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Full Length Research Paper

Association between *Trichomonas vaginalis* infection in pregnant, non-pregnant and HIV-positive women

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Trichomonas vaginalis **is a parasitic protozoan responsible for the sexually transmitted disease trichomonosis. The objective of the present study was to evaluate and compare the frequency of the parasite in groups of pregnant, non-pregnant, and HIV-positive womenaccording with five different techniques and establishing a putative correlation between trichomonosis and immunodeficiency status.The study population was pregnant women receiving care at *Dona Iris Hospital and Maternity Unit* (100), non-pregnant attending health care at the *Federal University of Goiás' Teaching Hospital* (106) and HIV-positive women attending an outpatient clinic at the Tropical Diseases Hospital in Goiás (103) in Goiânia, Goiás, Brazil. The polymerase chain reaction (PCR) wet mount microscopy, culture, Papanicolaou (Pap) smears, and a stained preparation from cultured *T. vaginalis* were the five techniques used. In this study, HIV-positive women presented a higher chance of acquiring trichomonosis (*OR= 2,26*). There was a higher risk of association between the infection and pre-cancerous lesions diagnosed by Papanicolaou's method. PCR was considered the gold standard. The comparative study showed the frequency of *T. vaginalis* in three groups of women in Goiânia, Goiás, Brazil.**

Keywords: *Trichomonas vaginalis*, pregnant women, HIV, diagnostic techniques, uterine neoplasms, immunossupression.

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INTRODUCTION

Trichomonas vaginalis is a parasitic protozoan responsible for the sexually transmitted disease trichomonosis. This parasitic infecton is reachinghigher incidence in groups of pregnant women and women with the human immunodeficiency vírus (HIV). HIV was detected in 201 women infected by *T. vaginalis*diagnosed by wet mount microscopy (Salawu and Esume, 2016). Additionally the parasite was detected in 6.2% of pregnant women attending care at the Obstetrics Clinic at the University Hospital of Buenos Aires. The gene 18S

rRNA was amplifyed by the polymerase chain reaction (PCR) (Testardini et al., 2016).

Detecting *T. vaginalis* infecton in HIV-positive women using different diagnostic techniques is highly relevant from a clinical point of view, bearing in mind that no single currently avaiable technique combines complete efficacy with simplicity. PCR is considered the gold standard technique according to papers recently published in the literature. PCR resulted in high specificity with primers TVK3/TVK7 amplifying the conserved region of the gene, with a mutation risk that was considered minimal or zero (Crucciti et al., 2003; Khalaf and Kadhim, 2010).

Wet mount microscopy is a highly specific technique however with sensitivity rates around 40% compared to the gold standard (PCR) (Leli et al., 2016; Testardini et al., 2016). Culture was considered gold-standard in the past however has been replaced by the nucleic acid amplification techniques (Menezes et al., 2016). In a study conducted with pregnant women culture in modified thioglycollate médium succesfully detected 75% of *T. vaginalis* infections and 100% of specificity compared with PCR (Testardini et al., 2016). Papanicolaou (Pap) smear is the diagnostic method mostly used in detecting the parasite. In the present study this stainned method was performed under microscopic examination with a magnifyance of a hundred and 400 times. *T. vaginalis*' DNA had been amplified from 130 archived Papstained cervical and vaginal smears which contained the parasite. A total of 98 Pap smears were found positive by nested PCR giving a sensitivity and specificity of 75.4% and demonstrating that the results obtained by the microscopic examination were confirmed by the PCR method (Lemos e Amaral, 2016; Ziael Hezarjaribi et al., 2015).

The objective of the present study was to establish the frequency of *T. vaginalis'* infection in three population groups: pregnant, non-pregnant and HIV-positive women by comparing five different techniques verifying the risk of co-infection and comparing the accuracy of the techniques to detect *T. vaginalis* taking PCR as the gold-standard.

METHODS

Population

This cross-sectional, prospective study involved 309 women, 100 of whom were pregnant, 106 non-pregnant and 103 women were HIV-positive. Low-risk pregnant patients of any age who were requesting gynecological examination comprised the group of pregnant women. The non-pregnant group consisted of sexually active in an appropriate phase of the cycle to permit samples to be taken for testing. In the case of HIV-positive women a confirmed diagnosis of HIV infection (ELISA and Western-blot) and the patient's awareness of their primary conditions were required.

