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ARTIGO

Associação *T. vaginalis* e *G. vaginalis*: variações nos efeitos epiteliais

"Cytological changes associated to *Trichomonas vaginalis*: the influence of a second agent"

Maria Alice Guimarães Gonçalves[1], Grimaldo Carvalho [2], Fernando Costa e Silva Filho[3]

RESUMO

Fndamentos: A ocorrência de Doenças Sexualmente Transmissíveis (DST) tem sido aventada por diversos autores como co-fatores na promoção de câncer cervical. Alguns agentes infecciosos como o *T. vaginalis* tem sido frequentemente encontrados em pacientes com diagnóstico de câncer cervical.

Objetivos: O presente estudo foi conduzido com o objetivo de correlacionar dois grupos de pacientes a fim de avaliar diferenças nas células escamosas entre mulheres albergando apenas *T. vaginalis*, e outro grupo *T. vaginalis e G. vaginalis* com sua flora anaeróbia associada. Alterações citoplasmáticas, nucleares, e celulares foram detectadas. O papel da *G. vaginalis* como um fator protetor na progressão das alterações citológicas causadas pelo *T. vaginalis* foram analisadas, assim como os sintomas relacionados com ambas as infecções.

Resultados: Pseudoeosinofilia (64%), halos perinucleares (53%), hiperceratose (18%) e núcleos aumentados (35%) foram os achados mais freqüentes. Enquanto que a hiperceratose foi duas vezes mais frequente no grupo de infecção dupla. Perda de bordas citoplasmáticas e alterações nucleares aumentaram paralelamente ao inóculo do *T. vaginalis*. Aumento do tamanho do núcleo mostrou associação com tricomoníase aguda (51%), enquanto que nos casos crônicos pseudoeosinofilia foi mais freqüente. Prurido foi mais freqüente no grupo do *T. vaginalis* (74%), e odor mostrou-se exacerbado nas pacientes albergando ambos os agentes.

Conclusões: Os autores concluiram que não somente a presença de *G. vaginalis*, mas também a quantidade de *T. vaginalis*, a duração do corrimento, prurido e odor fétido podem estar relacionados com alterações citológicas importantes, as quais algumas vezes podem ser confundidas com anormalidades encontradas no câncer cervical ou virais.

Palavras-chave: *T. vaginalis; G. vaginalis;* protozoários parasitas; tricomoníase; alterações citológicas; avaliação citológica; câncer cervical.

1. *Faculdade de Saúde Pública da Universidade de São Paulo, Brasil.*
- *Faculty of Public Health, State University of São Paulo, São Paulo, Brazil.*
2. *Serviço de Citologia Grimaldo Carvalho, Rua Dias da Cruz, 185/215, Meier, Rio de Janeiro, Brazil*
- *Senior Cytopathologist, Chief of his particular Laboratory of Cytology.*
3. *Insituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil.*
- *Associate Professor, Department of Parasitology and Cell Biophysics, Biophysics Institute, Federal University of Rio de Janeiro, Brazil.*

ABSTRACT

Background: The ocurrence of sexually transmitted diseases have been focused by several authors as co-factors on the promotion of cervical cancer. Among infections agents like *T. vaginalis* has been frequently found in patients exhibiting cervical cancer.

Objectives: The present study was conducted to correlate two patients groups, in order to evaluate squamous cytological differences between women harboring only *T. vaginalis,* and a group harboring *T. vaginalis* and *G. vaginalis* with its associated anaerobic flora. Cytoplasmatic, nuclear, and cellular changes were focused. The role of *G. vaginalis* as a protective factor in the development of cytological alterations caused by *T. vaginalis* was analysed, as well as the symptoms implicated with both infections.

Results: Pseudoeosinophilia (64%), perinuclear halos (53%), hiperceratosis (18%) and increased nuclei (35%) were the most usual findings. Meanwhile, hiperceratosis was two-fold more frequent in the double infection group. Fuzzy border and nuclear alterations increased accompagning *T. vaginalis* inoculum rise. Increased nuclei showed a positive association with acute trichomoniasis (51%), besides the chronic cases that pseudoeosinophilia was more commom. Itching was more significant in the *T. vaginalis* group (74%), and odor was exacerbated in the patients harboring both agents.

Conclusions: The authors concluded that not only the presence of *G. vaginalis*, but also the *T. vaginalis* amount, discharge duration, itching and malodor can be related to several cytological and clinical changes, which sometimes could be confounded with cervical cancer or viral abnormalities.

