SHORT REPORT

Use of urine polymerase chain reaction to define the prevalence and clinical presentation of *Trichomonas vaginalis* in men attending an STD clinic

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Sex Transm Infect 2003;**79**:151–153

Objective: To determine the prevalence and clinical features of *Trichomonas vaginalis* (TV) infection in men.
Methods: Men attending a public STD clinic in Baltimore, Maryland, were evaluated between March and July 2000. Clinicians recorded a standardised history and clinical examination. Urethral swab specimens were collected for Gram stain and *Neisseria gonorrhoeae* culture. First fraction urine samples were evaluated with TV culture and chlamydia and TV polymerase chain reaction (PCR). True positive TV was defined as a positive TV culture or a positive TV PCR confirmed with a second primer set.
Results: 355 men were evaluated in 363 visits. The prevalence of gonorrhoea, TV, and chlamydia were 19%, 13%, and 11%, respectively. In men over 28 years, the prevalence of TV was significantly higher than chlamydia. Age and urethritis by Gram stain were associated with a positive result on TV culture (p=0.03 and p=0.02, respectively) but not associated with TV infection as defined by a positive TV culture or a confirmed TV PCR. Discharge or dysuria was reported in 47% and 22% of men with TV, respectively.
Conclusions: TV prevalence in an urban STD clinic setting was high. Older age and urethritis were not significantly associated with TV infection as defined by a positive TV culture or a confirmed TV PCR.

Trichomonas vaginalis (TV) has been associated with complications of pregnancy and an increased risk of HIV acquisition in women.[1 2] Some studies have associated TV infection in men with prostatitis, urethral strictures, and elevated seminal HIV RNA in men with HIV infection and symptomatic urethritis.[3–5] Yet controversy continues over the prevalence and morbidity of TV infection in men.

Studies using TV culture have shown TV prevalence rates from 3–12% in men attending STD clinics in the United States and have associated TV infection with older age, symptoms of urethritis, and diagnostic criteria for non-gonococcal urethritis (NGU).[6–8] Recently, polymerase chain reaction (PCR) has emerged as a powerful tool for TV diagnosis, but studies using TV PCR have provided limited information about the prevalence of infection, the epidemiology, or the clinical presentation of disease in men.[9 10] This study used urine TV PCR and culture to determine the prevalence and clinical features of TV infection in men in an urban STD clinic in the United States.

METHODS

This study was approved by the institutional review boards of the Johns Hopkins Medical Institutions and the Baltimore city health department. Men attending a Baltimore City Health Department STD clinic were evaluated between March and July 2000. A standardised clinical form was used to record the history and directed examination.

Table 1 Patient characteristics and risk factors

	Men	
	(n=355)	(%)
Race (AA)	350/355	(99)
Mean age (years)	31	
IDU ever	23/353	(7)
Cocaine use ever	61/354	(17)
Needle sharing ever	10/354	(3)
Known to be HIV infected	17/355	(5)
History of trichomonas*	30/353	(9)
	Male visits	
	(n=363)	(%)
Average No partners in past month (n=356)	1.56	
New partner in past month	97/349	(28)
Alcohol intoxication in past week	56/344	(16)
Cocaine or heroin use in past week	11/344	(3)
Reported recent trichomonas exposure*	30/361	(8)

AA = African-American; IDU = injecting drug use.
*By self report.

Laboratory evaluation

Intraurethral secretions were collected for Gram stain and *Neisseria gonorrhoeae* culture using modified Thayer-Martin media. First fraction urine (15 ml) was centrifuged for 10 minutes. The sediment was inoculated into an InPouch TV test (Biomed Diagnostics, Santa Clara, CA, USA) for TV culture and incubated at 37°C in 5% carbon dioxide. Microscopic examination for motile trichomonads was performed at 24 hours, 48 hours, and at 5 days. Chlamydia PCR (Amplicor; Roche Diagnostic Systems, Branchburg, NJ, USA) was performed on 7 ml of urine according to the manufacturer's directions.

After aliquoting patient urine for TV culture and chlamydia PCR, the remaining quantity (1–7 ml) was stored at −70°C and tested 11–17 months later by TV PCR. After thawing, 1–7 ml of stored urine were centrifuged. The pellet was resuspended, mixed with chelating resin (Chelex 100; Sigma, St Louis, MO, USA), and incubated.[11] The PCR primer set BTUB 9/2 was used to target 112 bp conserved regions of the three β tubulin genes of TV using the PCR method described previously.[11] If results of BTUB 9/2 TV PCR and TV culture were discordant, PCR primer sets TVK 3/7 and AP65 A/B were used for adjudication.[11 12]

Definitions

TV infection was defined as either a positive culture by InPouch TV test or a positive TV PCR with primer set BTUB 9/2

Table 2 Prevalence of STDs by age group (median age 28 years)

	Age ⩽28 years			Age >28 years			Total		
		(%)	95% CI		(%)	95% CI		(%)	95% CI
GC	41/174	(24)	17 to 31	27/182	(15)	10 to 21	68/356	(19)	15 to 24
Chlamydia	32/177	(18)	13 to 25	7/184	(4)	2 to 8	39/361	(11)	8 to 14
Trichomonas*	23/178	(13)	8 to 19	24/185	(13)	8 to 19	47/363	(13)	10 to 17

CI = confidence interval; GC = gonorrhoea; Trichomonas* = defined as culture positive for *T vaginalis*, or a positive *T vaginalis* PCR confirmed with a second primer set.

confirmed by a second PCR primer set, TVK 3/7 or AP65 A/B. Urethritis was defined as a urethral swab Gram stain with ⩾5 polymorphonuclear cells per oil immersion field.

