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Original Article

Molecular Epidemiologic Study of Male Trichomoniasis in Hamadan, Western Iran

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Abstract

Background: Trichomoniasis, caused by *Trichomonas vaginalis* protozoan, may lead to clinical or subclinical urethritis or prostatitis in men. Despite the importance of men in the epidemiology of trichomoniasis, there is little information about this topic. This epidemiological study was performed on men in Hamedan, western Iran.

Methods: During Oct 2018 to Mar 2019, 214 male individuals, presenting to the Urology Clinic of Shahid Beheshti Hospital in Hamadan, were enrolled and evaluated for trichomoniasis. First-voided urine specimen was used for detection of *T. vaginalis* infection using molecular and parasitological methods.

Results: Trichomoniasis was detected in 10 of 214 male participants (4.7%, 95% CI: 7.5-1.8%) using PCR assay. Culture and wet mount preparation of urine sediment were unable to isolates any *T. vaginalis* parasite. Nine of the 10 infected men were married, and six of them were ≥49 yr of age. Urinary frequency and dysuria were the most complaints (80%) among infected individuals.

Conclusion: Given the notable prevalence of the infection, the prevalence of male trichomoniasis will be underestimated if only conventional diagnostic methods are used. Therefore, the risk of infection as well as the molecular survey of *T. vaginalis* infection should be considered in men with or without clinical symptoms.

Introduction

Trichomonas vaginalis, a protozoan parasite of human urogenital tract, is the most common non-viral agent of sexually transmitted infection (STI) worldwide. According to the WHO (2008), trichomoniasis with 276.4 million cases annually is more

prevalent than syphilis (10.6 million cases), chlamydial (105.7 million cases), and gonorrheal (106.1 million cases) infection. The global estimation of trichomoniasis is 8.1% for women and 1.0% for men. These estimates are often underestimated because of the neglect of asymptomatic infections and the less sensitive diagnostic methods (1-2). Vaginitis and vaginal discharge in women are common manifestations of trichomoniasis. Some complications are associated with trichomoniasis including adverse pregnancy outcomes, increased risk of HIV transmission and acquisition, and infertility. Remarkably, ~50% of women and more than 75% of men with *T. vaginalis* infection are asymptomatic. Although men are carriers, trichomoniasis may cause urethritis, which can lead to epididymitis, prostatitis, and temporary infertility (3-5). In the United States, the prevalence of male trichomoniasis was reported from 2.8% to 17%, and a high prevalence of the infection (71.7%) in male partners of the infected women (6-10).

Symptomatic trichomoniasis in women can last for years but the symptoms of infection in men usually resolve spontaneously within 10 days (5). Therefore, early detection and treatment of male trichomoniasis, particularly the asymptomatic infection, is essential as a public health concern. The prevalence and morbidity of *T. vaginalis* infection in men, as an etiologic agent of nongonococcal urethritis, are not fully understood.

Little research conducted on men, often has been focused on marital partners of infected women. Similarly, in Iran, little is known about the epidemiological aspects of the male infection. Therefore, this molecular epidemiologic study was designed to investigate the prevalence of trichomoniasis in men attending a urology clinic in Hamadan, west of Iran.

Materials and Methods

This cross-sectional study was conducted to investigate the prevalence of trichomoniasis in men attending the Urology Clinic of Shahid Beheshti Hospital in Hamadan, western Iran. Two hundred and fourteen men who attended the clinic for screening or treatment of genitourinary disorders were enrolled to participate in this study, during Oct 2018 to Mar 2019.

The study was approved by the Research Ethics Committee of the Hamadan University of Medical Sciences (IR.UMSHA.REC.1397.566). Written informed consent was provided by the all participants before sampling and demographic and clinical data was recorded.

In this study, the inclusion criteria were 18 yr and older. Moreover, men who had taken antibiotics in the past two weeks were excluded from the study.

