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Comparison of Methods for the Identification of *Trichomonas vaginalis* in HIV-Positive and Negative Women

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Abstract: This study compared wet mount microscopy, culture and cytology for the diagnosis of *Trichomonas vaginalis* in HIV-positive and negative women. In addition, the inflammatory alterations caused by the presence of the parasite were evaluated in the cervical smear test. Samples were collected from patients attending 3 healthcare centers, using swabs for wet mount microscopy and culture. For cytology, an endocervical cytobrush specimen was collected in all patients except for the pregnant women. A total of 237 samples were examined, 125 in the HIV-positive test group and 112 in the HIV-negative control group. The highest number of positive samples was identified by culture (13.9%), followed by cytology (13.5%) and wet mount microscopy (11.4%). Of the 33 culture-positive specimens, 26 (78.8%) were positive for *T. vaginalis* in all 3 techniques. There was no statistically significant difference between the 2 groups of patients with respect to inflammatory alterations associated with the parasite in the cervical smear test. Culture was the most effective diagnostic method (gold standard) for evaluation of the presence of *T. vaginalis* and there was no statistically significant difference between the 2 groups with respect to inflammatory alterations related to the parasite.

Key words: *Trichomonas*, HIV, diagnostic, women, cervical smear test, culture, wet mount microscopy

INTRODUCTION

Trichomonas vaginalis, the agent that causes trichomoniasis, is a flagellate protozoan that infects the human vagina (Mundodi *et al*., 2006). Trichomoniasis is the most common nonviral sexually transmitted infection worldwide, with an estimated 250 million cases. Prevalence estimates vary in accordance with the population studied but have been reported to range from 5-74% (Swygard *et al*., 2004).

A higher incidence of *T. vaginalis* is found in women around the time of menstruation. This may be caused by the capacity of the parasite in the phagocytic blood cells to acquire iron from hemoglobin, which, according to López *et al*. (2000) constitutes an essential nutrient to its metabolism. The mechanism by which *T. vaginalis* causes damage to the cells remains unknown; however, Mirhaghani and Warton (1998) found that the presence of glycoconjugate components in the external membrane of the parasite enables it to attach to and damage the host epithelial cells, thus, conferring its pathogenicity.

According to Wiese *et al*. (2000) wet mount microscopy is considered a highly specific diagnostic method (99.8%); however, sensitivity is lower (58-82%). A review carried out by Patel *et al*. (2000) highlights the advantage of its low cost and considers this the most convenient and widely used method for the investigation of *Trichomonas vaginalis*. Clark *et al*. (2007) reported that women with positive wet mounts tend to have a high infection load and signs of inflammation are more evident in the cervical smear.

Culture remains the gold standard for the diagnosis of *T. vaginalis* in view of the high rates of sensitivity and specificity offered by this method (Maciel *et al*., 2004). Mabey *et al*. (2006) affirmed that the majority of culture samples will read positive within 48 h; however, they must be kept under observation for at least 10 days before being discarded. The review performed by Patel *et al*. (2000) showed that Diamond's medium results in maximum parasite growth *in vitro*.

In the Papanicolaou smear test, sensitivity for *T. vaginalis* is approximately 61% with specificity of around 97%. Lara-Torre and Pinkerton (2003) reported a higher rate of positive samples of *Trichomonas* in patients in whom liquid-based Papanicolaou smear detected inflammation.

The aim of the present study was to compare the 3 diagnostic techniques, wet mount microscopy, culture

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and cytology, in groups of HIV-positive and negative women and to describe the principal inflammatory alterations in the Papanicolaou smear test related to the presence of this parasite.

MATERIALS AND METHODS

Setting and patients: The study population consisted of 237 women, 125 of whom were HIV-positive, while the remaining 112 were HIV-negative. Patients were selected consecutively from those attending 3 different healthcare institutes: a tropical disease hospital and 2 maternity hospitals, all situated in Goiania, Goiás, Brazil.

