CONCISE COMMUNICATION

Double-Stranded RNA Viral Infection of *Trichomonas vaginalis* Infecting Patients Attending a Sexually Transmitted Diseases Clinic

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Trichomonas vaginalis **(TV) can be infected with double-stranded RNA (dsRNA) viruses that may have important implications for trichomonal virulence and disease pathogenesis. A cross-sectional study was conducted in a sexually transmitted diseases clinic to determine the prevalence and clinical significance of dsRNA viral infection of TV infecting men and women. Overall, dsRNA virus was present in 21 (75%) of 28 TV isolates (95% confidence interval [CI], 55%–89%). dsRNA viral infection of TV was not associated with the presence of discharge, dysuria, genital pruritus, or genital irritation or odor. However, patients with virus-positive isolates were significantly older than patients with virus-negative isolates (median age, 38 vs. 23 years; $P = .003$), and virus-positive isolates were more prevalent among women (19 [86%] of 22 isolates; 95% CI, 65%–97%) than among men (2 [33%] of 6 isolates; $P = .02$). The age and sex specificity of virus-positive isolates may aid in understanding the differences in chronicity and clinical presentation of TV in men and women.**

Trichomonas vaginalis (TV) is now appreciated as one of the most common nonviral sexually transmitted diseases (STDs) in the world. Infection by this parasite is associated with premature rupture of membranes and premature delivery in pregnant women [1–3]. Recently, the vaginitis has also been associated with an increased risk of human immunodeficiency virus (HIV) acquisition and transmission [4, 5]. Although TV infection occurs in men, its prevalence and associated morbidity appear to be significantly lower than those reported for women [6–10].

Little is known about TV virulence factors. The female predominance of infection, the variable severity of clinical vaginitis, and the pregnancy- and HIV-related morbidity of TV infection remain poorly understood. Infection of trichomonads by double-stranded RNA (dsRNA) viruses causes up-regulation of synthesis and surface expression of a highly immunogenic protein, P270 [11]. The presence of dsRNA viral infection of TV is also associated with differential qualitative and quantitative expression of cysteine proteinases [12]. Cysteine proteinases are linked with TV cytoadherence to vaginal epithelium, cytotoxicity, and degradation of basement membrane components [13]. Thus, dsRNA viruses induce various phenotypic changes that may impact TV virulence [12, 14]. Isolates composed of virus-negative trichomonads are termed "type I," whereas those with virus-positive parasites are termed "type II." In this study, we evaluated the prevalence of type II isolates and the clinical implications and demographic factors associated with dsRNA viral infection of TV in men and women attending a Baltimore STD clinic.

Methods

A cross-sectional study was conducted of men and women attending a Baltimore City Health Department STD Clinic between March and July 2000. Any person attending the clinic on days of active enrollment was eligible, except for persons who had taken an antibiotic in the prior 2 weeks. Patient demographics were collected at the time of patient registration. Clinicians recorded genitourinary symptoms, STD history, sexual behavior, and drug and alcohol use. A directed STD clinical examination that used a standardized medical encounter form was recorded.

Clinical laboratory evaluation. Standard laboratory evaluation for women included vaginal pH measurement, amine test, potassium hydroxide preparation with light microscopy for evaluation of yeast, wet preparation for microscopic detection of TV and bacterial vaginosis, and cervical swabs for Gram stain and gon-

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The study was approved by institutional review boards of the Johns Hopkins School of Medicine and the Baltimore City Health Department. Retrospective analysis was done after all personal identifiers were removed from the isolates, and new unique identifiers were assigned.

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orrhea culture. For men, urethral swabs were obtained for Gram stain and gonorrhea culture. Chlamydia polymerase chain reaction (Amplicor; Roche Diagnostic Systems) was done for cervical specimens from women and for urine specimens from men. TV culture was performed by InPouch TV test (Biomed Diagnostics). For women, a swab was used to inoculate fluid from the posterior fornix of the vagina directly into the TV culture test for isolation of TV. For men, 10 mL of first-void urine was spun for 15 min, and the pellet was inoculated into the TV culture test. The pouches were incubated at 37°C. Direct microscopic examination of the plastic pouch for the presence of motile trichomonads was done at 24 h; if the results were negative, evaluation was repeated at 48 h and at 5 days.

Research laboratory evaluation. Positive TV culture tests were subcultured in trypticase–yeast extract–maltose medium [15] supplemented with 5% heat-inactivated horse serum, streptomycin sulfate, and penicillin G potassium and incubated at 37°C. Samples were harvested in the late-logarithmic or stationary phase of growth and at a total organism count of $\geq 1.6 \times 10^7$ organisms. Isolates were centrifuged at 300 *g* for 8 min and resuspended in 1 mL of cold trypticase–yeast extract–maltose medium with 20% heat-inactivated horse serum and 10% dimethyl sulfoxide. Resuspended samples were placed in a −70°C freezer and transferred to liquid nitrogen storage prior to batch evaluation for dsRNA viral infection.

