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The Prevalence of *Trichomonas vaginalis* in HIV-Positive and Negative Patients in Referral Hospitals in Goiania, Goiás, Brazil

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Abstract: The frequency of *Trichomonas vaginalis* infection in HIV-positive and negative women attending hospitals in Goiania, Brazil was evaluated using the gold standard diagnostic method of culture. A total of 237 vaginal swab specimens were examined: 125 (52.7%) comprising the HIV-positive group and 112 (47.3%) the HIV-negative control group. *T. vaginalis* was detected in 13.5% of the women, 23 (18.4%) of whom were HIV-positive while 9 (8.0%) were HIV-negative. This difference was statistically significant however, infection by this parasite was not found to be associated with immune status. *T. vaginalis* was found in 23.1% of the pregnant women and there was a statistically significant difference in the rate of infection by this parasite between the pregnant HIV-positive and the pregnant HIV-negative women (25.8% versus 12.5%). *T. vaginalis* was more prevalent in HIV-positive compared to HIV-negative women however, no association was found between the infection and the immune status of the patients.

Key words: *Trichomonas vaginalis*, human immunodeficiency virus, infection, women, pregnancy, immune status

INTRODUCTION

Trichomonas vaginalis is a flagellate parasite that infects 170-200 million individuals worldwide (Wiese *et al*., 2000). In Brazil, it affects 10-20% of the female population. Few studies have been published on the prevalence of *T. vaginalis* (Sorvillo *et al*., 2001). Magnus *et al*. (2003) reported a greater prevalence of this infection (13.1%) compared to other Human Immunodeficiency Virus (HIV-related), Sexually Transmitted Infections (STIs) however, the infection was not found to be associated with lower CD_4 counts eliminating the hypothesis of an opportunistic condition.

Approximately 1, 230 individuals currently live with HIV in Brazil. In the city of Goiánia in the state of Goiás with approximately 1,281,793 inhabitants, there have been 4,085 cases registered since 1984.

According to some researchers, *T. vaginalis* increases the risk of acquiring HIV, since it provides pools of leukocytes and macrophages that intensify the shedding of HIV in the genital area.

This infection may also provoke disruption of the epithelial barrier and may cause micro-ulcerations in the genital tract increasing the portal of entry and exit of the virus (Niccolai *et al*., 2000; Sorvillo *et al*., 2001). Another factor that favors transmission is the capacity of the infection to degrade secretory leukocyte protease inhibitor, a product capable of blocking the virus from attacking the cells (Mirhaghani and Warton, 1998). *T. vaginalis* may also activate the immune cells increasing TNF-α cytokine production in the presence of this parasite (Guenthner *et al*., 2005).

Buve *et al*. (2001) confirmed that the risk of *T. vaginalis* is higher in women reporting a greater lifetime number of sexual partners in those with poorer education levels and in women with alcohol dependency while McClelland *et al*. (2007) reported the infection was also more common in women with concomitant cervicitis or bacterial vaginosis. On the other hand, the use of condoms and progesterone-only contraceptive methods (depot-medroxyprogesterone acetate or Norplant) was found to be associated with a lower risk of infection in a multivariate analysis model.

T. vaginalis is one of the most frequent sexually transmitted infections worldwide (Magnus *et al*., 2003). Its presence in the vagina increases predisposition to HIV seroconversion (Guenthner *et al*., 2005).

Since *T. vaginalis* infection is considered an important cofactor in HIV transmission, the objective of this study was to evaluate and compare the frequency of *T. vaginalis* in groups of HIV-positive and HIV-negative women.

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MATERIALS AND METHODS

Setting: This study was conducted in three major hospitals in the city of Goiania, Goiás, Brazil: the Hospital of Tropical Diseases, a tertiary hospital for infectious diseases situated in the mid-west of Brazil which forms part of the National Health Service network and has been a referral center for the care of HIV-infected individuals since 1980; the Maternal and Child Healthcare Hospital, a tertiary healthcare center for pregnant women that also includes a pediatric healthcare center and a municipal maternity hospital which is a tertiary, community-based healthcare center for pregnant women.

Ethics: This research was conducted within the required ethics guidelines of the Declaration of Helsinki and under the terms of the Resolution 196/96 of the Brazilian Ministry of Health. The ethical committee of the hospitals involved had previously approved the study and inclusion of subjects followed the understanding and the consent of each participant.

Subjects: A total of 237 women were enrolled to the study between August 2005 and November 2006, 125 of whom were HIV-positive and 112 HIV-negative. Within this study population, 39 of the patients were pregnant, 31 HIV-positive and 8 HIV-negative women. Demographic and clinical data were collected by the investigators at enrollment using an assessment questionnaire. All patients provided vaginal smears for culture. Samples were obtained in the hospitals where the study took place and were analyzed by the investigators.

