Improving Diagnosis of *Trichomonas Vaginalis* Infection in Resource Limited Health Care Settings in Sri Lanka

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ABSTRACT

Objective: This study was designed to compare diagnosis of trichomoniasis by culture, wet smear examination, and Giemsa stain. A modified technique was used to transport and prepare the specimen to ensure parasite viability prior to Giemsa staining. **Materials and Methods:** A clinic-based prospective study was carried out in association with the National STD/AIDS Control Programme over a period of 18 months. Three swabs were collected from the posterior fornix of 346 newly registered female patients for diagnosis of trichomoniasis. A wet smear was prepared using the first swab. The second swab was placed in 5 mL of 0.9% saline with three drops of 5% glucose at room temperature and centrifuged twice at a low speed prior to preparation of a Giemsa stained smear. The third swab was for culture. The three tests were performed independently. The specificity and sensitivity of the wet smear and Giemsa stain were compared to culture. **Results:** With culture, the prevalence of trichomoniasis was 6.9% (95% CI: 4.1–9.3%). The Giemsa-stained smear was found to be highly sensitive (100%, 95% CI: 86.2–100%) and specific (99.69%, 95% CI: 98.26–99.95%) compared to culture. The wet smear was less sensitive (95.83%, 95% CI: 79.76–99.26%) but equally specific (100%, 95% CI: 98.82–100%).**Conclusion:** In developing countries, facilities for using culture are limited and wet smear examination in the field is also difficult due to the immediate need for laboratory facilities. Our study demonstrated that, in this setting, using a transport medium prior to Giemsa staining is a feasible alternative, with a high-diagnostic yield.

Key words: Culture, Giemsa stain, Sensitivity, Specificity, *Trichomonas vaginalis*, Wet smear

INTRODUCTION

Trichomonas vaginalis, a flagellated protozoan parasite, is the causative agent of trichomoniasis, which is the commonest treatable sexually transmitted infection in industrialized countries.[1] In women, trichomoniasis has a range of presentations, from asymptomatic infection to an acute inflammatory disease with a copious and malodorous vaginal discharge. Infection is also linked to preterm labor and prenatal morbidity.[2,3]

Studies carried out in Sri Lanka have shown different prevalence rates for trichomoniasis. As expected, the clinic-based studies report a higher prevalence than community-based studies. Hemachandra[4] reported a prevalence of 1% in a community-based study in the Sabaragamuwa Province, while Herath[5] reported a community prevalence of 0.6% in the Colombo district. Perera[6] has observed a prevalence of 4.4% among women presenting to two gynecological clinics. More recently, Banneheke *et al.*[7] have demonstrated a prevalence of 4.2% of trichomoniasis in women attending a sexually transmitted disease clinic.

Diagnosis of trichomoniasis is usually made by microscopic examination of a saline wet smear preparation of vaginal secretions. This technique has not changed since it was first described by Donne.[8] A positive wet smear is diagnostic because of its high specificity, whereas a negative test cannot exclude trichomoniasis because of the low sensitivity of 30–80%.[9-11]

Use of stained vaginal smears with Giemsa stain to diagnose *T. vaginalis* infection has been compared to other diagnostic methods.[12-14] In these studies, the vaginal swabs were smeared on the slides, air dried and fixed with methanol prior to staining with Giemsa. The percentage

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of *T. vaginalis* infections detected by using Giemsa stain has always been lower as compared to culture (19% vs 22% reported by Ojuromi *et al*., 21.7% vs. 29.8% reported by Mason *et al*., 5.5% vs. 10.5% by Radonjic *et al*.).[12,13,15]

The gold standard for the diagnosis of trichomoniasis is culture, which has a high sensitivity of 71–100%.[16] Unfortunately, culture requires an incubator with a constant electricity supply, relatively expensive culture media, and an experienced microscopist. Furthermore, it can take up to 5–7 days for results to be obtained; these issues limit its use in resource-limited settings.[17,18]

This study compares the diagnostic yield of trichomoniasis by culture, wet smear examination, and Giemsa stain. It also assesses the feasibility of employing the Giemsa-stained smears (after preparing the sample with a modified technique) as a routine diagnostic tool in resource-limited settings.

MATERIALS AND METHODS

Study site and study population

A clinic-based prospective study was carried out at the National STD/AIDS Control Programme (NSACP) over a period of 18 months from October 2007 to April 2009. The study population comprised all newly registered female clinic attendees aged 15–60 years. This included symptomatic patients and asymptomatic patients who presented to the clinic to rule out a sexually transmitted disease. Chronically ill women and those who did not give consent for speculum examination were excluded from the study.

Ethics approval for the study was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Colombo.