Keywords: *T. vaginalis; G. vaginalis;* parasitic protozoa; trichomoniasis; cytological Changes; cytological evaluation; cervical cancer.

1. INTRODUCTION

Cervical cancer is one of the most important pathologies afflicting women in Brazil (1,11). Experts have described related risk to develop cervical cancer due to nutritional aspects (31), sexual behaviour (6), among others factors (6, 26, 30). In addition of all, it is important to point out that the periodic cytological evaluation in female populations from Third world countries hasn't already a satisfactory preventive coverage (1).

Cervical cancer is one of the most important pathologies afflicting women in Brazil

The ocurrence of sexually transmitted diseases (STD) have been focused by several authors as co-factors on the promotion of cervical cancer. Among infections agents *Trichomonas vaginalis* has been frequently found in patients exhibiting cervical cancer. This parasitic protozoa is spread worldwide, counting about 180 million people contamined yearly (3, 22, 24, 25).

International literature relating this protozoa to cellular atypias is enormous, but a detailed study is not yet avaiable. Further data has been accumulated on the *Trichomonas vaginalis* habilities to invade host cells (3,4,5). Also is not clear if the parasitic hability to invade could be modified due to the influence from an associated anaerobic bacteria group (32).

Up to now, significant information concerning *Trichomonas vaginalis* infection still remain unclear: 1) women harboring active trichomoniasis on delivery time have two fold chance to display prolonged discharge or frank endometritis on the postpartum period than those not infected (23); 2) *Trichomonas vaginalis* may probably masquerade cytological diagnosis and clinical treatment of other coexisting infections (14, 24, 27); 3) Trichomoniasis can cause reversible sterility (8, 23, 32, 37); 4) 90% of patients with chronic trichomoniasis diagnosis have cervical lesions, which is a predisposing factor to malignant cellular transformation. Sometimes squamous cells abnormalities found in patients infected with *Trichomonas vaginalis* can be usually confounded with pre-neoplastic cells by experts (5, 16, 20); 5) parasits can carry other microorganisms like virus or *Neisseria gonorrhoaea* through genital tract (15, 18, 38) or be frequently found in the same slide to *Gardnerella vaginalis* (14).

The present study was conducted to correlate two patients groups, in order to evaluate squamous cytological differences between women harboring only *Trichomonas vaginalis* and a group harboring *Trichomonas vaginalis* and *Gardnerella vaginalis* with its associated anaerobic flora. Attempts were also made to investigate the role of *G. vaginalis* and associated flora as protective factors from developing cytological abnormalities suggestive of pre-neoplasic stages.

2. MATERIAL AND METHODS

Patients

Thirty women attending outpatient clinic of Institute of Ginecologia (Federal University of Rio de Janeiro, Brazil) were clinically and cytologically evaluated. They were divided into two groups: 1) 15 with *T. vaginalis* diagnosis and 2) 15 women with both *T. vaginalis* and *G. vaginalis* infection.

Cellular squamous alterations have been examined at each 100 cells per field using a 40X magniffying lens

All patients were at reproductive age (mean age: 32 years, range 20-50 years). The follow criteres were excluded from study: pregnant or menopausic women, those who used oral antibiotics or vaginal medications on the previous 14 days; women mentally incapacitated or if they had undergone histerectomy (34).

All the women interviewed answered questionaires about vaginal discharge, duration, amount, odor, colour and the presence of itching. After that all of them were examined by the same gynecologist.

Chronic disease was considered since the complains of vaginal discharge persisted for more than six months, and acute disease those with vaginal diacharge or other symptoms for less than six months (13, 34, 39). When the patient could not answer with accuracy the discharge duration we considered this uncertain.

Morphologic Assessment

All patients cell samples were collected in different menstrual cycle phases. From each patient cervicovaginal smears were obtained from three sites (endocervical, exocervical and posterior vaginal fornix), spread on glass slides in the usual manner for cytological smears, and stained below Papanicolaou technique for morphological assessment.

Cellular squamous alterations have been examined at each 100 cells per field using a 40X magniffying lens. The findings were distribuited into three groups: cytoplasmic, nuclear and cellular (7). For each slide the following abnormalities were examined:

1) Cytoplasmic: fuzzy border, pseudoeosinophilia, metacromasia, perinuclear halos, active vacuolization, hiperceratosis, paraceratosis, frail cytoplasm and Koilocytosis.
2) Nuclear: increased size, multinucleation, membrane rupture, cariorrexis, cariopicnosis and cariolisis.
3) Cellular: aberrant shapes.

