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Original Article

Trichomonas Vaginalis Infection in Men with High-Risk Sexual Behaviors

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Abstract

Background: *Trichomonas vaginalis* is a protozoan pathogen of the human genitourinary tract, which is the cause of trichomoniasis. The disease is one of the most important non-viral sexually transmitted infections in the world. Many studies have been performed on the molecular identification of *Trichomonas* in men in different parts of the world, but in Iran, such a study has been very limited. We aimed to detect *T. vaginalis* in urine of men with unusual sexual behavior by wet mount microscopic observation, culturing in TYM-33 and molecular method in Tehran, Iran.

Methods: Totally, 47 urine samples were collected from men with high-risk sexual behaviors referred to Health Care Centers in South of Tehran, Iran during 2017-2018. After urine centrifugation and wet mount sediment preparation, the samples were cultured in TYM-33 medium and examined microscopically. Then DNA was extracted from urine sediment samples and finally the 18srRNA gene was amplified by PCR.

Results: Thirty-one (65.95%) urine samples were positive for *T. vaginalis* by PCR. Of these, only 6 (12.76%) were detected positive by wet mount method and 25 (53.19%) by culture and successive passages.

Conclusion: The high prevalence of trichomoniasis among men with high-risk sexual behavior in Tehran indicates a potent health risk condition for families. Of course, this percentage is not the real infection rate in ordinary men in Tehran. This situation could be controlled by promoting concurrent diagnosis and treatment of *T. vaginalis* infection in men and women, as well as strengthening religious beliefs and health education in the individuals.



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Introduction

T *richomonas vaginalis* is a flagella protozoan in the urogenital system of men and women. Trichomoniasis is one of the most important non-viral STDs that annually affects a large percentage of the world's population. Clinical signs of this infection in women include vaginal itching, yellowish, green mild discharge, redness and inflammation in the vaginal wall, burning of urine, risk of infertility, susceptibility to cervical cancer (1). During pregnancy, this parasite can cause complications such as preterm labor and low birth weight. The most common way of transmitting this parasite is through sexual intercourse. However, parasites may transmit via the use of toilets in public places, use of underwear in people who do not pay enough attention to health issues.

This infection in men is usually symptomless or in severe cases causes urethritis usually associated with cystitis and prostatitis and epididymitis. The oxidative nature of men's genital fluids and the presence of zinc in the prostate fluid is a cytotoxic agent for this parasite, which is why men are considered carriers of the parasite (2).

Different laboratory methods are used to diagnose *T. vaginalis*. The most important of these are the microscopic examination of the wet-mount, the culture of the parasite and the molecular methods.

Diagnosis by microscopy examination of wet mount is low-sensitivity and parasitic cultures, especially for male specimens, may take up to 5 days. However, methods based on nucleic acid amplification tests (NAATs) are fast and have a sensitivity of 85 to 100% (5–3). Urine, genital tract secretion, and semen samples can be used in molecular methods.

PCR provides a rapid, sensitive, and specific approach for detection of *T. vaginalis*. *T. vaginalis* genes that have been targeted by specific PCR assays include the ferredoxin gene (6, 7), the β -tubulin gene (8, 9), a highly re-

peated 2-kb DNA sequence (10, 11), the 18S ribosomal RNA (rRNA) gene (12), and the adhesion protein gene (13).

In addition to numerous researches on the molecular detection of *Trichomonas* in different regions of the world, several research has been carried out in Iran. The 18s rRNA gene (14, 15) or the ferredoxin and actin genes (16, 17) or the actin gene alone (19, 18), or the P270 gene (20) have been used for either detection of the infection or genotyping the parasite.

However, all this research has been limited to trichomoniasis infection in women. Since, report on *T. vaginalis* infection in men is rare in Iran. Therefore, we aimed to investigate the molecular diagnosis based on the 18S ribosomal RNA (rRNA) gene of *T. vaginalis* isolated of high-risk men and multiple sexual partners in Tehran.

Materials and Methods

Sampling

Totally, 47 sera and urine samples were collected from men with unsafe sexual behaviors referred to Health Care Centers in South of Tehran, Iran during 2017-2018. The individuals were expected trichomoniasis due to having multiple sex with infected women.

Ethical approval

This study was authorized by the Ethics Committee of Tarbiat Modares University, Tehran, Iran. We conducted this study in accordance with the guidelines proposed by Helsinki Declaration.

Wet mount

Immediately after taking 10 ml morning urine samples, the specimen were poured into the test tube and centrifuged at 2000 RPM for 5 min, then the supernatant discarded, and a drop of sediment was put on the slide and ob-

served with 10x and 40x objective magnification.

