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Prevalence of Trichomonas Vaginalis in Women with Precancerous and Indeterminate Lesions at Cytology

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***Abstract*— *Trichomonas vaginalis* was investigated by cervicovaginal cytology in pregnant and non-pregnant women receiving care at two public referral hospitals in Goiânia, Goiás, Brazil. The frequency of *Trichomonas vaginalis* infection, the correlation between its presence and a diagnosis of precancerous and indeterminate lesions, and potential inflammatory changes in the vaginal epithelial cells in the presence of the parasite were evaluated.Overall, 359 Papanicolaou smears from 157 pregnant women and 202 non-pregnant women were evaluated. The principal inflammatory changes associated with the presence of *T. vaginalis* were registered.A second examiner reevaluated the slides. *T. vaginalis* infection was found in 46% of the slides, withprecancerous lesionsbeing found in 4.2% of the infected women. Perinuclear halos were the most common inflammatory change associated with the presence of the parasite. Adherence of the parasite to the vaginal epithelial cellsand ill-defined cytoplasmic borders were more commonly found in the group of pregnant patients. The prevalence of *T. vaginalis* was high in the present study, both in the pregnant and non-pregnant women. The socioeconomic level of the study population and the examiners' experience played a particularly relevant role in the results obtained.**

***Index Terms*—*Trichomonas vaginalis*; Papanicolaou smear; uterine neoplasms.**

I. INTRODUCTION

Trichomonas vaginalis (T. vaginalis)is an extracellular protozoan parasite that infects the vagina,affecting around 170 million women worldwide. The presence of the parasite triggers inflammatory processes in the vaginal epithelial cells that range from moderate to severe (according to the Papanicolaouclassification). However, there is speculation that these inflammatory processesmay consequently lead to a diagnosis of precancerous lesions (classified according to the Bethesda system for reporting cervical cytology) [1].

Cytology is the method of choice for diagnosing*T. vaginalis*because of its low cost and ease of use as well as its wide range of advantages in cervical cancer screening. *T vaginalis* has been associated with the neoplastic process, particularly following the discovery of its characteristic adherence to the vaginal epithelial cells, which is responsible for the interaction between these cells and the parasite, with an exchange of chemical signals between them [1, 2].

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The present study evaluated the detection frequency of T. vaginalisby cytology. Cytological abnormalities and inflammatory changes were evaluated as a function of the presence of the parasite in pregnant and non-pregnant women receiving care at referral hospitals in Goiânia, Goiás, Brazil.

II. METHODS

A. Study Population and Site

The study population consisted of 359 healthy women (157 pregnant women and 202 non-pregnant women) receiving care at two public hospitals in Goiânia: the Dona Iris Maternity Hospital, a reference center for women's healthcare associated with the Goiânia Municipal Health Fund, Goiânia, Goiás, Brazil, and the Teaching Hospital of the Federal University of Goiás, a reference health center for the state of Goiás.

B. Ethical Aspects

The internal review board of the Federal University of Goiás Teaching Hospital approved the study protocol. The study was conducted in accordance with the 2008 amendment of the Declaration of Helsinki [3]. The women signed an informed consent form.

C. Data Collection and Processing

During gynecological consultations, the medical teams at the participating hospitals collected the cervicovaginal smearsusing cervical brushes and spatulas.The smears were immediately fixed in 70% alcohol. The conventional method of Papanicolaou (Pap) smear preparation was used and the slides were analyzed using an Olympus optical microscope, model CBA (Figures 1, 2 and 3).

D. Papanicolaou Smears

The slides were examined by the principal investigator of the study, who has a postgraduate degree in clinical cytology from the School of Pharmacy, Federal University of Goiás. Quality control consisted of rescreening 10% of all slides examined. A technician trained at the *José Alencar Gomes da Silva National Cancer Institute* conducted a test-retest of all smears that tested positive for *T. vaginalis* in order to allow a consensus to be reached regarding results.

The cytopathology reports were prepared in accordance with the 2001 Bethesda system for reporting cervical cytology [4].