Frozen cultures were grown overnight in fresh trypticase–yeast extract–maltose medium with 5% serum [11–13]. At least 2×10^7 organisms were washed twice in ice-cold PBS, and pellets were suspended in 500 μL of PBS containing 10% SDS. Total nucleic acids of trichomonads were obtained as described elsewhere [11]. In brief, after mixing with an equal volume of phenol, the preparation was microfuged at maximum speed for 3 min. The aqueous phase was transferred to microfuge tubes containing 500 μL of phenol and mixed, and this procedure was repeated. The aqueous phase was then mixed with an equal volume of chloroform, after which the new aqueous phase was mixed with 50 μL of 3 *M* sodium acetate and 1 mL of 100% ethanol. The nucleic acids were precipitated at −70°C for 30 min and then pelleted by centrifugation for 20 min. The pellet was washed with 70% ethanol in diethylene pyrocarbonate (DEPC)–treated water. After drying, the pellet was dissolved in 20 μL of DEPC-treated water containing electrophoresis loading dye. Nucleic acids were stained with ethidium bromide after electrophoresis in 1% agarose gel. TV isolates with and without dsRNA virus were used as controls, as described elsewhere [11, 12, 14].

Statistical analysis. Statistical analysis was performed by using EpiInfo (version 6.04; CDC) and STATA (version 6.0; StataCorp) software. A 2-sided $P \leq .05$ was considered to be significant. We used Fisher's test to evaluate categorical variables and the Wilcoxon rank sum test for continuous variables.

Results

Over the course of 5 months, 361 women and 525 men were evaluated with TV culture. The cohort was 98% African American. The median age was 28 and 26 years for men and women, respectively. TV was isolated from 85 (24%) of 361 women (95% confidence interval [CI], 19%–28%) and 21 (4%) of 525 men (95% CI, 2%–6%). Seventy-nine isolates were subcultured (65 from women and 14 from men). This subculture step yielded an organism count of $> 1.6 \times 10^7$ organisms in 52 of 79 trichomonal isolates.

Failure of subculturing was due to poor growth kinetics with progressive loss of organisms or to bacterial or fungal overgrowth that was unresponsive to antifungal and antibacterial additives in the original culture medium or subculturing broth. Successful subculturing was not associated with the patient's age, sex, or HIV status, the results of wet preparation, or the presence of penile or vaginal discharge. However, a history of alcohol intoxication within the last week was associated with successful subculturing of TV isolates (10/10 vs. 40/65 isolates; $P = .025$). Of the 52 trichomonal isolates that were amplified to $\geq 1.6 \times 10^7$ organisms, significant remaining fungal contamination precluded dsRNA virus evaluation of all but 28 isolates.

Of the 28 isolates evaluated for dsRNA viral infection, 22 were from women, and 6 were from men. All members of the final cohort were African American. The median age of the 22 women was 36 years (interquartile range [IQR], 27–41 years). The median age of the 6 men was 31 years (IQR, 23–39 years). Ten (48%) of 21 women had a history of TV infection, but no men did. HIV infection was documented in 1 (5%) of 22 women and in 1 (17%) of 6 men. Table 1 lists risk characteristics of the cohort.

Type II trichomonads were present in 21 (75%) of 28 isolates (95% CI, 55%–89%; figure 1). Patient sex and age were significantly associated with dsRNA viral infection of TV. Type II isolates were more prevalent among women (19 [86%] of 22 isolates; 95% CI, 65%–97%) than among men (2 [33%] of 6 isolates; $P = .02$). Patients with type II isolates were also significantly older than those with type I isolates (median age, 38 vs. 23 years; $P = .003$). This age predilection of type II isolates was also seen when we compared women with type II versus type I isolates (median age, 38 vs. 18 years; $P = .009$).

Although symptoms were not significantly associated with dsRNA viral infection of TV, patients with type II isolates tended to report more genital irritation and odor than did patients with type I isolates (5 [25%] of 20 vs. 0 of 7 patients, respectively; $P = .283$). Alternatively, dysuria tended to be

Table 1. Behavioral characteristics of patients evaluated for double-stranded RNA (dsRNA) virus–positive versus dsRNA virus–negative *Trichomonas vaginalis* isolates.

	No./total (%)	
Risk factor	Women ($n = 22$)	Men ($n = 6$)
New sex partner in last month	3/21 (14)	0/6
Heterosexual	18/20 (90)	6/6 (100)
Injection drug use	1/20 (5)	1/6 (17)
Drunk in last week	3/20 (15)	1/6 (17)
Cocaine/heroin use in last week	2/20 (10)	0/6
Prostitution	5/21 (24)	0/6

NOTE. Differences between men and women did not reach statistical significance by Fisher's exact test.