Site

The patients receiving care at three principal referral public healthcare centers in the state of Goiás, Brazil: the *Dona Iris Hospital and Maternity Unit*, a reference in women's healthcare and linked to the Municipal Health Fund, the *Federal University of Goiás' Teaching Hospital* and the *Tropical Diseases Hospital*, a referral center affiliated to the Goiás State Health Department situated in the city of Goiânia.

Sampling procedures

Secretion samples were collected by the medical teams at the respective hospitals in the period of a year (from August 2015 to August 2016). During a gynecological examination, cervicovaginal secretion were collected using a sterile swab against ectocervix and endocervix.

Wet mount

For this exam, the swabs were immediately soaked in sterile saline solution (0,85%). Microscopic examination was performed within half an hour of sampling.

Culture

Modified thioglycollate médium was used for the culture of *T. vaginalis*. Sampling was performed as for wet mount microscopy, with the vaginal swabs immediately being transferred into the liquid médium, The samples were incubated at 37ºC until the parasite peaked in growth (48 hours) and then examined on a glass slide under a coverslip.

Stained preparation from cultured T. vaginalis

After a 72-hour period, the cultures in liquid médium were centrifuged at 3,500 rpm and their pellets were smeared onto glass slides. The smears were placed at 37ºC for air-drying fixation. Next, the preparations were stained using the Quick Panoptic method.

Pap smears

Fine and homogeneous smears distributed evenly over the slides and identified in pencil at the extremities and fixed using 70% etanol to prevent inadequate air drying. This slides were then submitted to the Papanicolaou staining process.

Table 1. Distribution of *T. vaginalis*' frequency taking PCR as the gold standard in three groups of women, in Goiânia, 2016.

T. vaginalis	Non-pregnant	95% CI		Pregnant	95% IC		HIV positive	95% IC	
Present	19 (17,9%)	11,1	26,2	19 (19,0%)	11,8	71,9	34 (32,2%)	23,6	42,2
Absence	87 (82,1%)	73,4	88,8	81 (81,0%)	71,9	88,6	69 (67,8%)	57,8	76.4
Total	106 (100%)			100 (100%)			103 (100%)		
				[1]OR= 1,07	*0,53*	*2,17*	*[2]OR= 2,26*	*1,18*	*4,30*

[1, 2]ODDS RATIO:[1]Pregnant *versus* Non-pregant; [2]HIV positive *versus* Non-pregnant women.

Table 2. Distribution of *T. vaginalis'* frequency among five techniques in pregnant, non-pregnant, and HIV-positive women in Goiânia, 2016.

Diagnostic techniques	Non-pregnant	CI %	CI%	Pregnant	CI %	CI %	HIV-positive	CI %	CI %	Total
PCR	19 (17,9%)	11,15	26,57	19 (19,0%)	11,84	28,07	34 (33,0%)	24,06	42,97	72 (100,0%)
Wet mount	3 (2,8%)	0,59	8,05	9 (9,0%)	4,20	16,40	21 (20,3%)	24,94	43,97	33 (100,0%)
Culture	32 (30,2%)	21,65	39,87	37 (37,0%)	27,56	47,24	35 (33,9%)	13,09	29,46	104 (100,0%)
Pap smear	58 (54,7%)	44,75	64,41	38 (38,0%)	28,48	48,25	69 (66,9%)	57,03	75,94	165 (100,0%)
Cultured *T.vaginalis* stained	95 (89,6%)	89,19	94,70	80 (80,0%)	70,82	87,33	87 (84,4%)	76,00	90,85	262 (100,0%)
Total	106 (34,3%)			100 (3,2%)			103 (33,3%)			309 (100,0%)

CI%: 95% Confidence Interval

DNA extraction

For this exam the swabs were immediately soaked into distilled water. *T. vaginalis* was extracted from the vaginal samples using the QIAamp DNA mini kit (QIAGEN®).