Study sample: The study population consisted of a group of HIV-positive and a group of HIV-negative sexually active females of reproductive age. The HIV-positive patients were required to provide laboratory confirmation of their condition. There was a small subset of pregnant women in each group, at an appropriate phase of their pregnancy to permit the collection of samples for the 3 diagnostic methods. All patients gave their signed informed consent after having received information on the procedures and risks involved in the study. The protocol was approved by the institutional review board of the participating hospitals and the study was conducted in accordance with the Helsinki Declaration (1964), as revised in 1983.

The data on these patients were collected by the study physician and recorded on a form specifically designed for this aim. The sampling procedures and analysis of samples were performed in the 3 institutes participating in the study and were analyzed by the investigators. Any cases failing to comply with the study criteria were excluded.

Clinical and epidemiological aspects: A diagnostic questionnaire was designed and an interview was conducted with the patient to obtain her demographic data, at which time a clinical examination was also performed. This form constitutes the case instrument on which the patient's epidemiological characteristics and laboratory results were recorded in order to permit the frequency of *T. vaginalis* and the degree of efficacy of each diagnostic method to be calculated.

Diagnostic methods: Cervicovaginal samples were obtained by the investigators at the clinics and clinical data were recorded on the appropriate form. Vaginal samples were taken using an Ayre's spatula for cervical smears and a cytobrush for fine and homogeneous smears, distributed evenly over the slides and identified in pencil at the extremities and fixed using a mixture of polyethylene glycol and 70% ethanol to prevent inadequate air drying. These slides were then submitted to the Papanicolaou staining process.

For wet mount microscopy, a vaginal swab was placed in a tube containing 2 mL of saline solution. A drop of this homogeneous mixture was immediately placed on a slide and observed under direct microscopy at 10× and 40× magnification.

Diamond's culture medium, which is considered the gold standard, was prepared. The medium was 1st tested in a pilot study performed on a positive culture obtained from the Hospital of Tropical Diseases and the efficacy of Diamond's medium was confirmed. A vaginal swab from each patient was transferred directly into the medium and sent to the Department of Microbiology. Cultures were maintained at 37°C and observed under direct microscopy daily (24, 48 and 72 h).

The inflammatory alterations associated with the presence of *T. vaginalis* were defined by consensus in 30 positive samples between 2 specialist cytologists working in the cytology quality control program at the Federal University of Goiás. Of these 30 samples, analysis was unable to be performed in 3, since in 2 cases the slides had been broken and collection had been inadequate in the remaining case. The criteria defined as follows all result from cell degeneration:

- The presence of a perinuclear halo, which indicates that the cytoplasm or the nucleus or both are enlarged and contain excess fluid
- The presence of hyperkeratosis, occurring when squamous cells accumulate keratin in cytoplasms leading to the rupture of the cell nucleus
- Findings of increased nuclei, which may appear in response to a traumatic irritation and represent hydropic degeneration
- Identification of invisible cytoplasmic borders, which are a result of pallor of the nucleus and are considered to represent the loss of parts of the cytoplasm (Naib, 1996)
- Cervical cytology was conducted in accordance with the Bethesda system

Data analysis: The original study data were stored in a database using the EPI INFO software program, version 3.4 (2000). Due to the nature of the study, analysis was performed using non-parametric tests (Fisher's exact test and the Mantel Haenszel test).

RESULTS

Of the 3 diagnostic methods, the highest number of positive samples was obtained using culture (33; 13.9%);

however, the difference between the results found with this method compared to the other 2 diagnostic techniques used was not statistically significant. Wet mount microscopy resulted in the lowest number of positive samples (11.4%), while cytology resulted in 13.5% of positive samples for *T. vaginalis*, a result similar to that found with culture (Table 1).

The percentage of *T. vaginalis* found using wet mount microscopy in the HIV-positive test group was 15.2% compared to 7.14% in the HIV-negative control group, while cytology detected 17.6% in the test group and 8.9% in the control group and culture identified 18.4% in the test group as positive for *T. vaginalis* compared to 8.9% in the control group, also confirming that culture was the most effective method for diagnosing the presence of *T. vaginalis* (Fig. 1).

