoal treatments for protozoal infections very common in rural areas. Although, positive wet mount is diagnostic, a negative test cannot exclude Trichomoniasis because of low sensitivity.

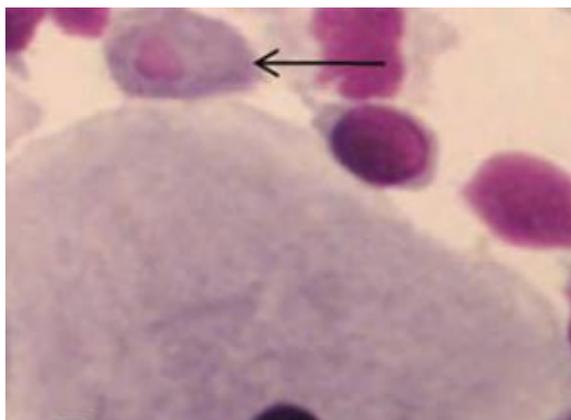
Table.1 Age wise distribution of patients clinically suspected of Vulvovaginitis included in the study for detection of *Trichomonas vaginalis*

Females	15 to 25 years	26 to 35 years	36 to 45 years	
	8	12	10	30

Table 2: Detection of *T.vaginalis* using Wet mount, Giemsa stain and Acridine orange stain

Staining techniques performed	Positive cases(%)	Negative cases (%)
Wet Mount (n=30)	Nil (0%)	30(100)
Giemsa staining (n=30)	1 (3.33)	29(96.6)
Acridine Orange staining(n=30)	2(6.66)	28(93.3)

Fig.1 Giemsa staining showing *Trichomonas vaginalis*



Study done by Razia Khatoon, NoorJahan *et al.*, (2014) shows, the performance of Giemsa staining was good and it was able to detect

Trichomonas vaginalis in 30 (4.9%) out of 615 patients. Studies done by different workers have reported varying range of

sensitivity (41-56%) of Giemsa stained smears for the diagnosis of *Trichomonas vaginalis* infection. In set up lacking immediate microscopic facilities Giemsa staining is very useful, where duly prepared and fixed smears can be transported to the laboratory for diagnosis. Also, with large number of patients attending gynaecology outpatient department, an immediate examination of a vaginal swab is virtually impossible. Unlike, wet mount examination, delay in transport has no significant impact on its reliability for diagnosing *Trichomonas vaginalis*. However, it is time consuming and needs technical expertise.

In our study, out of 30 cases examined, 2 cases were positive by Acridine orange staining. In similar study done by Razia Katoon, Noor Jahan *et al.*, (2014), Acridine orange staining was positive in 37 (6.0%) out of 615 patients, with sensitivity and specificity of 100% and 98.0% respectively. Whereas, in one study Acridine orange staining showed a high sensitivity and specificity of 71.43% and 99.44% respectively, in another study its sensitivity was only 47.5% in comparison to culture (Radonjic *et al.*, 2006). Also, it has been reported that the sensitivity of Acridine orange staining is high (67%) in women with *T.vaginalis* infection alone, and low (53%) in women with multiple infections (Bickley *et al.*, 1989).

In the present study, maximum number of cases of vaginal trichomoniasis was detected by Acridine orange staining. This is in agreement with a previously done study which showed that the infection rate detected by Acridine orange staining was higher than either culture or other vaginal swab examinations.

Although Acridine orange staining is easy to perform and rapid, its major disadvantage is

that it requires special microscopic facilities, trained personnel and availability of the dye. Also, the smears lose their fluorescence with the passage of time, so permanent record is not possible. However, the rapidity, ease and reliability of Acridine orange staining justify its use in routine laboratory diagnosis of Trichomonal infection.

In conclusion, although wet mount examination is the most commonly used test in routine diagnosis of *Trichomonas vaginalis* infection, but staining techniques should be used as an additional diagnostic test in order to diagnose cases missed either due to unavailability of immediate microscopic facility or delay in the transport of samples to the laboratory for culture. Acridine orange staining was found to be the best microscopic method when compared with wet mount examination and Giemsa staining. Hence, it should be used in routine diagnosis of *Trichomonas vaginalis* infection in places where fluorescent microscopic facility is available. This will also provide rapid screening of vaginal smears in patients suffering from vaginal discharge and thus, help in early diagnosis and prompt treatment of patients. As Giemsa staining performed well in comparison to wet mount examination and can easily be done in routine, it should be used as an adjunct to wet mount microscopy in small setups lacking facilities for fluorescent microscopy. This will ultimately prove beneficial to patients in reducing morbidity and associated adverse health outcomes in the form of pelvic inflammatory diseases and increased risk of acquiring other sexually transmitted diseases including human immunodeficiency virus infection

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