Infection was scored in accordance to the amount of trichomonads cells per 100 squamous cells (8):

1) + = < 5 *T. vaginalis*/100 cells
2) ++ = 5 to 10 *T. vaginalis*/100 cells
3) +++ = > 10 *T. vaginalis*/100 cells

Slides were suspected positive for *G. vaginalis* when they showed "clue cells" plus associated Gram-negative and Gram-variable bacilli (14, 36). Papanicolau stained smears were screened by various experts cytopathologists, who did not know each other results previously. They were reviewed by one of the authors. Infection diagnosis were all confirmed by culture.

Culture media

Sterile cotton swabs moistened with vaginal exsudate from each patient were inoculated into screeped sterile tubes containing 9 ml TYM medium (12) plus 10% heat-inativated bovine serum and added appropriated antibiotics. After 24 h incubation at 37°C. Cultures were examined and parasites re-inoculated into fresh culture medium. Axenic cultures for *T. vaginalis* could be obtained after five passages in TYM medium. Addition to antibiotics to the medium was abolished after the fifth passage and parasites remain in acellular cultures or frozen in liquid nitrogen as previously recomended (28,33).

3. RESULTS

Diagnostic criteria

A group of 30 patients with trichomoniasis was selected by wet mounts diagnosis, from cervico-vaginal smears, and also acellular cultures carried out in Diamond's medium.

Cervical and vaginal pool smears were studied and considered positive for *T. vaginalis* presence in 15 women (50%) and 15 women had positive preparations for both *T. vaginalis* and *G. vaginalis.*

Cytological Correlations

Cytological aspects evaluation have demonstrated three cellular abnormalities different types (Table 1). In both groups the most usual cytological changes were fuzzy border and metacromasis. It was observed that fuzzy borders were concomitant to the increase

Table 1 - Correlation between cytoplasmic abnormalities and the presence of microorganismis

Microorganismis and inoculum size (+ to +++)	fuzzy border (%)	metacro masia (%)	active vacuoliza-tion (%)	parece-ratosis (%)	frail cytoplas m (%)	koilo cy-tosis (%)
T. vaginalis (+)	08	14	03	00	00	00
(++)	16	11	11	09	02	00
(+++)	27	07	11	05	02	00
Total*	17	11	08	05	01	00
T. vaginalis(+) IG. vaginalis	10	09	04	00	00	00
T. vaginalis (++) IG. vaginalis	13	08	07	02	01	00
T. vaginalis (+++) IG. vaginalis	15	06	03	10	02	00
Total*	13	08	05	04	01	00

* mean rate

of *T. vaginalis* inoculum, contrariously of the decrease in metacromasia. The mentioned criteria were more significant in the group of patients presenting only trichomoniasis.

Comparing the mainly significant data concerning cytoplasmic and nuclear groups it is clear that the prior was more frequent than the latter (Table 2). Pseudoeosinophilia was the most commonly cytoplasmic abnormality found in the studied smears (64% of squamous cells of *T. vaginalis* hosts), while among patients with double infection 52% of squamous cells were affected. Hyperceratosis was found in 18% of squamous cells obtained from patients with trichomoniasis against 47% in the other group.

In addition, Table 2 also shows that the most ordinary nuclear alteration in both groups was increased nuclei associated with hipercromasis (35% of squamous cells from *T. vaginalis* patients and 33% from mixed infected group).

Scoring the inoculum amount in +, it was noticed that an increase in nuclei did follow proportionally the inoculum augmentation.

Table 2 - Cytological abnormalities associated with *T. vaginalis*.

Microorganismis and inocumum size (+ to +++)	number of patients	pseudoeosinophila (%)	cytoplasmic perinuclear thalos (%)	hyperceratosis (%)	increased nuclei (%)
T. vaginalis (+)	02	45	33	08	15
T. vaginalis (++)	04	80	56	28	38
T. vaginalis (+++)	09	67	70	17	51
Total*	15	64	53	18	35
T. vaginalis (+)/ G. vaginalis	06	25	35	06	23
T. vaginalis (++)/ G. vaginalis	06	50	65	21	35
T. vaginalis (+++)/ G. vaginalis	03	80	50	60	40
Total*	15	52	50	47	33

* mean rate

The presence of other cellular abnormalities was not appreciable. Finally, aberrant shapes were systematically noticed as resumed in Table 3.