Samples and Parasitological Detection

First-voided urine (VB1) sample was used for detection of *T. vaginalis* infection (11). First, approximately 30 ml of the VB1 specimen from participants were collected in sterile screw-lid urine container. As soon as possible, the total volume of the samples was pelleted by centrifugation at 1,000 × g for 5 min. The supernatant was decanted and the pellet resuspended in 250 µl of sterile distilled water (12). Immediately, one drop of the suspension was microscopically examined, at low (100 ×) and high (400 ×) magnifications, for detection of motile trichomonads. In the next step, 50 µl of the prepared suspension was inoculated into Dorset's culture medium and incubated at 35.5 °C. Then, the rest of the urine sediment suspension was rinsed with 1 ml of sterile phosphate buffered saline (PBS) and recentrifuged at 2,000 × g for 10 min, decanted, and resuspended in 100 µl of sterile PBS. Finally, the processed samples were transferred to -70 ºC for further molecular analysis. Moreover, the *Trichomonas* cultures were daily monitored for the growth of the parasites under light microscope for up to five days (13).

Molecular detection

The highly sensitive primer set TVK3/TVK7 (F: 5'-ATTGTCGAACATTGGTCTTACCCTC-3'/R: 5'-TCTGTGCCGTCTTCAAGTATGC-3') was selected to amplify a multicopy target sequence in the genome of *T. vaginalis* which yield a product of 261 bp (14). DNA extraction was performed according to the Chelex-100 DNA extraction method previously described (11, 15). Briefly, thawed samples were mixed with 100 µl of a 10% suspension of chelating resin (Chelex 100; Sigma, St. Louis, Missouri, USA) in 0.01 M Tris buffer (pH 8.0), and then followed by incubation at 56 ºC for 15 to 30 minutes. After gently mixing the preparations, they were boiled (8 to 10 min) and centrifuged (12,000 × g for 1 min). Finally, the supernatant was carefully removed and stored at -70 °C as DNA template for PCR assay.

PCR amplification was performed at final volume of 50 µl, including 3 µl gDNA (50-100 ng), 25 pmol of each primer (Bioneer, Daejeon, South Korea), 5 µl buffer 10X, 250 µM of each dNTP's, 3.5 mM MgCl2 (25mM), 1 units Taq DNA polymerase (Cinnagen, Karaj, Iran) and sterile distilled water up to the final volume. Positive control (containing *Trichomonas* DNA) and negative control (PCR master mix containing no DNA) were included in each PCR run. The PCR conditions was as follows: primary denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation (60 sec at 90 °C), annealing (30 sec at 60 °C), extension (120 sec at 70 °C), and then final extension at 72 °C for 7 min. Then, the amplification product was visualized by 1.5% agarose gel electrophoresis in 1X Tris-borate/EDTA buffer stained with SYBR Safe DNA gel stain (Invitrogen).

The representative amplicons of the target sequence were purified and subjected to sequencing using Applied Biosystems Automated 3730xl DNA Analyzer (Bioneer Inc., Korea) and the same primers as used in the PCR amplification. The electropherograms were visually inspected using Chromas software (version 2.6), and the sequences were edited and aligned by BioEdit software (version 7.2). Identification of the sequences and comparison with reference sequences in GenBank were done using the BLASTn program.

Results

T. vaginalis infection was diagnosed in 10 of 214 male participants (4.7%, 95% CI: 7.5-1.8%) using the PCR assay (Fig. 1).

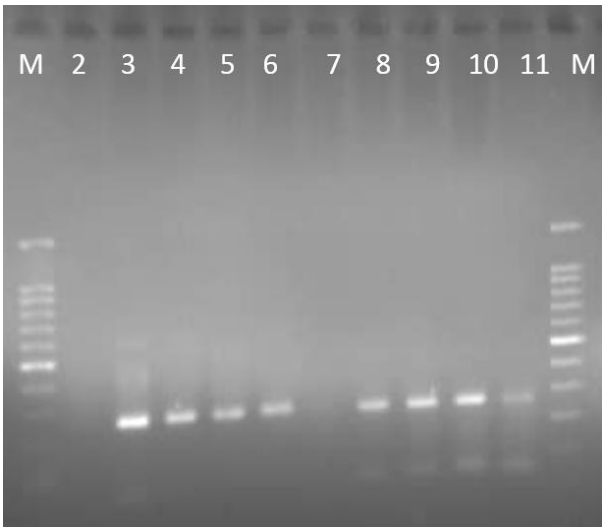

Fig. 1: Agarose gel electrophoresis of PCR amplification of the 261-bp target fragment from *T. vaginalis isolates*. Lane M: DNA marker (100 bp); Lane 2 & 3: negative & positive control; Lane 4-6 & 8-11: positive samples; Lane 7: negative sample

