Serum antibodies to *Trichomonas vaginalis* in invasive cervical cancer patients

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Abstract

Objective—To evaluate, by seroepidemiology, the possible role of the sexually-transmitted flagellate, *Trichomonas vaginalis*, in invasive cervical cancer.

Subjects and method—Sera from 121 invasive cervical cancer patients and 242 random age-matched female controls. Antibodies to *T. vaginalis* were detected by the western blot technique.

Results—Antibodies to *T. vaginalis* were detected in the sera of 41.3% (50/121) of invasive cervical cancer patients compared with only 5.0% (12/242) of female controls. All the reactive sera reacted strongly with the immunogenic surface membrane proteins of *T. vaginalis* of molecular weights of about 92 and 115 kDa, with variable reactivity to other immunogenic proteins of *T. vaginalis*.

Conclusion—The significantly increased relative risk, RR = 3.42 (95% CI = 1.73-6.78), is comparable to the RRs derived in seroepidemiological studies of human papillomavirus, suggesting that *T. vaginalis* may be even more closely associated with invasive cervical cancer than previously realized.

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Keywords: *Trichomonas vaginalis*, Antibodies, Cervical cancer

Introduction

Cervical cancer is one of the most common malignant diseases worldwide. The epidemiological profile suggests that the aetiological agent/s are genital pathogens, probably acting synergistically.¹ Many sexually-transmitted organisms have been associated with it, principally human papillomavirus (HPV), herpes simplex virus (HSV) type-2 and *Chlamydia trachomatis*. Current evidence tends to support a causal role of high risk oncogenic HPV types in cervical cancer, although only a small number of those with HPV infection develop invasive cervical cancer. In the island republic of Singapore where about 200 cases of cervical cancer are reported annually, we have found that 95.1% of biopsies from patients contained HPV 16 and/or 18 DNA.² A possible association of *Trichomonas vaginalis*, a sexually-transmitted parasite, with cervical cancer was reported in 1969.^{3,4} In the present study, we report the high prevalence of antibodies to *T. vaginalis* in the sera of patients with invasive

cervical carcinoma compared with that of healthy female controls, and discuss the possible role of *T. vaginalis* in cervical cancer.

Materials and methods

Sera were obtained from 121 invasive cervical cancer patients at the Singapore General Hospital during the period 1987-1989, and from 242 local age-matched healthy adult females. Sera were stored at -70°C. Antibodies were detected by the western blot technique⁵ using antigen prepared by the trichloroacetic acid-precipitation⁶ of an axenized local isolate of *T. vaginalis* cultured in Hollander medium.⁷ Briefly, the antigen was electrophoresed in a 10% SDS-PAGE gel. After blotting, the nitrocellulose was placed in a blocking solution made up of 5% non-fat skim milk for 1 h at 37°C. After incubation overnight in a 1:50 dilution of the human serum, the nitrocellulose was washed twice for 10 min each with Tris-buffered saline-0.05% Tween 20. Following washing, a 1:200 dilution of rabbit antihuman IgG, IgA, IgM, conjugated to horseradish peroxidase (DAKO) was added and incubated at 37°C for 1 h.

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(Left) Coomassie brilliant blue-stained SDS-PAGE of trichloroacetic acid-precipitated *T. vaginalis*. (Right) Western blot of serum antibodies to *T. vaginalis* in patients with cervical cancer (Lanes 1-5) and in normal female controls (Lanes 6-7).

Table Western blot analyses of serum antibodies to *T. vaginalis* in cervical cancer patients and age-matched female controls

<i>T. vaginalis</i> antibodies in	Age group (yr)							Total
	20-29	30-39	40-49	50-59	60-69	70-79	≥ 80	
Cervical cancer patients	1/2	8/16	8/22	17/40	10/24	6/12	0/5	50/121
(Percentage positive)	50.0	50.0	36.4	42.5	41.7	50.0	0	41.3
Age-matched controls	0/4	1/32	2/44	6/80	2/48	1/24	0/10	12/242
(Percentage positive)	0	3.1	4.6	7.5	4.2	4.2	0	5.0

After washing, the nitrocellulose was developed in 0.015% H₂O₂ and 4-chloro-1-naphthol.