Of the 33 culture-positive specimens, 26 (78.8%) were positive for *T. vaginalis* according to all 3 techniques. Of the remaining 7 culture-positive specimens, 1 was also positive when evaluated by wet mount microscopy (1/33; 3%) and 6 were also positive when evaluated by cytology (6/33; 18.2%) (Table 2).

Table 1: Frequency of *T. vaginalis* according to the 3 diagnostic techniques

Diagnostic technique	Diagnosis	Frequency	(%)	Cumulative (%)	95% CI
Wet mount	*T.vaginalis*+	27	11.4	11.4	7.6-16.1
	T.vaginalis-	210	88.6	100.0	83.9-92.4
	Total	237	100.0	100.0	
Culture	*T.vaginalis*+	33	13.9	13.9	9.8-19.0
	T.vaginalis-	204	86.1	100.0	81.0-90.2
	Total	237	100.0	100.0	
Cytology	*T.vaginalis*+	32	13.5	13.5	9.4-18.5
	T.vaginalis-	205	86.5	100.0	81.6-90.6
	Total	237	100.0	100.0	

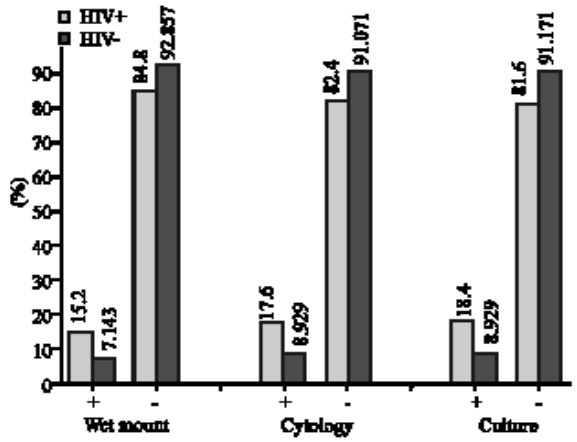

Fig. 1: Presence of *T. vaginalis* according to the 3 diagnostic techniques in HIV-positive and negative women

In the groups of HIV-positive and negative women, the detection rate of *T. vaginalis* was also higher when the 3 diagnostic techniques were considered altogether. Eighteen (78.3%) of the 23 culture-positive samples in the HIV-positive test group were positive for *T. vaginalis* according to all 3 diagnostic techniques and 8 (80.0%) of the 10 culture-positive samples in the HIV-negative control group were *T. vaginalis* positive in all 3 techniques. Of the remaining 5 samples that were culture-positive in the HIV-positive group, 1 was also positive according to wet mount microscopy and 4 were also positive at cytology.

Of the remaining 2 samples that were culture-positive in the HIV-negative group, both were also positive at cytology, whereas *T. vaginalis* failed to be identified at wet mount microscopy in these 2 cases (Table 3).

Figure 2-4 show *T. vaginalis* in Diamond's medium, following Giemsa staining and following Papanicolaou staining, respectively.

Perinuclear halos were the most frequent inflammatory alteration related to the presence of *T. vaginalis*, followed by invisible cytoplasmic borders and increased nuclei. There was no statistically significant difference in

Table 2: Frequency of *Trichomonas vaginalis* according to a combination of diagnostic techniques

Techniques	Frequency	(%)	Cumulative (%)	95% CI
Wet mount, culture and cytology	26	78.8	78.8	61.1-91.0
Wet mount and culture	1	13.9	13.9	0.1-15.8
Culture and cytology	6	18.2	100.0	7.0-35.5

Table 3: HIV-positive and HIV-negative women according to the presence of *T. vaginalis* and a combination of diagnostic techniques.