Culture of *T. vaginalis*

The complete TYM-33 medium was used for culture of the parasite. To prepare the medium, 180 ml of basic medium, 20 ml of serum, 1ml of antibiotic mixture (Penicillin G 20,000 U/ml, Streptomycin sulfate 20 mg/ml and amphotericin B 0.04 mg/ml) added to each tube. The tube store in the refrigerator at 4 °C until used.

The urine precipitates were added into a pre-prepared culture tube in completely sterile conditions and the tube placed in an incubator at 37 °C. The culture medium kept in the incubator for 24–48 hours. If the parasite did not grow, the culture was kept for up to 6 days.

To investigate the growth of the parasite, the culture medium containing the parasite was removed from the incubator and, in completely sterile conditions, with a sterile Pasture pipette about 2-3 ml of the liquid removed and transfer into fresh tube, the tube centrifuged at 250 x g for 10 min, the supernatant discarded and the sediment was mixed and examined microscopically. If growth was poor, the sediment transferred into a fresh pre-prepared culture tube.

In addition, ELISA were run on serum samples for HIV (Pajohan Teb®, Iran). Rapid plasma reagin (RPR) (biorexfarf®, Iran) was performed for *T. pallidum*. TOYO gonorrhea kit (TOYO®, Turkey) was used for detecting *N. gonorrhoea* in urine.

DNA extraction

DNA was extracted from urine sediment samples using GENET BIO kit according to the manufacturer's instructions.

PCR test

A specific primer pair was used to amplify the 18S rRNA gene of *T. vaginalis*. Sequence of the specific primers used (14).

F: TAATGGCAGAATCTTTGGAG and R: GAACTTTAACCGAAGGACTTC

The final reaction volume was 15 µL and consisted of Master Mix (2X Sinaclone, Iran) 7.5 µL, primer (10 picomol) 2 µL, DNA sample 2 µL and distilled water 3.5 µL. The compounds were mixed, then spin down for a few seconds and, according to the schedule, using a thermocycler (Bio-Rad USA). For the first step, initial denaturation was done at 95 °C for 4 minutes, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing was performed at 58 °C for 45 s, extension at 72 °C for 45 s, and final extension at 72 °C for 10 min. The PCR products were electrophoresed in a 1% TBE (Tris base–boric acid–EDTA) agarose gel and stained with DNA safe stain solution (1 µg/ml). The PCR amplification is expected to produce 312 bp amplicons in positive reaction.

Data analysis

SPSS software version 16 (Chicago, IL, USA) were used for statistical analysis of the variables. All data were compared using chi-square test with a 95% confidence level and P value less than or equal to 0.05 statistically significant was considered.

Results

Of the 47 urine samples taken from men who had multiple sex with women in Tehran, 31 (65.95%) were found to be positive by molecularly detection (Table 1). Of these, only 6 (12.76%) were detected by the wet mount method and 25 (53.19%) were positive in parasite culture. The difference between the infection rates in different age groups was not significant. In the PCR assay, as a result of amplification in the 18 s region of the rRNA gene, 31 samples with 312 bp band were found positive (Fig. 1).

Table 1: Frequency distribution of *T. vaginalis* infection by age group and other sexually transmitted infections base on molecular assay in Tehran, Iran

| Age groups (yr) | No. examined | Infected with | | | | | | | |
|-----------------|--------------|------------------------------|-------|--------------|-------|-------------------|------|--------------------|------|
| | | Positive <i>T. vaginalis</i> | | Positive HIV | | Positive Syphilis | | Positive Gonorrhea | |
| | | No. | % | No. | % | No. | % | No. | % |
| 20-30 | 14 | 9 | 64.28 | 3 | 21.42 | 0 | 0 | 0 | 0 |
| 31-40 | 25 | 17 | 68 | 3 | 12 | 2 | 8 | 1 | 4 |
| >40 | 8 | 5 | 62.50 | 1 | 12.5 | 0 | 0 | 0 | 0 |
| Total | 47 | 31 | 65.95 | 7 | 14.89 | 2 | 4.25 | 1 | 2.12 |