PCR

The primers selected were TVK3 and TVK7, as recomended by Khalaf and Khadim (2010). The PCR reaction mix yielded a final volume of 22 µL which consisted of 1 µL of each primer, 10 µL of DNA, 0,5 of Taq polymerase (5 IU/Invitrogen), 0,5 µL of buffer mix (10 mM) and 6.5 µL of water. The target DNA was amplified in accordance with the folowing protocol: 2 minutes at 96ºC, 33 cycles of 1 minute at 94ºC, 1 minute at 60ºC, 1 minute and 30 seconds at 70ºC and another 30 minutes for the final extension of the target DNA. The amplicons were submitted to eletrophoresis using a Mega BACE 1000 DNA-sequencer (GE Healthcare, USA). Genotyping analysis were performed using Fragment Profiler® software.

Ethical issues

The study protocol was approved by the institution's internal review board which is credited by the National Comissiono of Ethics in Reseach (CONEP). The study was conducted in accordance with the 2008 revised Helsinki Declaration and all the participants signed a consent form.

Statistical analysis

The chi-square test and Fisher's exact test were used in the statistical analysis which was conducted using the EpiInfo software program version 7. Significance level was defined as $p < 0,05$.

RESULTS

The present study showed HIV-positive women strongly associated with *T. vaginalis'* infection (OR= 2,26). Comparisom between pregnant and non-pregnant women showed OR close to be associated (OR=1,07).

PCR and wet mount techniques presented the highest percentages in the HIV-positive group (33,0% and 20,3%). Culture showed around 30% in the three groups and culture stained by Panoptic detected the highest frequencies of all, around 80%.

Table 3. Distribution of *T. vaginalis'* infection in non-pregnant, pregnant and HIV-positive women in Goiânia, 2016.

Diagnostic techniques	***PCR gold standard***			
	Non-pregnant women			
	% Sensitivity	% Specificity	PPV	NPV
Wet mount	23	99	80	85
Culture	47	73	25	88
Cultured T. vaginalis' stained with Panoptic	100	12	17	100
Pap smear	53	45	15	83
	Pregnant women			
	% Sensitivity	% Specificity	PPV	NPV
Wet mount	47	96	75	89
Culture	53	66	26	85
Cultured T. vaginalis' stained with Panoptic	89	23	20	90
Pap smear	50	65	24	85
	HIV positive			
	% Sensitivity	% Specificity	PPV	NPV
Wet mount	54	99	67	85
Culture	70	81	81	85
Cultured T. vaginalis' stained with Panoptic	94	19	36	87
Pap smear	75	37	35	74

Table 4. Distribution of *T. vaginalis'* frequency with the presence of cytological abnormalities in three groups of women in Goiânia, 2016.

Cytological abnormalities	**Non-pregnant**		**Pregnant**		**HIV positive**	
	T. vaginalis					
	+	-	+	-	+	-
Negative for Malignity	19 (100,0%)	82 (94,2%)	17 (89,4%)	79 (97,5%)	28 (85,35%)	60 (86,96%)
ASC-US	0 (0,0%)	1 (1,1%)	0 (0,0%)	0 (0,0%)	3 (8,82%)	3 (8,82%)
LSIL	0 (0,00%)	4 (4,60%)	2 (10,5%)	2 (2,4%)	5 (7,25%)	5 (7,25%)
			OR= 4,65 (IC % 0,61-35,34)		*OR= 2,14 (IC % 0,72-6,29)*	

*Frequency taking PCR as gold-standard;ASC-US: Atypical Squamous Cells of Undetermined Significance; LSIL: Low-grade Squamous Intraepithelial Lesions. *OR: ODDS RATIO*. CI%: 95% Confidence Interval.

Comparing diagnostic techniques in the detection of *T. vaginalis* taking PCR as gold-standard, this present study verified that the wet mount technique in non-pregnant women showed lower sensitivity (23%) than in pregnant women (47%) and in HIV-positives (54%). Culture specificity was higher than 80% among the three groups of women.

Stained methods (Papanicolaou and Quick Panoptic) presented the highest frequencies however with high false-positives rates.