Admission criteria: Women who met the following criteria were enrolled to the study: women of reproductive age and sexually active; if pregnant at a gestational age that permitted vaginal smear testing women who had agreed to participate in the study who had been informed of the procedures and risks involved and who had signed an informed consent form in the presence of a witness.

A control group of HIV-negative women was then formed based solely on the aforementioned criteria while in the case of HIV-positive women additional criteria comprised a confirmed diagnosis of HIV infection and the patient's awareness of her primary condition.

Diagnostic tests: Diamond's medium, considered the gold standard for the culture of *T. vaginalis* was prepared and previously tested in a pilot study performed in 12 samples acquired from the Tropical disease hospital. The culture medium was found to be effective. Cultures were maintained at 37°C and observed under direct microscopy daily for 3 consecutive days with observations at 24, 48 and 72 h.

Data analysis: The data collected in this study were stored in a database using the EpiInfo software program, version 3.4 (2000). In view of the nature of the study, the nonparametric chi-square test and Fisher's exact test were used in the analysis. Significance level was established at ($p<0.05$).

RESULTS AND DISCUSSION

T. vaginalis was found in 33 of the 237 vaginal smear samples (13.9%), the highest prevalence being in the group of HIV-positive women (18.4%; n = 23) compared to 8.9% (n = 10) in the HIV-negative control group (Table 1).

When the laboratory findings of CD4 cells, viral load and lymphocytes per mm^3 were correlated with the presence of *T. vaginalis* most of the women were found to have good immune status (Table 2). Of the 237 HIV-positive and HIV-negative women, 39 (20.5%) were pregnant. A statistically significant

Table 1: Presence of *T. vaginalis* in HIV-positive and HIV-negative women attending referral hospitals in Goiânia, GO and Brazil

	Trichomonas vaginalis		
HIV	Presence (%)	Absence (%)	Total (%)
Positive	23 (18.4)	102 (81.6)	25 (100.0)
Negative	9 (8.0)	103 (92.0)	112 (100.0)
Total	32 (13.5)	205 (86.5)	237 (100.0)

Fisher's exact test: p = 0.015

Table 2: Presence of *T. vaginalis* in relation to viral load, CD_4 and lymphocytes in the group of HIV-positive women

	T. vaginalis		
Groups	Presence (%)	Absence (%)	Total (%)
Viral load			
Missing	2 (25.0)	6 (75.0)	8 (100)
<Lower limit	12 (21.8)	43 (78.2)	55 (100)
<1,000 copies mm^{-3}	3 (15.0)	17 (85.0)	20 (100)
>1,000 copies mm^{-3}	6 (14.3)	36 (85.7)	42 (100)
Total	23 (18.4)	102 (81.6)	25 (100)
χ^2-test: 1.2876			
CD_4			
Missing	2 (22.2)	7 (77.8)	9 (100)
<200 cells mm^{-3}	3 (15.8)	16 (84.2)	19 (100)
>200 cells mm^{-3}	18 (18.6)	79 (81.4)	97 (100)
Total	23 (18.4)	102 (81.6)	125 (100)
χ^2-test: 0.1754			
Lymphocytes			
Missing	2 (15.4)	11 (84.6)	13 (100)
<25%	5 (14.3)	30 (85.7)	35 (100)
>25%	16 (21.1)	61 (79.2)	77 (100)
Total	23 (18.4)	102 (81.6)	125 (100)
χ^2-test: 1.0550			

Table 3: Pregnancy and the presence of *T. vaginalis* in HIV-positive and HIV-negative women

	T. vaginalis		
Groups	Presence (%)	Absence (%)	Total (%)
HIV-positive			
Pregnant	8 (25.8)*	23 (74.2)	31 (100)
Non-pregnant	15 (16.0)	79 (84.0)	94 (100)
Total	23 (18.4)	102 (81.6)	125 (100)
HIV-negative			
Pregnant	1 (12.5)*	7 (87.5)	8 (100)
Non-pregnant	8 (7.7)	96 (92.3)	104 (100)
Total	9 (8.0)	103 (92.0)	112 (100)

Chi-square for differing odds ratios by stratum (interaction): 0.0029; Chi-square for differing risk ratios by stratum: 0.0000

Table 4: Early sexual initiation and presence of *T. vaginalis* in HIV-positive and HIV-negative women

	T. vaginalis		
Age groups	Presence (%)	Absence (%)	Total (%)
HIV-positive/age			
Missing	1 (4.3)	2 (1.9)	3 (2.4)
<15	7 (30.4)	28 (27.2)	35 (27.8)
15-18	13 (56.5)	53 (51.5)	66 (52.4)
19-21	2 (8.7)	13 (12.6)	15 (11.9)
>21	0 (0.0)	6 (5.9)	7 (5.6)
Total	23 (100.0)	102 (100.0)	125 (100.0)
χ^2: 2.4273			
HIV-negative/age			
<15	1 (11.1)	27 (26.2)	28 (25.0)
15-18	5 (55.6)	50 (48.5)	55 (49.1)
19-21	1 (11.1)	16 (15.5)	17 (15.2)
>21	1 (11.1)	10 (9.7)	11 (9.8)
Total	9 (100.0)	103 (100.0)	112 (100.0)
χ^2: 12.4057			

difference was found in the rate of *T. vaginalis* infection between the group of 31 pregnant HIV-positive women and the group of 8 pregnant HIV-negative women (25.8% versus 12.5%; p = 0.0029) (Table 3).