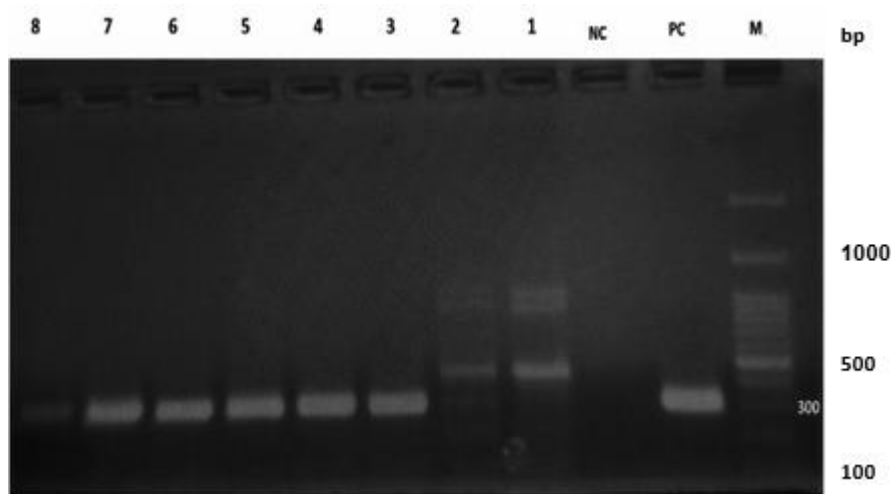


Fig. 1: Electrophoresis of the 18srRNA gene of *T. vaginalis*. M: 100 bp ladder, Pc: positive control, NC: negative control, 1-2 negative and 3 to 8 positive samples: 312 bp 18S rRNA gene bands

Discussion

Epidemiologically, the highest prevalence of trichomoniasis is in individuals who have the most sexual activity, usually between the ages of 16 and 35 years. Economical, social and cultural factors are of secondary importance. Trichomoniasis lacks a cystic stage, usually transmitted during sexual contact. It is also commonly seen in menopause age of women (21).

There have been numerous studies on the prevalence of trichomoniasis in Iran. The prevalence of trichomoniasis in women has been reported between 2 to 8% (22). However, there is no any report of infection rate of

trichomoniasis in men in the country so far. Of course, a man can get the infection from his partner, but this is not always the case. The rate of infection, usually in men was less than women were. Only 71.7% of infected men that have infected partner were diagnosed positive (23). This ratio was reported to be 45% (24). This may be due to the low intensity of parasites and the slow growth of male specimens in the media culture, which usually the growth takes 4 to 5 days. Molecular methods are known as a good assay for measuring infection rate of trichomoniasis in men. In the present study, out of 47 urine samples from men who had sex with multiple women in Tehran, 31 were molecularly diagnosed. Of

these, only 6 (19.35%) were found positive in wet mount and 25 (80.64%) on culture.

In one study, *T. vaginalis* infection was detected in the urine culture of 8% of male partners, whereas in urine PCR they showed 70% infection. By using combination of the results of urethral cultures, urine cultures, and urine PCR, the trichomoniasis was detected in 72% of male partners (2). In terms of age, the highest rate was 68% in the age group of 31 to 40 years. Although the differences between the age group was not significant. In another study (23) in men aged 20-39 and 30-39 years, the risk of *T. vaginalis* infection was 4 times higher than men aged 40 years. However, other studies have reported an association between men age 30 and trichomoniasis (25, 26).

The age group of 31 to 40 years were more likely to have gonorrhea, syphilis, and HIV infection alongside trichomoniasis. Actually, the concomitant STIs are frequently detected in men with trichomoniasis (27), and 10%–13% of women and their male partners with trichomoniasis in Sena et al. (2007) study was coinfecting with *Chlamydia trachomatis* and *Neisseria gonorrhoea* (23).

The important point is that in examining the beliefs of the men under study, they all had a negative view of religious beliefs. In divine religions, having sex with an unmarried, illegitimate, sinful and fornicator is considered adultery. A negative view of religion can lead to illicit unusual sexual behaviors in individuals that may lead to illicit the prevalence of unprotected sexual contact, which is followed by sexually transmitted diseases in the community. Particularly in trichomoniasis infection, which is mostly asymptomatic in men and easily affects wife and other women. Studies in different regions of the world have shown that the prevalence of sexually transmitted diseases is lower in religious populations than in other groups (28-32). Conversely, women with unusual sexual behaviors have higher rates of infection (33-35). Even in difficult conditions of life and poverty, the religious individual main-

tains his decency and avoids the anti-religious practice. Therefore, strengthen people's religious beliefs could be effective ways to prevent this disease.

Conclusion

Men with trichomoniasis could be diagnosed quickly and accurately by PCR. The high prevalence of trichomoniasis among men with unusual sexual behavior in Tehran indicates a potent health risk condition for families. This situation could be controlled by promoting concurrent diagnosis and treatment of *T. vaginalis* infection in men and women, as well as strengthening religious beliefs and health education in the individuals. On the other hand, screening of *T. vaginalis* should be considered for men and women at risk.

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Conflict of interest

The authors of this study confirmed that we have no any kind of conflict of interest.

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