None of the parasitological methods, urine wet mount preparation and culture, were able to detect the parasite. The age of participants ranged from 18 to 83 with a mean age of 55.4 years. The average age of infected men was 50.8 yr, with an age range of 27 to 78 years. The results indicated that 60% of trichomoniasis occurred in people ≥49 yr old (Table 1). Most infected men were married (90%) and were educated below diploma (70%). Urinary discomfort, including dysuria and urinary frequency, was the most complaint (80%) among

men with *T. vaginalis* infection. Moreover, according to employment status, the highest rate of trichomoniasis (9%) was observed in the retired group. Other characteristics of the participants are listed in Table1.

Table 1: Prevalence of male trichomoniasis among participants by clinical and demographic characteristics

Characteristics	***Trichomoniasis***		***Total***	***P-value***
	Positive No. (%)	***Negative No. (%)***	***No. (%)***	
Age (yr)				
18-27	1 (8)	12 (92)	13 (6)	0.994
28-37	1 (3)	31 (97)	32 (15)	
38-47	2 (5)	38 (95)	40 (19)	
48-57	2 (6)	35 (94)	37 (17)	
58-67	2 (5)	38 (95)	40 (19)	
68-77	1 (3)	30 (97)	31 (14)	
78-87	1 (5)	20 (95)	21 (10)	
Marital status				
Single	1 (4)	22 (96)	23 (11)	1
Married	9 (5)	182 (95)	191 (89)	
Education				
Primary school	1 (2)	41 (98)	42(20)	0.735
Junior high school	6 (6)	87 (94)	93 (43)	
Senior high school	3 (5)	57 (95)	60 (28)	
University education	0 (0)	19 (100)	19 (9)	
Urogenital Symptoms				
None	0 (0)	43 (100)	43 (20)	0.096
Dysuria	4 (13)	26 (87)	30 (14)	
Urinary frequency	4 (6)	60 (94)	64 (30)	
Penile discharge	0 (0)	13 (100)	13 (6)	
Kidney pain	2 (3)	62 (97)	64 (30)	
Employment Status				
Employee & Student	0 (0)	11 (100)	11 (5)	
Unemployed	0 (0)	21 (100)	21 (10)	0.514
Retired	2 (9)	20 (91)	22 (10)	
Others	8 (5)	152 (95)	160 (75)	

The six representative amplicons of target sequence were subjected to nucleotide sequencing and yielded sequences about of 240 bp. The confirmed sequences compared with the reference sequence, *T. vaginalis* repeated DNA target for PCR identification (accession number L23861), and other available sequences in GenBank. The six sequences were similar and formed one haplotype (Fig. 2). The analysis of the consensus sequence indicated 98.3% homology with the reference sequence and up to 95.2% with other sequences in GenBank.