HIV	Wet mount, culture and cytology	Wet mount and culture	Culture and cytology	Total
Positive	18.0	1.0	04.0	23
Percent	78.3	4.3	17.4	100
Negative	08.0	0.0	02.0	10
Percent	80.0	0.0	20.0	100
Total	26.0	1.0	06.0	33
Percent	78.8	3.0	18.2	100

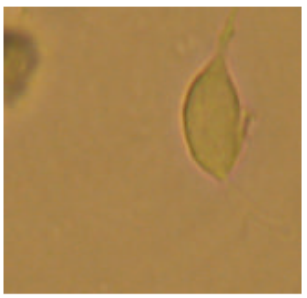

Fig. 2: *T. vaginalis* in Diamond's medium (1000×)

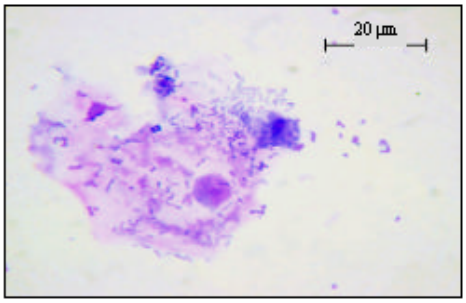

Fig. 3: *T. vaginalis* following Giemsa's stain (1000×)

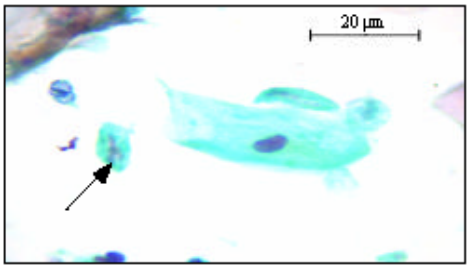

Fig. 4: *T. vaginalis* in cervical cytology (1000×)

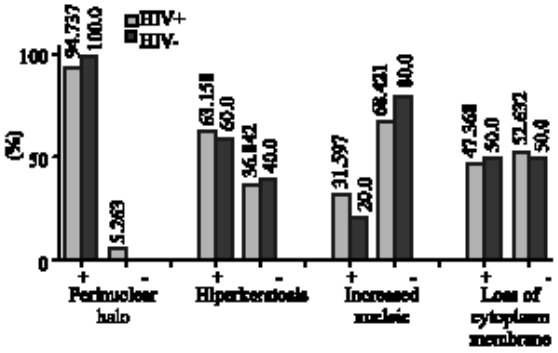

Fig. 5: Inflammatory alterations in women with *Trichomonas vaginalis* according to HIV status

Table 4: Presence of *T. vaginalis* according to cytology findings

Presence of	Inflammation					
T. vaginalis	Moderate	Intense	ASC-US	LSIL	HSIL	Total
Positive	16.0	17.0	0.0	0.0	0.0	33
Percent	48.5	51.5	0.0	0.0	0.0	100
Negative	141.0	42.0	9.0	11.0	1.0	204
Percent	69.1	20.6	4.4	5.4	0.5	100
Total	57.0	59.0	9.0	11.0	1.0	237
Percent	66.2	24.9	3.8	4.6	0.4	100

alterations between the HIV-positive and negative groups (Fig. 5). In this study, the presence of *T. vaginalis* was associated with a high rate of intense inflammatory findings but not with a positive cytology smear for squamous intraepithelial lesions or atypical squamous cells of undetermined significance (Table 4).

DISCUSSION

Sensitivity with Diamond's medium is high, around 95% (Patel *et al*., 2000) and as expected, this method detected the highest number of positive samples in culture. Culture is considered the gold standard for the diagnosis of *T. vaginalis* since specificity and sensitivity are high and interpretation is simple (Maciel *et al*., 2004). However, this method requires some days for the parasite to be identified. Mabey *et al*. (2006) emphasized the importance of maintaining the culture medium for 7-10 days after seeding. In the present study, the culture medium was maintained for 72 h (3 days), after which time the *Trichomonas* population began to decline. Positive cultures for the parasites were observed in the first 12 h following sampling and the largest number was identified within 48 h. These results are similar to those presented by other investigators such as Patel *et al*. (2000).