Table 3 - Correlation between aberrant shapes and the presence of microorganisms

Microorganismis and inocumum size (+ to +++)	Aberrant shapes (%)
T. vaginalis (+)	02
T. vaginalis (++)	07
T. vaginalis (+++)	08
T. vaginalis (+)/ and vaginalis	01
T. vaginalis (++)/ and vaginalis	07
T. vaginalis (+++)/ and vaginalis	10

Clinical correlations

We did try to correlate some clinical aspects among patients (discharge duration and amount, itching, odor, and patient age) with the most frequent cytological findings (fuzzy border, metacromasis and increased nuclei).

Considering vaginal discharge duration, 35% of patients had acute trichomoniasis while 45% had chronic disease (Table 4/5). This clinical data is the only criteria associated with cytological changes.

In total population studied cytoplasmic alterations presented similar numbers in the cases of acute and chronic trichomoniasis (Table 5). Cells collected from acute trichomoniasis patients had greatest nuclei.

We weren't able to correlate discharge amount with neither trichomonads score nor double infection presence. Itching was more prevalent in patients with trichomoniasis (74%) than in the other group (20%), being the characteristic yellowish discharge present in 80% of the studied patients.

Odor was a complaint in 67% of patients with trichomoniasis, and in 40% of patients harboring *G. vaginalis* and *T. vaginalis*. All the patients with *T. vaginalis* and odor had acute infection, and a third of them related fishy odor. In the double infection group, it was observed that 50% of patients had chronic disease, and exhibited

malòdor. All clinical symptoms were even more widespread in *T. vaginalis* patient group. This became more evident when symptoms were related to cytological findings.

Table 4 - Correlation between discharge duration and associated agent

Discharge duration	T. vaginalis n	(%)	T. vaginalis and G. vaginalis n	(%)	Total	
Acute	06	40	03	20	09	35
Chronic	07	47	06	40	13	45
Uncertain	02	13	06	40	08	20
Total	15	100	15	100	30	100

Table 5 - Correlation between discharge duration and cytological findings

Discharge duration	Microorganism	Pseudoeosinophilia (%)	Increased nuclei and Sypercromasia	Aberrant shapes (%)
Acute	T. Vaginalis	69	51	07
	T. vaginalis and G. vaginalis	70	40	10
Chronic	T. vaginalis	71	36	06
	T. vaginalis and G. vaginalis	25	23	01
Uncertain	T. vaginalis	50	38	09
	T. vaginalis and G. vaginalis	55	35	08

4. DISCUSSION

Relatively few studied have examined or discussed bacterial flora involved in trichomonads infection, and there is up to now a great deal of variability on the reported results (4,15,18,32). However, some reports have focused that *G. vaginalis* is the most frequent microorganism associated with trichomoniasis presence (7,14,23,41).

We wondered if *T. vaginalis* and *G. vaginalis* would determine synergistic effect or if the second agent would compete against the first hampering the maintenance of trichomonads infection. How this interaction reflects in the stained Papanicolaou smears is the why this study was conceived.

ROBINSON and MIRCHANDANI (32) noticed that an almost complete association between symptomatic trichomoniasis and hemolytic streptococci presence. Actually, it is possible that these ultimate organisms play indeed *G. vaginalis* (14). Also HONIGBERG proposed several stages on the trichomoniasis infection development which could explain variations on its accompagning bacterial flora. He divided natural history of trichomoniasis into five phases: acuta, culminating, chronic, latent and the last one (23).

Bacterial vaginosis is recollected to be present on the stages culminating and chronic, both very similar microscopically. Furthermore, there are involutional lactobacilli forms that resemble *G. vaginalis* either on morphological or in biochemical aspects. On latent phase, when association with lactobacilli is predominant, *G. vaginalis* is also found (24). As these agents are so associated on infection, it has been hard to define agent role.

Other investigators have afirmed before that microorganisms may use different kind of mechanisms to invade and cause cellular injury. HEATH and HOGUE observed that *T. vaginalis* can be highly cytophatic in culture cells (20,21). The onset of interaction host-cells begins with clusters, cytoadherence and culminating with cytotoxic effects which are initially characterized by cytoplasm contraction and increase in nuclei diameter (20,21). GARBER and cols. Examined the behaviour of *T. vaginalis* in cell culture and they have shown that the parasitic cytotoxic effect was dependent upon the inoculum size and the duration of the interaction between *T. vaginalis* and cell monolayers (17).

