

Infection Rate and Clinical Symptoms of Trichomoniasis among Women Referring to the Hospital in Mahshahr City in Khuzestan Province, Southwest of Iran

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ABSTRACT

Backgrounds: Trichomoniasis is one of the most common sexually transmitted infections in the world. The main aim of the present descriptive study was to determine the prevalence rate and clinical symptoms of trichomoniasis among women referring to the hospital in Mahshahr city in Khuzestan province, southwest of Iran.

Materials & Methods: Urine samples were collected from 2200 women referring to Imam Musa Kazim hospital in Mahshahr city. In addition, 500 Pap smear samples were used for early detection of *Trichomonas vaginalis*. At first, parasitological tests were performed to detect *T. vaginalis* in urine and dissolved Pap smear samples using microscopic examination. Finally, DNA extraction was performed on 34 parasites isolated from positive urine and Pap smear samples. Then the 18s rRNA gene of the parasite was amplified by PCR method. The PCR products of the 18s rRNA gene were finally sequenced.

Findings: The prevalence rate of this parasite was determined to be 1.54%. The highest prevalence rate of infection and clinical symptoms were observed in women aged 31-40 years. Totally, clinical symptoms were observed in 64.70% of infected women, including vaginal itching and irritation (64.70%) and abnormal discharge (26.47%).

Conclusion: The prevalence rate of *Trichomonas* infection was relatively low in women living in Mahshahr. In addition, about 35.29% of infected women were found to be clinically asymptomatic.

Keywords: *Trichomonas vaginalis*, Infection rate, Clinical symptoms, Mahshahr.

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Introduction

Trichomonas vaginalis as a causative agent of trichomoniasis is one of the most common protozoa in the world [1]. The global prevalence of this parasite is estimated to be 5% (4.0–6.4%) among women aged 15 to 49 years [2]. The prevalence and incidence of this infection varies depending on the geographical area and gender of the patients [2]. The prevalence of trichomoniasis among women ranges from 1.9 to 7.8% [2]. The global incidence rate of trichomoniasis is 3.8% (0.8–8.3%) in women and 4% (0.9–9.4%) in men [2]. Studies on trichomoniasis infection rate have been conducted mostly based on microscopic observations; thus, the actual rates are likely to be higher than those reported.

In clinical laboratories, *T. vaginalis* infection is usually diagnosed by microscopic observation of clinical specimens (wet mount or Papanicolaou smear) or by culture and staining with Giemsa to find pathogenic flagella with specific characteristics. The growth of *T. vaginalis* in culture usually takes 3 to 5 days, and microscopic identification of the parasite should be done carefully because *T. vaginalis* trophozoites are similar to those of *T. hominis*, and it is important to identify the two.

Due to the morphological similarity between different *Trichomonas* species and the relatively low sensitivity of the microscopic method [3, 4] and the inability of some *T. vaginalis* isolates to grow in culture [5], serological and molecular techniques have been recommended.

Among the serological methods reported to be useful for the diagnosis of trichomoniasis are ELISA, IFA, and latex agglutination [6–9]. In recent years, the use of polymerase chain reaction (PCR) to diagnose trichomoniasis has been recognized as a rapid, sensitive, and specific approach. *T. vaginalis* genes targeted by specific PCR assays include the

18S ribosomal RNA (rRNA) gene [10], the adhesion protein gene [11], a highly repeated 2-kb DNA sequence [12, 13], the ferredoxin gene [14, 15], and the β -tubulin gene [16, 17].

Clinical symptoms of infection caused by this parasite in women include abnormal vaginal odor; vulvovaginal itching; redness, inflammation, and burning in the vaginal area; and abdominal pain, which may be provoked during menstruation [18]. In addition, this parasite causes preterm labor, low birth weight infants, HIV transmission, and infertility in women [18]. However, in a large number of asymptomatic women and men, the infection is identified only when they refer to diagnostic laboratories for other purposes [1].

Although several studies on the epidemiology of *T. vaginalis* have been carried out in different parts of the country (Iran) [19, 20], no study has been conducted in this field in Mahshahr city so far.