Results

Figure shows the Coomassie brilliant blue-stained SDS-PAGE profile of the trichloroacetic acid-precipitated *T. vaginalis*. By western blot analyses, it was found that there was more than eight-fold increase (41.3% vs 5.0%; RR = 3.42; 95% CI = 1.73-6.78) in prevalence of antibodies to *T. vaginalis* in Singapore patients with invasive cervical cancer compared with healthy age-matched female controls (table). The increase was evident in all age groups from 20-79 years, except those in the cohort ≥80 years old. Using the same technique, we had also found that a high percentage, 58.9% (33/56), of sera from patients with cervical cancer in Thailand had antibodies to *T. vaginalis*. This data was not included in the table because no control serum was available. All the reactive sera from Singapore and Thailand reacted with *T. vaginalis* surface antigens of about MW 92 and 115 kDa, with variable reactivities against other antigens ranging from about 23 to 65 kDa (figure). Antibodies to *T. vaginalis* were not detectable in normal and patient sera of those who were 80 years or older.

Discussion

This is the first seroepidemiological study demonstrating a significant association of *T. vaginalis* with invasive cervical cancer. The significantly increased relative risk (RR = 3.42) obtained in our study is of the same order as that derived in similar studies for HPV and cervical cancer where the RRs of 3.1 (for IgG to HPV 16- or 18-derived antigens from the L1 reading frame), 2.8 (for antigen from E2) and 3.8 (for antigens from E7) had provided further seroepidemiological support for an aetiological role of HPV in cervical cancer.⁸ In the same study, an increased risk was also found for IgG to *Chlamydia trachomatis* albeit with a low RR of 1.7 (95% CI = 1.0-2.7), but antibodies against cytomegalovirus, HSV type-2, Epstein-Barr virus or bovine papillomavirus were, on their own, not significantly associated with cervical cancer.

In Singapore, the prevalence of *T. vaginalis* infection is relatively low, being isolated in only 3.5% (3/85) of patients attending a local STD clinic.⁹ This compares favourably with the 5.0% of our random female controls who had antibodies to this parasite. In contrast, a

significant 43.1% of our patients with cervical cancer had detectable antibodies by immunoblot, suggesting exposure to *T. vaginalis*. Patients with negative immunoblots could have been previously infected with the organism since serum antibodies to this genital pathogen is not likely to persist for very long. Host plasma proteins are able to bind avidly to live parasites, providing a mechanism by which the trichomonads might evade immune surveillance mechanisms occurring in the urogenital environment.¹⁰

During 1964-1966, in the Swedish county of Ostergotland, the frequency of *T. vaginalis*, in a series of more than 13,000 normal women, was found to be 7.4%.³ There were 348 cases of pre-invasive and 47 of invasive cervical carcinoma. The frequencies of *T. vaginalis* in these cases were 29.3% and 29.8% respectively—values that were about 4 times that for the normal series. In Maryland, USA, studies involving a series of women with *T. vaginalis*, showed that “abnormal cytologic findings” were twice as common and the frequency of cervical carcinoma nearly 3 times as high as for subjects with smears negative for the species.¹¹ A similar observation was made in Quebec, Canada, where cytologic diagnosis revealed that 27.5% (36/131) of invasive cervical cancer patients had *T. vaginalis* compared with 10.5% (6,528/62,284) of the normal female population.⁴ Recently, in Italy, it was reported that there was a markedly increased frequency of *T. vaginalis* in patients of cytological class III and IV according to the Papanicolaou classification.¹² *T. vaginalis* was also found with a more significant frequency in patients showing erosion of the cervix. Is it possible that the disintegrating cancer tissue favours growth of the trichomonad?