The lower rates observed by wet mount microscopy are to be expected in view of the poor sensitivity (58-82%) of this method (Wiese *et al*., 2000). In the present study, sensitivity with this method was 81%.

Although, the highest percentage of detection was found with culture in this study, cervical smear testing, for which the detection rate was 13.5%, had a sensitivity rate of 96% and the performance of this diagnostic method was only surpassed by that of culture, which detected a rate of *T. vaginalis* of 13.9%. Lobo *et al*. (2003) carried out a comparative study to evaluate cervical smear testing for the diagnosis of *T. vaginalis* and reported that of the 61 cases confirmed by polymerase chain reaction, 48 were found to be positive by Diamond's medium and 37 by cervical smear cytology, with 24 false-negatives and 23 false-positives. These results emphasize the limitations of this test with respect to the detection of *T. vaginalis*. It is known that fragments of cytoplasm may assume an irregular format similar to that of the parasite; however, the samples were analyzed by the investigator using the three techniques, commencing with wet mount microscopy followed by culture and finally cervical smear cytology, with more attention being paid to the final test with respect to the presence of the parasite. The fact that all samples classified as positive by cytology were also found to be positive by culture eliminated any possibility of false-positive results.

Of the 26 samples classified as positive in the 3 techniques, 18 (69.2%) consisted of samples from HIV-positive women. Guenthner *et al*. (2005) pointed out the capacity of *T. vaginalis* to activate immune cells and increase the response of HIV. Considering that a high parasite load results in a more accurate diagnosis, it may be possible that the parasite multiplies faster in the presence of the virus.

In this study, perinuclear halo was the most common inflammatory alteration found in samples that were positive for *T. vaginalis* and this finding is consistent with data already reported in the literature by Fonseca (1975) and Naib (1996) as well as in other more recent studies (Gonçalves, 1999). Perinuclear halo, hyperkeratosis and invisible cytoplasmic borders were more prevalent in the HIV-negative group compared to the HIV-positive women. The difference in hyperkeratosis between the groups was less evident compared to the other alterations. The disappearance of cytoplasmic borders may be caused by the effect of cytoplasmic enzymes or enzymes of *T. vaginalis* itself that according to Mirhaghani and Warton (1998) possess glycoconjugate components in their external membrane that confer the parasite the ability to attach to and damage the cells.

Gonçalves (1999) reported that pseudoeosinophilia was the most frequent alteration associated with the parasite (64%); however, in the present study this alteration was not taken into consideration since it constitutes mild inflammation and may result from inappropriate manipulation of the smear samples. The majority of smears in the present study were fixed using a mixture of polyethylene glycol and 70% ethanol in the form of a spray although, 95% ethanol is known to be more effective for fixing samples.

Increased nuclei constituted the single most common alteration found in samples from HIV-positive women and have been reported by other investigators as being present in samples of *T. vaginalis*, alone or together with other agents or co-factors. Ayres de Campos *et al.* (1997) reported that 4/34 inflammatory cervical smears were found to be infected with *T. vaginalis*, suggesting a correlation between the infection and inflammation; however, this correlation was not statistically significant. Consolaro *et al.* (2000) reported an association between an exacerbated inflammatory process and the presence of the parasite in 59.3% of samples and demonstrated that this correlation was more prevalent in women of 26-30 years of age. In the present study, *T. vaginalis* was more common in women of 26-35 years of age and was also associated with intense inflammation in 51.1% of samples.

CONCLUSION

Laboratory diagnostic techniques are the most effective way of identifying *T. vaginalis* in vaginal secretion, since clinical signs may mimic other vaginal infections. Moreover, testing for the presence of this flagellate is also relevant because it may act as a carrier of HIV. In conclusion, definitive diagnosis will always lead to better control and adequate treatment of *Trichomonas vaginalis*.

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