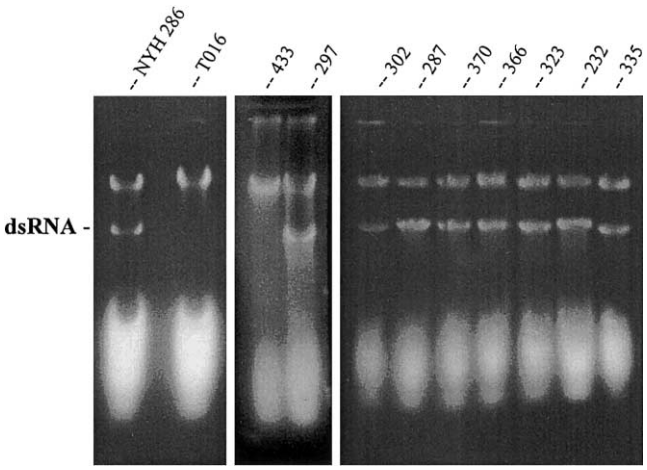

Figure 1. Presence or absence of double-stranded RNA (dsRNA) virus among fresh *Trichomonas vaginalis* clinical isolates. "dsRNA," on the left, refers to the detection of a band by ethidium bromide staining after electrophoresis in 1% agarose. Isolates NYH 286 *(lane 1)* and T016 *(lane 2)* are type II and type I isolates (dsRNA virus–infected or –uninfected positive and negative controls), respectively. These controls were always used for comparative analysis with representative type I and type II clinical isolates, as shown for lane 3 and lanes 4–11, respectively. Additional experiments were done on representative fresh isolates to confirm viral infection. Indirect immunofluorescence assay was done by use of a monoclonal antibody to a phenotypically varying immunogen and detection of fluorescent type II trichomonads [11, 13]. The absence of dsRNA was further confirmed by the inability to purify virus by CsCl banding centrifugation, compared with virus-positive isolates [11, 13]. Nos. at the tops of the gels are isolate designations assigned after cultivation.

more frequent among patients with type I isolates than among patients with type II isolates (3 [43%] of 7 vs. 3 [15%] of 20 patients; $P = .29$). Genital pruritus was present in 4 (20%) of 20 patients with type II isolates and in 1 (14%) of 7 patients with type I isolates ($P = 1.0$). Genital discharge was present in 13 (65%) of 20 patients with type II isolates and in 4 (57%) of 7 patients with type I isolates ($P = 1.0$).

Discussion

We believe that this report is the first to focus on the prevalence and clinical features associated with infection by either dsRNA virus–negative (type I) or dsRNA virus–positive (type II) trichomonads. We evaluated clinical infections in men and women with type I or II isolates and found that the majority of isolates were type II. We did not find an association between the presence or absence of dsRNA viral infection of trichomonal isolates and patient symptoms, although the numbers of patients and isolates evaluated were small. We recognize that more research is needed to determine whether the clinical presentation or morbidity of trichomoniasis is affected by dsRNA viral infection of trichomonads.

There was a higher prevalence of type II isolates in women than men ($P = .02$). This finding may be of note, given the higher prevalence of trichomonal disease and more significant morbidity associated with this infection in women [1–5]. The gender differences in the prevalence of type I and type II TV may reflect differences in the virulence of these 2 types of isolates, as influenced by the micronutrients and pH of the male genitourinary tract versus the vagina. Urine, prostatic secretions, and semen may be detrimental to growth and sustained infection with virus-positive TV, resulting in short periods of infection and transmission of type II isolates. Transmission of both isolate types must occur, but prevalence may be most strongly influenced by the ability of isolates to persist in a particular host environment. These results nonetheless strongly suggest that the difference in prevalence of virus-positive TV between men and women requires further investigation and confirmation.

Younger women in our clinic were less likely to be infected with type II isolates ($P = .003$). This finding may reflect a difference in sexual exposures. Alternatively, it may suggest a relationship between age and susceptibility to infection with type I or type II isolates. Host immune responses may play a role in the age-related prevalence of either type I or type II infections. It could be hypothesized that older women have developed more-extensive immune surveillance toward type I isolate infections, which itself may be indicative of decreased virulence of type I isolates in women. Likewise, the continued vulnerability of women to infection with type II isolates may indicate that properties conferred to trichomonads after dsRNA viral infection enhance overall virulence of the parasite in the microenvironment of the vagina.

Our study was limited by the small sample size because of the significant loss of isolates secondary to poor growth kinetics and fungal superinfection commonly experienced in the propagation of fresh clinical trichomonads. Further efforts to investigate the clinical significance of type II isolate infections may require the addition of more-potent antifungal agents to the culture media to optimize recovery of isolates. Nonetheless, we feel that these observations are significant. Future research should focus on the age and sex specificity of type II infections and further elucidate the impact of the virus-harboring isolates on patient clinical presentation.

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