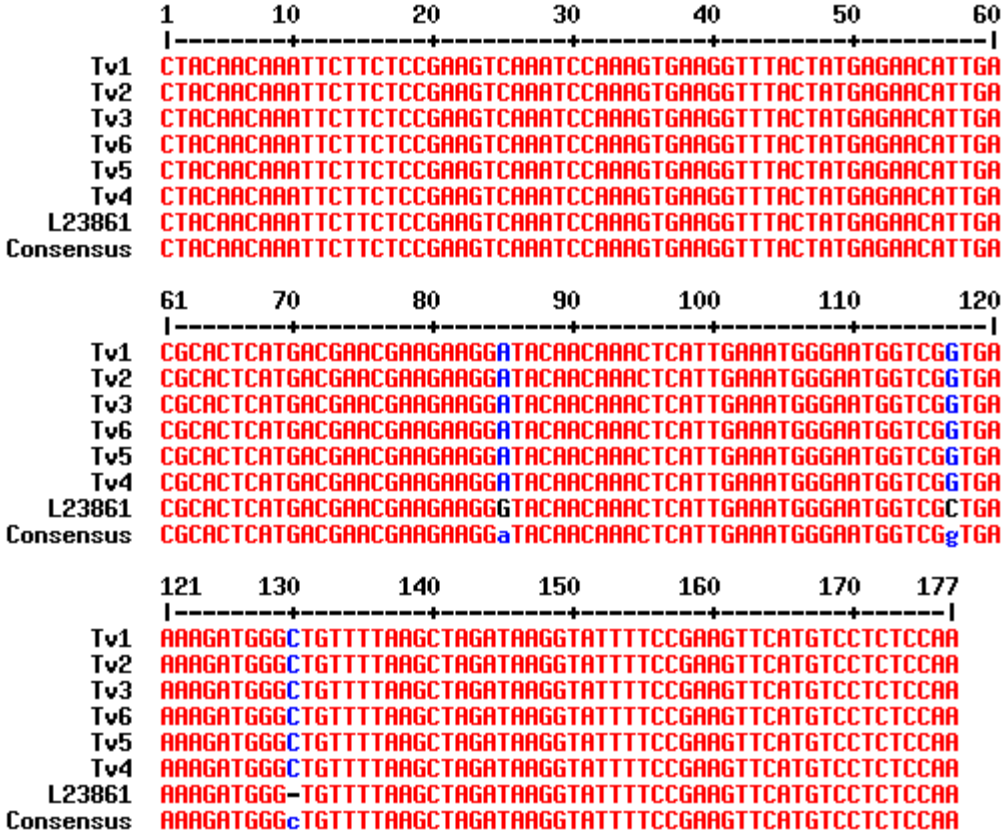

Fig. 2: Partial sequence alignment of the sequence target of *T. vaginalis* isolates was compared with reference sequence *(T. vaginalis* repeated DNA target for PCR identification, Accession No. L23861)

Discussion

Trichomoniasis, as an easily curable STI, is still widespread in the world. Unfortunately, despite the association of the infection with increased risk of HIV acquisition and adverse pregnancy outcomes, little emphasis has been placed on the importance of infection control. A sensitive diagnostic method is one of the main strategies for controlling STIs. Currently, wet mount preparation is the most common method for diagnosis of trichomoniasis and culture method was considered as a gold standard method. The sensitivity of wet mount preparation is limited, up to approximately 70%. In addition to being time consuming, *Trichomonas* culture requires expertise and knowledge, as well as a sufficient number of viable cells. Therefore, it is not widely used. More frustratingly, male trichomoniasis is often overlooked and various aspects of the infection such as duration, clinical consequences, and epidemiological features are largely unknown. Moreover, the licensed screening method for detection of *T. vaginalis* infection in men has not been introduced yet, which could be an important barrier to controlling the infection (4, 10, 16).

According to our knowledge, the present study was the first comprehensive study of male trichomoniasis in Iran, performed independently of women's trichomoniasis status. Although this study was limited to 214 men attending one clinic in Hamadan, a considerable prevalence of trichomoniasis was detected among participants with a positive rate of 4.7%. Sixty percent of infected men were aged ≥49 yr with the mean age of 50.8 yr old. Unexpectedly, the positive results were obtained only based on the PCR method and the para-

sitological methods were resulted negative that is inconsistent with most studies on trichomoniasis in men (7, 15, 17, 18). Although, this finding once again emphasizes that detection of *T. vaginalis* in men is encountered with a diagnostic challenge. The limited studies conducted on male trichomoniasis confirm almost our finding. Sena et al investigated trichomoniasis in 256 male sexual partners of infected women in the United States. The rate of positivity among subjects was various based on specimen type and diagnostic method. A total of 71.7% of the male partners were infected according to assay method (urethral culture, urine culture, and urine PCR). Urine PCR assay was more sensitive than urine culture, with positive rates of 70% and 8%, respectively (10). In this study, 46% of the infected men had clinical evidence of urethritis compared with 80% in the present study. Contrary to our study, the risk of *T. vaginalis* infection in men <40 yr was two to four times higher.