Objectives: The main purpose of this study was to determine the prevalence rate and clinical manifestations of trichomoniasis in infected women in Mahshahr city in Khuzestan province, southwest of Iran. In addition, the relationship between age and clinical symptoms of patients was investigated.

Materials and Methods

Area of study: Mahshahr port is situated in the extreme northeast of Khuzestan province (Iran). Its inhabitants are indigenous and Arabic-speaking, and immigration to this city is less common.

Sampling: In this cross sectional study, a total of 2200 urine samples and 500 Pap smear samples were collected from 22 to 52-year-old women referring to Imam Musa Kazem hospital and private laboratories in Mahshahr, respectively.

Parasitology test: At first, parasitological tests were performed to detect *T. vaginalis* in

urine and dissolved Pap smear samples by centrifugation of the samples at 3000 rpm for 10 min and microscopic examination of the sediment. Then positive samples were stored in the freezer at -20 °C for DNA extraction.

Molecular study: DNA extraction was performed on the specimens using the GENET BIO kit (South Korea) according to the steps recommended by the manufacturer. To amplify the 18s rRNA gene fragment, PCR was performed using a specific pair of *T. vaginalis* primers: TV-F: 5'- TGG ATA GCA GCA GCA ACT CTG - 3'; and TV-R: 5'-ACA AAC TCC TGT CGG CAT CG-3 '.

Amplification was performed in a reaction mixture containing 5 µL of DNA template, 1 µL of each primer (20 pmol), 8 µL of 2x master mixes (Sinaclon, Iran), and 5 µL of distilled water. For each reaction, a positive control (DNA extracted from *T. vaginalis*) and a negative control (dual distilled water) were considered. PCR amplification was conducted under the following thermal cycling conditions: an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 40 s (denaturation), annealing at 60 °C for 30 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 10 min. PCR products were electrophoresed on 1.5% agarose gel stained with safe stain (Sinaclone, Iran) and visualized under ultraviolet trans-illumination.

Nucleotide sequence analysis: Four positive PCR products were sequenced by Sequetech. The sequences were edited with BioEdit sequence alignment editor [21], aligned with *T. vaginalis* partial sequences by ClustalX2.12 [22], and compared with *T. vaginalis* sequences registered in GenBank. Phylogenetic tree was inferred, and evolutionary analyses were conducted using MEGA6 software (<http://www.megasoftware.net/>) by employing neighbour-joining model [23]. The bootstrap scores were calculated for 1000 replicates [23].

Data analysis: All data were analysed and compared using Chi-square test with 95% confidence level using SPSS software Version 16.

Findings

Prevalence rate of *T. vaginalis*: Based on microscopic examination, out of 2200 urine samples and 500 Pap smear samples, 34 (1.54%) and 2 (0.4%) cases were found to be positive, respectively. The frequency of infected women based on age and clinical symptoms is shown in Fig. 1 & 2. The highest prevalence rate of infection was observed in the age group of 31-40 years, which included 55.88% of infected women.

Frequency of symptoms: In general, clinical symptoms were observed in 64.70% of infected patients, of whom 64.70% had only vaginal itching and burning, 26.47% had only unusual discharge, 26.47% had unusual itching, irritation, and discharge, and 35.29% did not have the aforementioned symptoms. The most prevalent symptoms were vaginal itching and burning (32.35%), followed by unusual discharge (14.7%), which were more prevalent in women aged 31-40 years (Fig. 1).

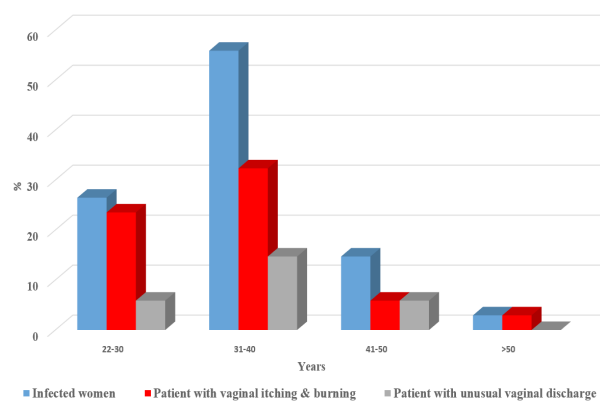


Figure 1) Frequency of *T. vaginalis* in terms of age and clinical symptoms in women living in Mahshahr city.