The above studies diagnosed *T. vaginalis* using cytologic, direct wet mounts and by culture of vaginal materials which reflect active infection. Serological diagnosis better reflect past as well as present infection, although the persistence of serum antibodies may be variable. That sera from two of our local patients with active trichomoniasis showed weak reactions in immunoblots compared to that seen in sera from the cervical cancer patients led us to believe that *T. vaginalis* may play a more active role than previously believed. The serum antibodies in our study reacted strongly with the immunogenic surface membrane proteins of *T. vaginalis* of molecular weights of about 92 and 115 kDa, with variable reactivity to other immunogenic proteins. These results support an earlier observation that different individuals respond immunologically to different *T. vagi-*

nal antigens.⁶ The high-titre antibodies reflect active invasion by the parasite which may promote malignancy. *T. vaginalis* exists in two forms, the ovoid-motile (OM) form and its variant, the amoeboid-adherent (AA) form.¹³ The cytotoxic AA form predominates in fresh cultures. In contrast, *T. vaginalis* maintained for years in axenic cultures tend not to adhere to the epithelial cell monolayer, being cytotoxic at a concentration 100 times that of freshly-isolated trichomonads.¹⁴ Pathogenic *T. vaginalis*, but not the non-pathogenic *T. tenax*, selectively binds chemically stabilized HeLa cells which were originally derived from a cervical cancer patient!¹⁵ Binding involves at least 4 proteins of molecular weights ranging from 65 to ≤ 21 kDa. Not only are *T. vaginalis* able to adhere to cells, it has been observed to invade cells in a tissue biopsy of the cervix uteri.¹⁶ The cytopathic effect of *T. vaginalis* is mediated by close contact between the parasite and the epithelial surface with the release of substances such as the "cell-detaching factor" (CDF)¹⁷ and a variety of proteinases including metalloproteinases¹⁸ which is important in the progression of squamous cell carcinomas and metastasis.¹⁹

Live *T. vaginalis* has also been found to induce immunosuppression with the more pathogenic strains inducing a greater degree of immunosuppression.²⁰ This could lead to activation of carcinogenic viruses. Large numbers of the flagellate could alter the microenvironment and facilitate the growth of facultative and anaerobic bacteria which may explain the equivocal association of some bacteria with cervical cancer.²¹

A likely role for *T. vaginalis* is that of a vector for the spread of other pathogens since reovirus and genital HSV have been found to be able to survive intracellularly but not *Mycoplasma*, *Neisseria* or *Chlamydia*.^{22,23} Unique non-segmented double-stranded RNA virus-like particles (VLPs) have been described in some isolates of *T. vaginalis*.²⁴ The viral genome does not share significant homology with that of viruses of other parasites. Whether these VLPs have a role to play in cervical cancer may prove to be an intriguing investigation, although virus-harboring trichomonads synthesize lower amounts of adhesins essential for the binding to target cells, hence are less adherent and correspondingly less cytotoxic to HeLa cells *in vitro*.²⁵ The association, if any, between *T. vaginalis* and HPV, remains to be determined since their relative risks (RR) with cervical cancer are comparable. Whether *T. vaginalis* is merely a surrogate marker of exposure to HPV, influenced by promiscuous sexual behaviour which has been the most consistent epidemiological finding,²⁶ is a moot point. After all, a close association between *T. vaginalis* infection and presence of vaginal papillomas had been reported.²⁷

A possible application of our study would be to identify women with serum antibodies to *T. vaginalis* as being at higher risk of developing cervical cancer. A prospective study can be initiated to determine whether eliminating infes-

tation with *T. vaginalis* may reduce the incidence of cervical carcinoma. A potential diagnostic test could be developed by testing for both serum anti-*T. vaginalis* as well as anti-early (E) and late (L) proteins of HPV types 16 and 18 antibodies.

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