E. Scanning Electron Microscopy

For illustrative and comparative purposes, scanning electron microscopy was performed using a samplefrom a pregnant patient cultured in Diamond'smodified medium. The smear was fixed under a cover slip measuring 24 x 24 cm, coated with 0.1% gelatin, fixed in 4% paraformaldehyde and stored overnight in a humidified chamber. Dehydration was performed in increasing solutions of ethanol.The slide was dried in hexamethyldisilazane and deposition of gold films was performed under vacuum usinga Denton vacuum system. The specimen was examined using a Jeolscanning electron microscope (JSM-610), equipped with energy-dispersive spectrometry and Thermo Scientific NSS spectral imaging, operated at 4 kV, and installed in the microscopy laboratory of the Physics Institute, Federal University of Goiás (Figure 4).

F. 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 $p<0.05$.

III. RESULTS

Overall, 46% of the women investigated in the present study were infected with *T. vaginalis*. The frequency was higher in the group of non-pregnant women compared to the group of pregnant patients (54% versus 37%) and this difference was statistically significant ($p<0.05$) (Table 1).

Table 1: Frequency of *T. vaginalis* infection in pregnant men receiving care in referral hospitals in Goiânia.

T. vaginalis	**Pregnant Women**	**Non-Pregnant Women**	**Total**
Positive	58 (37%)	109(54%)	167(46%)
Negative	99 (63%)	93 (46%)	192(53%)
Total	157(100%)	202(100%)	359(100%)

the patients with *T. vaginalis* (Table 2).Fisher's exact test 0,001

Table 2: Association of precancerous lesions with the presence of *T. vaginalis* in women receiving care at referral hospitals in Goiânia

	Positive	**Negative**	**Total**
ASC-US	4 (2.4%)	1 (0.5%)	5(1.4%)
LSIL	3 (1.8%)	6 (3.1%)	9 (2.5%)
HSIL	0	1 (0.5%)	1 (0.3%)
Negative for malignancy	160(95.8%)	184(95.8%)	344(95.8%)

ASC-US: Atypical squamous cells of undetermined significance
LSIL: Low-grade squamous intraepithelial lesion
HSIL: High-grade squamous intraepithelial lesion

The main inflammatory change found in the patients infected with *T. vaginalis* consisted of perinuclear halos in both groups, including 100% of the 24 slides analyzed in the group of non-pregnant women. Enlarged nuclei and hyperkeratosis were also more common in the group of non-pregnant women (91% versus 79%); however, the percentages with ill-defined cytoplasmic borders and adherence of the parasite to the vaginal epithelial cells were higher in the group of pregnant women at the Dona Iris Maternity Hospital (Table 3).

Table 3: Principal inflammatory changes found in the presence of *T. vaginalis* in pregnant and non-pregnant women receiving care at referral hospitals in Goiânia.

Inflamatory changes	**Pregnant Women**	**Non-Pregnant Women**	**Total**
Perinuclear halos	24(100%)	43 (98%)	67 (98%)
Enlarged nuclei	19 (79%)	40 (91%)	59 (87%)
Ill-defined cytoplasmic border	23 (96%)	36 (82%)	59 (87%)
Adherence to vaginal epithelial cells	21 (87%)	37 (84%)	58 (85%)

IV. FIGURE

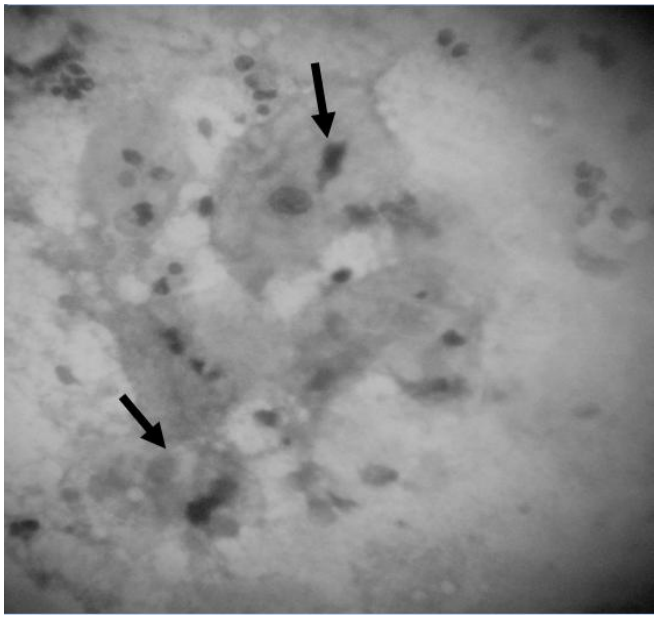

Fig.1 Adherence of *T. vaginalis* to the vaginal epithelial cells and perinuclear halo (Pap smear).