Statistical analysis

Statistical analysis was performed using EPI-INFO Version 6.04 (Atlanta, GA, USA) and STATA 6.0 (College Station, TX, USA). A two sample *t* test or Wilcoxon rank sum test was used, as appropriate, for comparison of continuous variables. Differences among categorical variables were assessed with either Pearson's χ^2 test or Fisher's exact test for strata with less than five observations in a cell.

RESULTS

In all, 506 men were evaluated with the InPouch TV test at 525 visits. Urine volume provided was inadequate for TV PCR testing in 151 men at 162 visits, leaving a final study cohort of 355 men with 363 visits. Demographic characteristics of the cohort are recorded in table 1. Forty seven men met criteria for TV infection. TV culture detected 13/47 cases (28%). TV PCR detected 44/47 cases (94%). Of the 46 cases that were only positive by TV PCR, 34 were confirmed with a second PCR primer set. Three cases were only positive by TV culture. The prevalences of gonorrhoea, TV, and chlamydia are shown in table 2.

Older age was associated with a positive TV culture (p=0.03) but was not associated with TV infection based on the criteria of either a positive culture or a positive adjudicated PCR. The median ages of men with positive TV cultures and men with negative TV cultures was 38 years and 28 years, respectively. TV infection was not significantly associated with condom use, a reported history of exposure to a sex partner with TV, injecting drug use, alcohol abuse, a history of previous STDs, duration of symptoms, or number of sexual partners.

In the absence of gonorrhoea or chlamydia co-infection, 17/36 men (47%; 95% CI, 30 to 65) with TV reported discharge and 8/36 men (22%; 95% CI, 10 to 39) had dysuria. Complaints of discharge, dysuria, or genital irritation or odour were present in 24/36 men (67%; 95% CI, 49 to 81) with TV. In men without gonorrhoea or chlamydia co-infection, a positive TV culture was associated with a diagnosis of urethritis (TV culture positive in 8/96 men (8%) with urethritis versus 3/163 men (2%) without urethritis; p=0.02.) However, TV infection, as defined by culture and adjudicated PCR, was not associated with urethritis (TV positive in 16/96 men (17%) with urethritis versus 20/163 men (12%) without urethritis; p=0.36). In the absence of gonorrhoea or chlamydia co-infection, only 16/36 men (44%) with TV infection had evidence of urethritis.

DISCUSSION

The prevalence and features of male TV infection in the United States have been previously defined using relatively insensitive TV culture techniques. Recently, studies in Africa have used TV PCR to improve detection of infection in men.[5 9 10] In this study in an urban STD clinic, the prevalence of TV by urine PCR and culture (13%) was less than gonorrhoea (19%) but similar to chlamydia (11%). In men over 28 years of age, the prevalence of TV (13%) was significantly higher than chlamydia (4%).

Older age was associated with a positive TV culture but not with TV infection as defined by culture and more sensitive TV PCR testing. This finding suggests that higher organism loads may occur in older men than in younger men. Age related differences in urinary pH, micronutrients, or toxins may impact TV survival in vivo.

Other reports have documented an association between TV culture results and urethritis.[6 8] Studies using PCR techniques have been inconclusive.[9 10] In our study, in the absence of gonorrhoea or chlamydia co-infection, a positive TV culture was associated with urethritis (p=0.02), but TV as defined by culture and PCR was not associated with urethritis. The increased inflammation in these men with TV isolated by culture may be a marker of a higher organism burden.

The primary limitations of this study are the retrospective application of TV PCR, the cross sectional design, and the variable volumes of urine available for TV PCR testing, which may have affected the sensitivity of the assay. PCR testing for *Mycoplasma genitalium* and *Ureaplasma urealyticum* was not performed, and co-infection with these agents might have affected associations between TV infection and the presence of symptoms or signs of urethritis.

This study demonstrated a significant burden of TV infection in men attending an urban STD clinic. Age and the presence of urethritis were not associated with TV infection as defined by PCR and culture. Additional research using TV PCR is needed to further clarify the risk factors, clinical features, and natural history of TV infection in men.

ACKNOWLEDGEMENTS

We are indebted to the staff of the Baltimore City Health Department Druid STD Clinic. We thank Justin Hardick, BA, of the Johns Hopkins School of Medicine International Chlamydia Laboratory, and Della Duncan, BS, and Vincent Marsiglia, BA MT (ASCP, DLM, MHA), of the Baltimore City Health Department Druid STD Clinic, for their assistance and laboratory expertise.

CONTRIBUTORS

Conception and design KW, EE, AR; analysis and interpretation of the data KW, CG; drafting of the article KW; critical revision of the article for important intellectual content: EE, CG, AR; final approval of the article KW, EE, CG, AR; provision of study materials or patients EE, AR, CG; administrative, technical, or logistic support EE, AR, CG; collection and assembly of data KW.

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Accepted for publication 10 October 2002

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