In the United States during 2012–2013, medical records of 2514 men and 3821 women were reviewed who had received nucleic acid amplification tests (NAAT) for detection of trichomoniasis. The prevalence rates of trichomoniasis were 9.8% in men and 27% in women. In this review, the association between infection and older age in men (age >40 yr) was observed (8). In Japan, 313 men were evaluated for trichomoniasis by Seike et al. *T. vaginalis* infection was detected in men with or without urethritis with prevalence rate of 1.4% and 1.0%, respectively. In this study, the relation between trichomoniasis in men and clinical symptoms was not observed (19). In South Africa, during six annual STI surveys from 2007 to 2012, investigated 1218 men and 1232 women. Trichomoniasis was identified in 6.1% of men and in 23.6% of women with the highest prevalence in those aged ≥40 yr (9). The last two studies consistent with our study, indicate older age as a risk factor. However, this relationship was not significant in the present study.

Another molecular investigation of trichomoniasis in men was conducted in Birmingham, Alabama. In this study, 300 urine sample were tested by PCR method of which 52 samples (17%) were positive for *T. vaginalis*. The prevalence of trichomoniasis did not differ significantly from the prevalence of gonorrhea (17.7%) and chlamydial (19.6%) infection (7). The other study was conducted on men attending a Baltimore City Health Department STD clinic from Mar to July 2000. InPouch TV culture and PCR method were used for detection of male trichomoniasis in 506 and 335 men, respectively. Of the forty-seven men who met the criteria for Trichomoniasis, 28% (13/47) and 94% (44/47) were positive for *T. vaginalis* infection by culture and PCR methods, respectively. In this study, the mean age of men with positive and negative *Trichomonas* culture was 38 and 28 yr, respectively, which was significant (P=0.03). The discrepancy may be due to the high parasitic burden on older men (15).

Findings of another study were emphasized multiple specimens for detection of trichomoniasis in men. The sensitivities of urethral swabs and urine culture were the same. In this research, semen was the most sensitive specimen for *Trichomonas* culture, and semen culture alone was positive in 25% of cases (18). Trichomoniasis were studied in 33 men with chronic prostatitis and urethritis. In this research, urine culture failed to identify any infection while urine PCR assay detected 21.2% (7/33) infection among the subjects (20). In this regard, Seo et al. investigated the relationship between male trichomoniasis and prostatitis. Of the 201 urine PCR in men, presenting to a primary care urology clinic in South Korea, 4% (8/201) were positive. Prostatic disease was detected in 87.5% (7/8) of men with *T. vaginalis* infection and the average age of infected men was 52 yr (11). Among the few studies conducted on male trichomoniasis in Iran, we can mention the study conducted in Kashan. In this study, 970 women and 235 men were investigated for trichomoniasis by

wet mount and culture method. In general, a prevalence rate of 2% of trichomoniasis was reported among the participants, without discrimination between sexual groups (21).

Recent parasitological investigations of female trichomoniasis in Hamadan show relatively low prevalence in the region ranging from 0.6%, in pregnant women, to 2.2%, in non-pregnant women (13, 22-24). However, the present epidemiologic study in men indicates a higher prevalence of the infection detected by more sensitive methods. The prevalence rate of trichomoniasis could have been higher if we could evaluate multiple specimens. Another possible implication of the findings is that silent infection may persist in men. This inference is reinforced by the fact that the average age of infected men was over 50 years.

Due to the study limitations, trichomoniasis detection in their sexual partners was not done. Given that people with negative parasitological tests have lower parasite loads, the potential for infection transmission remained unknown in this study. Therefore, further studies need to be conducted to clarify these ambiguities.

Conclusion

This study indicates a considerable prevalence of trichomoniasis in men attending in a urology clinic in Hamadan, higher than that detected by conventional methods. PCR assay, as a sensitive method for detection of male trichomoniasis, can be implemented in clinics, and the sensitivity of NAAT assay should be improved. Inevitably, the development of diagnostic methods should be considered as a necessity in controlling trichomoniasis in community.

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Conflict of interest

The authors declare that there is no conflict of interest.

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