A correlation was found between the presence of *T. vaginalis* and a history of early initiation of sexual activities defined as the initiation of sexual life prior to 18 years of age, 86.9% of the women in the HIV-positive group and 66.7% of the women in the HIV-negative group having had early sexual initiation (Table 4).

Regarding the presence of *T. vaginalis* and condom use, a higher frequency of the parasite (80%) was found in patients of the HIV-negative group who did not report condom use compared to the HIV-positive group (26%) (Table 5).

Analysis of the laboratory exams (viral load and CD_4 lymphocyte count) showed no correlation between *T. vaginalis* and immunodeficiency in HIV-positive women, a result that is in agreement with the findings prepared in the study conducted by Magnus *et al.* (2003). However, it must be taken into consideration that most of the infected women are being followed up in one of the two specialized hospitals; therefore, immunosuppression is not an issue. The high rate of *T. vaginalis* found

Table 5: Use of condoms and the presence of *T. vaginalis* in HIV-positive and HIV-negative women

	T. vaginalis		
Use of condoms in womens	Presence (%)	Absence (%)	Total (%)
HIV-positive			
Missing	2 (8.7)	12(1.9)	4 (3.2)
Yes	14 (60.9)	55 (53.4)	69 (54.8)
No	6 (26.1)	31 (30.1)	37 (29.4)
Sometimes	1 (4.3)	14 (13.7)	15 (12.0)
Total	23 (100.0)	102 (100.0)	125 (100.0)
χ^2: 4.5412			
HIV-negative			
Missing	0 (0.0)	1 (100.0)	1 (100.0)
Yes	0 (0.0)	13 (12.6)	13 (11.6)
No	7 (77.8)	80 (77.7)	87 (77.7)
Sometimes	2 (22.2)	9 (8.7)	11 (9.8)
Total	9 (8.0)	103 (92.0)	112 (100.0)
χ^2: 2.7556			

in HIV-positive women in the present study is in agreement with findings shown other studies (Panaretto *et al.*, 2006) and reveals the existence of a relationship between the virus and the parasite, since the latter may cause erosion and bleeding in the cervix (Maciel *et al.*, 2004) facilitating entry by the virus in view of its capacity to bind the leukocytes capable of phagocyting infected virus particles and lymphocytes (Rendon-Maldonado *et al.*, 2003) or according to Guenthner *et al.* (2005) activating the immune cells and increasing the response of the virus by increasing the production of the TNF-α cytokine. However, Chang *et al.* (2004) observed that after 8 h of incubation activation of NF-kB (nuclear factor kappa B) which produces TNF-∝, declines. NF-KB stimulates and provokes the transcription of TNF-α which is involved in the regulation of cell growth, inflammatory response and apoptosis (anti-apoptotic) (Iwalewa *et al.*, 2007).

T. vaginalis was detected in the vaginal smears of 9 of the 39 pregnant women (23%) in the present study. Some investigators (Wilson *et al.*, 1996; Simhan *et al.*, 2007) have also reported a high rate in this type of population. The difference between the rate found in the group of HIV-positive women compared to that found in the HIV-negative group was statistically significant. Considering that pregnancy is a period in which immunity is low, pregnant women run a greater risk of acquiring sexually transmitted infections and the diagnosis and treatment of *T. vaginalis* is indispensable since the parasite acts as a carrier of the virus into the organism (Rendon-Maldonado *et al.*, 2003). Moreover, vertical transmission may lead to severe respiratory problems in the newborn infant (Redman and Johnson, 2003; Temesvári and Kerekes, 2004). It is important to point out that, according to Simhan *et al.* (2007), premature rupture of membranes is a consequence of the activation of neutrophils by *T. vaginalis* provoking an increase in defensins, principally IL-8 in amniotic fluid. Most of the women in this study were married or had a steady partner; therefore, promiscuity could not be directly related to the

presence of the parasite; however early sexual initiation (defined as referring to women under 18 years of age at the time of initiation of sexual activities) was associated with the presence of the infection.

In agreement with the findings of McClelland *et al.* (2007), in the present study poorer education levels were associated with the presence of *T. vaginalis*. The association between *T. vaginalis* and recreational drug use was not investigated in this study.

CONCLUSION

In this study, *T. vaginalis* was more prevalent in HIV-positive women compared to the control group of HIV-negative women; however no association was found between the infection and the immune status of the patients.

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