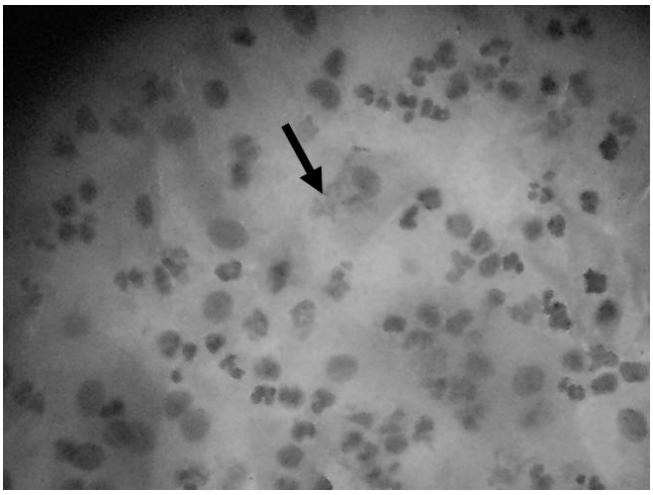

Fig. 2 Adherence of *T. vaginalis* to the vaginal epithelial cells and ill-defined cytoplasmic border.

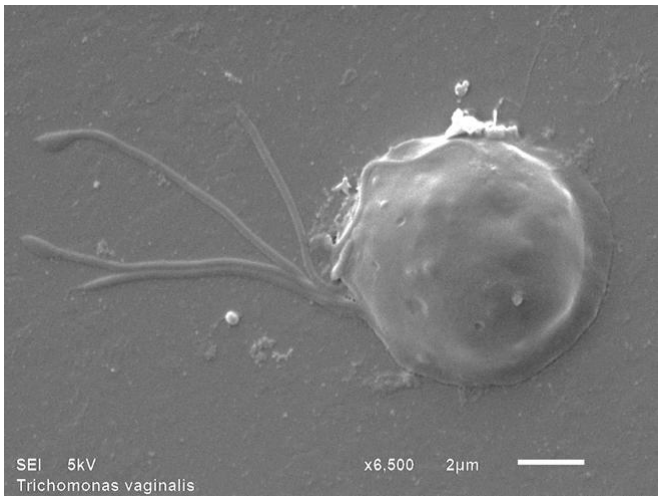

Fig. 3 Trophozoite form of *T. vaginalis* in scanning electron microscopy.

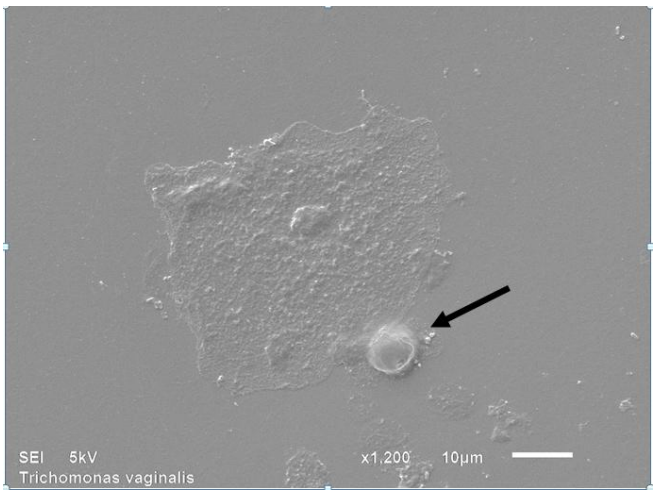

Fig. 4 Amoeboid form of *T. vaginalis*and vaginal epithelial cell in scanning electron microscopy.

V. DISCUSSION

The prevalence of *T. vaginalis* infection found in the present study was high compared to previous reports in the literature. A study conducted in the Manhiça district of Mozambique reported the presence of the infection in 31% of cases examined (78/254); however, in addition to the socioeconomic and cultural characteristics of that population, 47% of whom are illiterate, it is also important to take into consideration that the sensitivity of the diagnostic technique used (i.e. wet mount examination) is considered low [5,6].

predisposes women to infection by parasitic agents; however, the prevalence of *T. vaginalis* in the group of pregnant women in the present study was lower than that found in the non-pregnant group. The characteristics of the population receiving care in the latter group (women living in conditions of poverty) may in part justify thisfinding. Another factor may be the diagnostic technique used, bearing in mind that the specificity of the Pap smear is lower than that obtained with other methods [7]. The frequency of *T. vaginalis* was evaluated in pregnant women in Papua New Guinea, where the infection was found in 21.3% of the 400 samples analyzed by polymerase chain reaction (PCR) [8]. In another study conducted in Zimbabwe, infection was detected by wet mount examinationin 11.8% of 691 pregnant women (≥ 36 weeks of pregnancy) [9].