Our data obtained *in vivo* clearly demonstrated cell injuries caused by *T. vaginalis* since most of

examined cells showed fuzzy border, which in turn, is a consequence of perinuclear edema ocurrence (22). Reinforcing this comment, we also observed increases in the described cell injuries directly related to parasitic charge.

Bacterial vaginosis is recollected to be present on the stages culminating and chronic, both very similar microscopically

Cellular changes profiles here studied claimed our attention because of the greater incidence alterations of cellular activity than the degenerative activity. Whereas, the former were more frequently found on the initial trichomonads infection phase, named by FROST as acute or florid phase (16). However, other authors have found cellular activity in cases of chronic infection (5) considering a great number of patients. Thus, it is reasonable to consider that we might have studied a homogenous group in statistical terms.

Discharge duration seems to be an important indicator of cytological alterations, since either cytoplasmic or nuclear changes were more common in acute infections. Conversely, the ocurrence of increased nuclei was not so evident, on chronic or uncertain duration diseases. BERTINI and HORNSTEIN (5) did not observed this aspect. They showed that the cellular alterations frequence was high in the persistence cases of trichomoniasis, but they were unable to determine the real concept of persistent so it is not possible to compare with our findings.

The rate of perinuclear halos also became greater as the inoculum size increased among *T. vaginalis* group. CHAPPAZ and BERTRAND (9) described the ocurrence of perinuclear halos in cells infected by *T. vaginalis*. It has been suggested that perinuclear halos are caused by perinuclear edema. Thus, this cellular alteration must be differentiated from koilocytosis (7, 35).

When we focused aberrant shapes we observed that they has a predominance on groups presenting great trichomonads parasitic charge and also from the patients groups who complaint of uncertain duration discharge. It is important to recollect that the so-called hyperplasia and squamous cell metaplasia with various atypia grades may occur concomitant with chronic trichomoniasis (22).

Finally, our findings led us to suggest that the cytoplasmic aspects here discussed result from both microorganisms effects. Otherwise, nuclear effects were more frequent in patients harboring only *T. vaginalis*.

More recent investigations have suggested that the high invasiviness determined by human parasite *T. vaginalis* (17,19,33) may be due to its proteolytic and glycosidic activities (2, 10,29). We could draw that cytological effects found in studied groups were probably the reflection of cytopathic processes performed by *T. vaginalis*.

HONIGBERG and EWALT (22, 23) according to KULDA (28) also demonstrated that "suppression of cell division and the appearance of abnormal nuclear and cytoplasmic changes evoked by exposure of cell culture to parasite-free filtrates of trichomonad-rich cultures constitute a response to some toxic substances presumably eliminated by the parasites". In addition, they stated that this may play a less important role in degeneration of the vertebrate cell cultures infected with mild strains.

It is not clear if *T. vaginalis* virulence is determined by the co-existing microflora in human urogenital cavities. We observed that the cytophatic results were basically the same among both groups studied. Further, the patterns of cytological changes detected in patients with either trichomoniasis or trichomoniasis plus bacterial vaginosis were very close.

In our groups all patients with trichomoniasis and fishy odor had acute discharge duration. Conversely, in double infection group with malodor all women had chronic or uncertain discharge duration. Clinical symptoms were more intense in patients harboring only *T. vaginalis*, while cytological findings were basically the same in both groups. Some possibilities deserve to be considered. *T. vaginalis* as the great majority of aerotolerant microorganisms has sialic acid as one of the end products of carbohydrate metabolism (23,24). Lactate *per se* may occur as a component of vaginal discharge or as a substratum for the anaerobic and/or aerotolerant bacteria metabolism. The presence of high levels of *T. vaginalis* acid products in vaginal environment has been implicated as one of the generators of fishy amine odor. The oncogenic potential of nitrosamines produced by certain bacteria inhabitants of women vagina may be also considered as important (40). Obviously, the presence of potential feeders of lactic acid as *G. vaginalis* for example, may produce low levels of free acetate in vaginal fluid. Therefore, it is possible that clinical or cytopathic effects could be related to *T. vaginalis* and *G. vaginalis* metabolic relationship, or maybe it is proportional to both microorganisms active charge. For the moment we can infer the superficial cells effects, but we do not know what happens in deep layers. It would be probably a key to the understanding of the initiation or promotion of an incipient pre-neoplastic process in totipotent cells.

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Endereço para correspondência:
R. Bergamota, 470/121A
Alto da Lapa - São Paulo
CEP: 05468-000
Telefax: (011) 839-2463