Specimen collection

After obtaining consent, serology for syphilis and human immunodeficiency virus (HIV), and vaginal / cervical samples to detect common reproductive tract infections are routinely collected in almost all clinic attendees at NSACP. In addition to these samples, at the time of collecting vaginal secretions, two extra swabs were obtained from the posterior fornix for diagnosis of trichomoniasis. The first swab was used to prepare a saline smear for examination at the NSACP clinic. The second swab was stored in a graduated test tube containing 5 mL of sterile physiological saline with three drops of 5% glucose solution at room temperature.[15] Later, this sample was used to prepare a Giemsa-stained smear. The third swab was inoculated into a glass tube containing 5 mL of culture media. The second and third swabs were transferred to the Department of Parasitology, Faculty of Medicine, Colombo within 6–8 h of collection.

Laboratory tests

The three laboratory tests, namely, examination of a wet smear of vaginal secretions, examination of a vaginal smear stained with Giemsa, and culture were carried out by three independent, trained personnel. The wet saline smear was examined immediately by an experienced microscopist as a part of the routine diagnostic procedure carried out at the NSACP. Culture and the Giemsa staining were carried out at the Department of Parasitology, Faculty of Medicine, Colombo. Individuals performing the three tests were blinded to each other.

Culture of *T. vaginalis*

Culture for *T. vaginalis* was carried out using Trichomonas medium (OXOID code; CMO 161B enriched with Horse serum and incorporated with Chloramphenicol) based on manufacturer's instructions.[19] The tube containing the culture media was incubated at 37°C for 3 days. Afterwards, for the next 7 days, a fresh smear was prepared every 24 h from the medium taken from the bottom of the tube and examined microscopically for the presence of motile flagellates.

The Trichomonas medium used for culture in this study was a medium based on that of Feinberg and Whittington for the detection of *T. vaginalis* and *Candida* species.[20] Currently, the Trichomonas medium has been slightly modified by the incorporation of 0.1% w/v of agar which leads to reduced oxygen tension and consequently more prolific growth of trichomonads.[19]

The diagnostic yield for trichomoniasis varies depending on the type of culture medium used. A systemic review has shown that the sensitivity for diamond medium (11 studies) varied from 88 to 99% while the same value for Oxoid medium was 76–89%. However, the Oxoid medium was used in only three studies that were assessed in this review.[16] Diamond culture medium was not used in our study due to limited financial and laboratory resources.

Preparation of the sample for staining with Giemsa

A modified technique was adopted to ensure efficiency in transport and preparation of specimens than the standard technique.[12] Instead of preparing vaginal smears and air

drying it, the swabs were preserved in saline with three drops of 5% glucose for transportation.[21] This method was adopted from the procedure of preservation of samples for fine needle aspiration cytology where samples are not air dried but preserved in saline.[22]

Prior to staining with Giemsa, the tubes with the swabs were vigorously shaken to displace all the parasites into the saline solution. The swabs were then removed and the test tubes were centrifuged at a low speed (500–1000 rpm) for 30 min. The supernatant was carefully extracted using a Pasteur pipette leaving the deposit. Five milliliters of absolute methanol was added to the deposit and mixed well. The tube was centrifuged again at the same speed for 30 min. The methanol supernatant was then removed leaving a thin layer of methanol as a liquid phase. The deposit which contains the parasites and the remaining layer of methanol was mixed thoroughly. One drop of this mixture was put on a clean dry slide and three such slides were prepared from a single sample. The slides were air dried and then incubated at 37°C for 12 h.

The slides were stained with Giemsa diluted 1:9 with phosphate buffer solution (pH 7.2) for 7 min. Slides were washed carefully with running tap water, and air dried. Each slide was scanned for parasites at 10 × 100 magnification.

Data analysis

Prevalence was calculated to reflect the relative frequency of trichomoniasis with the corresponding 95% confidence intervals (CI). SPSS statistical software packages were used for data analysis. The variable measures were the numbers of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN). Sensitivity, specificity, negative, and positive predictive values were recorded.

RESULTS

A total of 359 women were recruited into the study. However, speculum examination could not be carried out in 13 (4%) women in the sample. Therefore, vaginal samples were collected only from 346 (96%) individuals. The mean age of women was 32.9 years [standard deviation (SD) ± 9.27]. The majority were within the 21–40 age range (254, 73.5%). The percentage of ever married women was 85.8% (297). Fifty-four percent (188) was unemployed while a further 15% (53) were commercial sex workers. Interestingly, 86.8% (300) had had a secondary education. Therefore overall, a majority in this cohort were young and middle aged, unemployed, married, and educated women.

Regarding the clinical symptoms, pruritus, vaginal discharge, and vulvovaginal soreness were significantly higher in women testing positive for trichomoniasis (χ^2 test, *df*–2, $P<0.05$). Other symptoms such as dysuria, post-coital bleeding, and visible inflammation of the cervix did not reach a level of statistical significance.

Using any one of the three methods, namely culture, wet smear examination or Giemsa stain, 7.2% were found to be infected with *T.vaginalis* [Table 1]. The results of parasite detection by culture, Giemsa stain, and wet smear are compared in Table 2. On examination of the smears prepared from the culture medium, 24 (6.9%) individuals tested positive for *T. vaginalis* and 25 (7.2%) tested positive with the Giemsa stain.