The present study evaluated the presence of cytological abnormalities in three groups of women correlating the rates found in pregnant women and the rates found inthe HIV-positive group with the rates found in the non-pregnant group. The purpose was to give further strength to correlate *T. vaginalis'* infection with a status of immunossupression.

The results founda strong association between trichomoniasis and cytological abnormalities in both groups of pregnant and HIV-positive women (*OR=4.65* and *OR=2,14*).

There was no significative association between *T. vaginalis'* frequency andvaginal microbiota predominated with cocci and short bacilli. Comparing pregnant group with non-pregnant women *OR=0,51* and HIV-positive women with non-pregnant group *OR=0,78*.

Table 5: Distribution of *T. vaginalis'* frequency among different vaginal microbiotain three groups of women in Goiânia, 2016*

T. vaginalis	Microbiota						Total
	Lactobacilli	Cocci and short bacilli	Mixed	Polymicro bial	Bacilli	Cocci	
Non-pregnant							
+	7 (36,8%)	9 (47,4%)	3 (15,8%)	0 (0,0%)	0 (0,0%)	0 (0,0%)	19 (100,0%)
-	30 (34,5%)	28 (32,2%)	22 (25,3%)	1 (1,1%)	5 (5,7%)	1 (1,1%)	87 (100,0%)
Total	37 (34,9%)	37 (34,9%)	25 (23,6%)	1 (0,9%)	5 (4,7%)	1 (0,9%)	106 (100,0%)
Pregnant							
+	7 (36,8%)	6 (36,6%)	3 (15,8%)	1 (5,3%)	2 (10,5%)	0 (0,0%)	19 (100,0%)
-	47 (58,0%)	13 (16,0%)	15 (18,5%)	5 (6,2%)	1 (1,2%)	0 (0,0%)	81 (100,0%)
Total	54 (54,0%)	19 (19,0%)	18 (18,0%)	6 (6,0%)	3 (3,0%)	0 (0,0%)	100 (100,0%)
HIV-positive							
+	10 (29,4%)	14 (41,2%)	6 (17,6%)	1 (2,94%)	0 (0,0%)	3 (8,8%)	34 (100,0%)
-	22 (31,9%)	20 (28,9%)	16 (23,1%)	5 (7,2%)	3 (4,3%)	3 (4,3%)	69 (100,0%)
Total	32 (31,0%)	34 (33,0%)	22 (21,3%)	6 (5,8%)	3 (2,9%)	7 (6,8%)	103 (100,0%)

*Frequency taking PCR as gold-standard (p > 0,05)

DISCUSSION

The higher frequencies of *T. vagnalis*' infection in pregnant and in HIV-positive women (33,0% and 19,0%) observed in this study demonstrated the better susceptibility of the parasite to cytological abnormalities, indicating also a risk fator for HIV infection (*OR=2,26*). Epidemiological studies similarly indicated a two to three-fold increase in HIV infection risk with concomitant *T. vaginalis* infection (Serwin and Koper, 2013).

The frequency of 19% found in pregnant women, taking PCR as gold-standard, was similar to the rates found by Testardini et al (2016) whose PCR detected exactly 19.2% in a similar group.

HIV has a tropism for the defense cells in the human host while *T. vaginalis* in the cervix and vagina leads to an accumulation of cells from the immune system. The phagocytic ability of *T. vaginalis*may lead to a dissemination of viral particles in HIV-positive women co-infected with trichomoniasis (Rédom-Maldonado, 1998; Benchimol and Pereira-Neves, 2007).

T. vaginalis causes the activation of neuthrophils and the release of a series of inflammatory cytokines (interleukin-8) producing toxicity (nitric-oxide) in the vaginal environment, altering the microbiota consequently leading to an oxidative stress in the mucosal tissue (Frasson et al., 2012; Nam et al., 2012).

PCR technique was considered gold-standard since the number of samples testing positive for *T. vaginalis* was higher principally in the HIV-positive group.Culture analysis conducted post-incubation at 37ºC, 48 hours later enabled *T. vaginalis*' visualization of trophozoite and pseudocyst's forms. Representing a normal portion of the life cycle rather than being degenerative forms, pseudocystsundergo mitosis by a different process from that of trophozoites (Pereira-Neves et al., 2003; Csw et al., 2014).