Pap smear is considered a highly sensitive technique compared to microscopy, culture and even PCR. Nevertheless, the specificity of this technique is arguable, since false-positive results have been reported in the literature as a consequence of possible diagnostic confusion between*T. vaginalis*and cell remnants [10]. In this case, the experience of the examiner becomes a relevant factor in determining the sensitivity and specificity of the exam. Howel et al. reported a better performance by cytotechnologists in the College of American Pathologists Interlaboratory Comparison Program in Gynecologic Cytology (CAPPAP program) for evaluation of the presence of the parasite in cervicovaginal smears [11]. Loo et al. reported sensitivity of 98%, specificity of 96% and a positive predictive value of 88% for detection of the parasite in the Pap smearin relation to the gold-standard culture in Feinberg's medium [12].

The indeterminate lesionsand the precancerous lesions detected in this study were associated with the presence of *T. vaginalis* in 4.2% of the 359 women analyzed. Donders et al. reported the presence of*T. vaginalis* in 1.3% of cases of atypical squamous cells of undetermined significance **(**ASC-US) (Bethesda) compared to 0.03% when no malignant lesions were found [13]. Misra and Singh found *T. vaginalis* in 8.1% of cases of low-grade lesions defined according to the Bethesda system [14].

The principal inflammatory changes associated with the presence of *T. vaginalis*in the present study are similar to those reported by Noël and Engohan-Aloghe[15]. There was a high rate of perinuclear halos, nuclear enlargement and adherence of the parasite to vaginal epithelial cellsin both studies.No case of ill-defined cytoplasmic border was reported in the aforementioned study; however, according to Lemos et al. that change is characteristic of the presence of the parasite, which was found in 50% of the smears analyzed [16].

In the present study, ill-defined cytoplasmic borders were present in 87% of the infected pregnant women and in 84% of the infected non-pregnant women. Greater importance is now being given to this changeas well as to the adherence of *T. vaginalis* to the vaginal epithelial cells, in view of the recent discovery of the pseudocystic or amoeboid form, the form acquired by the parasite when it comes into contact with the vaginal epithelial cells, at which time the signaling process begins [1]. Blurring of the cytoplasmic border and adherence to the vaginal epithelial cells were found principally in microbiota consisting of cocci and short bacilli.

Bär et al. highlighted the importance of commensal microbiota in providing protection from parasitic protozoan infections at the vaginal mucosa. In the present study, cocci and short bacilli predominated when*T. vaginalis*was present. The infectious agent that was most commonly associated with its presence was the Gram-negative bacteria *Gardnerella vaginalis*, a fact already reported in the literature when clue cells (vaginal epithelial cells coated with coccobacilli) are a constant part of the microbiota,in a pattern referred to as anaerobic [17,18].

T. vaginalis and lactobacilli may compete with one anotherin the microbiota and *Lactobacillusgasseri* significantly inhibitsadherence to the vaginal epithelial cells [17,18]. In the present study, the parasite was present together with lactobacilli in the microbiotaof 18% of the pregnant women compared to 8% of the non-pregnant women. This fact may indicate an advantage in favor of the former group, since the presence of lactobacilli could play a role in reducing the cytopathogenic effect of *T. vaginalis*.

VI. CONCLUSION

The detection frequency of *T. vaginalis* by cytology was high compared to previous reports in the literature. There is a considerable variation in sensitivity and specificity found in articles in which the different techniques are compared. The ability to detect *T. vaginalis* in vaginal smears was associated with the diagnostic technique used and with the experience of the examiner [12,19]. Evaluating *T. vaginalis'* presence in cases of precancerous lesions and illustrating the adherence of *T. vaginalis*to vaginal epithelial cells under optical and scanning electron microscopy the present studydemonstrates that this parasitemay potentiate or accelerate the neoplastic process through its contact with the vaginal epithelial cells [13,14]. Adherence of *T. vaginalis* to the host cell may be considered one of the principal inflammatory changes indicative of cytopathogenic development [15].

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