Following the modifications in the procedure for transport and preparation of the specimen, the Giemsa method was found to be highly sensitive (100%, 95% CI: 86.2–100) and 99.69% specific (95% CI: 98.26–99.95) for the diagnosis of *T. vaginalis* compared to culture with a positive predictive value of 96% and a negative predictive value of 100% [Table 3]. One false positive was found with Giemsa staining; a possible explanation is that the culture in this instance was falsely negative, since Giemsa stain is based on morphological identification of the parasite. In contrast, the wet smear was found to have a sensitivity of 95.83% (95% CI: 79.76–99.26) and a specificity of 100% (98.82–100) when compared to diagnosis by culture with a PPV of 100% and a NPV of 99.6%.

Table 1: Comparison of parasite detection by culture and wet smear examination

Wet smear for *T. vaginalis*	Trichomonas culture		
	Positive	Negative	Total
Positive	23	0	23
Negative	1	322	323
Total	24	322	346

Table 2: Comparison of parasite detection by culture and Giemsa's technique

Giemsa's stain	Trichomonas culture		
	Positive	Negative	Total
Positive	24	1	25
Negative	0	321	321
Total	24	322	346

Table 3: Performance of modified Giemsa's stain and wet smear examination for diagnosis of *T. vaginalis* relative to culture technique

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Giemsa's stain	100	99.7	96	100
Wet smear examination	95.8	100	100	99.6

DISCUSSION

As effective therapeutic agents for the treatment of trichomoniasis are widely available, diagnostic difficulties have become the limiting factor in reducing the disease burden.[23]

Wet preparation for microscopy is currently the preferred method for diagnosis of *T. vaginalis* infections in the sexually transmitted disease (STD) clinics in Sri Lanka because of its low cost. Even with skilled microscopists, this test shows a sensitivity of approximately 50–80% compared to culture.[17,24] Furthermore, the specimens must be examined immediately (within 20 min) after collection to enable the visualization of viable, motile protozoa. The sensitivity of the wet mount for microscopy declines substantially with even short time delays between collection and examination.[18,25]

Dry vaginal smears stained with Giemsa stain have been used for the diagnosis of *T. vaginalis* infections.[12-14] The optimum dilution of Giemsa stain used in the current study (1:9) and the duration of staining (7 min) were in accordance with the World Health Organization (WHO) criteria and different to that used earlier by Mason *et al.* (1:19 dilution for 10 min).[12,26] Radonjic *et al*.[13] have demonstrated a low sensitivity of 52.4% for diagnosis of *T. vaginalis* with Giemsa stain. In studies where dry vaginal smears were used for diagnosis, the smears were air dried and fixed immediately with absolute ethanol or methyl alcohol prior to staining in order to preserve the morphology of the parasites. This may not be feasible in clinical settings such as community-based clinics where there are no immediate laboratory facilities.

In this study, the swabs were inserted into a transport medium containing 5% glucose solution which acted as an extracellular energy source to preserve the viability of the organisms *in vitro*.[27] Thereby, smears could be prepared up to 24 h later (as established in two different field community studies carried out simultaneously).[4,5] The fact that smears could be examined after a lag period is a definite advantage over the immediate examination of a wet smear preparation. The modifications to the preparation of the sample prior to staining make diagnosis more efficient as the sample are concentrated and morphology of the parasite is preserved.

Prior to staining with Giemsa, the sample is centrifuged twice at low speed. The examination of a centrifuged deposit has the added advantage of concentrating a larger number of parasites. Further, centrifugation at a lower speed prevented the separation of the flagellum from the body of the parasite and preserved its structure for morphological studies. In addition, use of the modified technique allows general practitioners and even patients themselves to take blind vaginal swabs (without speculum use) and preserve the sample for later inspection.

Considering the cost of testing, in Sri Lanka the cost of a wet smear is USD 0.10, the cost of Giemsa staining is USD 0.30, and the cost of culture is USD 2.00. The modified method Giemsa staining will cost approximately USD 0.10 extra. Given the marginal extra cost, this makes it the most feasible and cost-effective test in our setting, considering its higher sensitivity when compared to the wet smear examination.

More recently, antigen detection tests to diagnose trichomoniasis have been used. They are more sensitive than wet preparation microscopy (78.5% and 72.4%, respectively; P=0.04) but were less specific (98.6 and 100.0%, respectively; P=0.01).[28] Cost is a prohibitive factor for wide spread use of rapid diagnostics, but it may be of value in settings where microscopy is not possible.

CONCLUSIONS

In a resource poor country such as Sri Lanka, where laboratory facilities and manpower are limited, and cost is a consideration, use of a low cost transport medium to preserve the high vaginal swabs and subsequent use of Giemsa-stained smears to diagnose *T. vaginalis* may be used as an alternative to wet smear examination. When compared with culture, both methods were equally specific, but the Giemsa stained smears were more sensitive in diagnosis.

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