Detection of *T. vaginalis* by Pap smear diagnostic test was lower compared with the other methods. The vaginal microbiota in pregnancy frequently associated with an intense vaginal dischargemay in part justify this finding. Stainning parasitological techniques (a cultured parasite and/or a positive wet mount stained with Giemsa or Quick Panoptic) were proved to be more sensitive than cytological methods in the diagnosis of *T. vaginalis* (Karaman et al., 2008).

Positivity at wet mount microscopy is associated with movement of the flagela of the parasitecorresponding to the most active stage. *T. vaginalis*' active stage may reflect its greater capacity for phagocytosis. Type 1 is the most commonly parasite type found at wet mount microscopy and corresponds to the wild type G3strain. Culture in the modified fluid thioglycollate médium detected only 47% of *T. vaginalis* infections in pregnant and non-pregnant women groups. Nevertheless in the HIV-positive group sensitivity was very close to that reported by Testardini et al (2016) using an identical culture médium.

The staining parasitological method used here (quick Panoptic) is the technique with the highest frequencies of detection; however, their false-positive rates were high. That may, however, suggest past untreated infections that generates na adaptive response of *T. vaginalis* in the host. The association between wet mount and permanent stainning methods can improve the sensitivity in the diagnosis of trichomoniasis (Menezes et al., 2016).

Immunodeficient women are at higher risk of developing precancerous than immunocompetent

women. The prevalence of precancerous cervical cancer lesions applied by visual inspection with acetic acid among HIV infected was found to be 22.1% (Gedefaw et al., 2013). A higher frequency of cytological abnormalities diagnosed with Papanicolaou's method was found in HIV-positive women compared with a control group (12.1% *versus* 5.4%) (Lemos et al., 2012).

Cytological abnormalities were strongly associated with both groups of pregnant and HIV-positive women (*OR= 4,65* and *OR=2,14* respectively). Indeterminate lesions and precancerous lesions were found in the presence of *T. vaginalis* with a frequency of 4.2%. Adherence of parasite in the host cell was considered the principal inflammatory change indicative of cytopathogenic development (Lemos and Amaral, 2016).

A pathogenic microbiota may occur during pregnancy when physiological changes disrupt this balanced ecosystem (Redelinghuys et al., 2015). Bacterial vaginosis is a microbiota consisting of cocci and short bacilli in a pattern referred as anaerobic. In this study, cocci and short bacilli did not predominate when *T. vaginalis* was present taking PCR gold-standard. However bacterial vagnosis has predominated in the parasite's presence taking only Pap smear diagnostic technique (Lemos and Amaral, 2016).

Commensal microbiota (lactobacilli) at the vaginal mucosa provides protection from parasitic protozoan infections (Bär et al., 2015). Increased vaginal discharge is typical of pregnancy when the higher estrogen levels stimulate the cervix to secrete more discharge predisposing pregnant women to bacterial vaginosis (Redelinghuys et al., 2015). In the present study the parasite was substantially present together with lactobacilli in the microbiota of pregnant women (36.8%).

This study found the prevalence rate of 41% for the co-infection trichomoniasis-bacterial vaginosis in the HIV-positive group. Gatski et al. (2011) found the co-occurence of HIV with bacterial vaginosis in 61% and 17.5% of prevalence between *T. vaginalis* co-infection with anaerobic bacteria.

CONCLUSION

The frequency of *T. vaginalis* in non-pregnant women was 18%, in pregnant women was 19% and in the HIV-positive group was 33%.

The physiological immunossupression condition during pregnancy was not a risk fator for trichomoniasis while HIV-co-infection was.

A strong association was found between trichomoniasis and cytological abnormalities in both groups of pregnant and HIV-positive women (*OR=4.65* and *OR=2,14*).

PCR was considered the gold standard for diagnosis of *T. vaginalis*.

The comparative study showed the frequency of *T. vaginalis* in three groups of women in Goiânia, Goiás, Brazil

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