Molecular analysis: The PCR assay yielded an amplicon of approximately 692 bp for all positive samples (Fig. 3). Nucleotide sequences were submitted to the GenBank database

(GenBank access number KX272849). The results showed that the sequences obtained in this study were 95-100% similar to those of other *T. vaginalis* strains registered in GenBank. The phylogenetic tree showed intraspecific differences between the strains isolated in this study and other *T. vaginalis* specimens (Fig. 4).

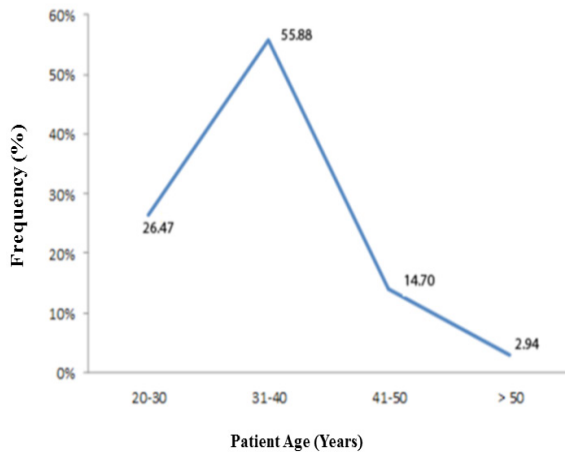


Figure 2) Frequency of *T. vaginalis* in infected women in different age groups in Mahshahr

Discussion

The prevalence rate of *T. vaginalis* infection varies widely in different geographic and demographic regions. Based on PCR analysis, Kim et al. (2016) reported a prevalence rate

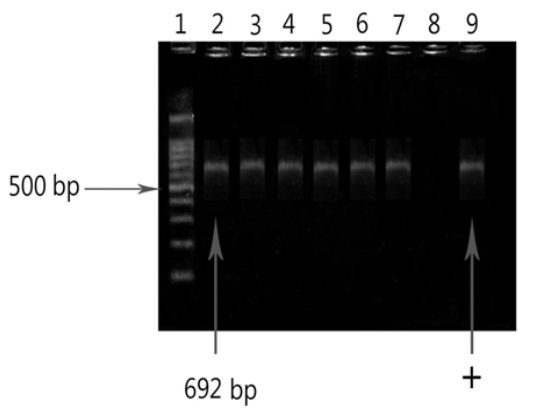


Figure 3) Electrophoresis gel of the 18s rRNA gene of *T. vaginalis* isolated from urine and pap smear samples. Lane 1: 100 kb DNA ladder, Lane 2-7: samples, Lane 8: negative control, and Lane 9: positive control.

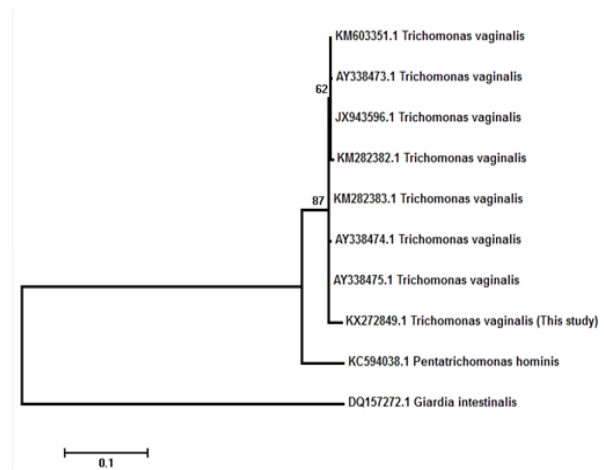


Figure 4) Phylogenetic relationships of 18s rRNA gene of *T. vaginalis*, inferred by neighbor-joining algorithm, in which genotypes of this study were registered with KX272849. (KM603351.1 from China, AY338473.1 from Austria, JX943596.1 from Philippines, KM282382.1 from Philippines, KM282383.1 from Philippines, AY338474.1 from Austria, AY338475.1 from Austria)

of 3.3% for trichomoniasis infection in women in Guri city, South Korea [24]. Kriesel et al. (2015) and Miranda et al. (2014) reported a prevalence rate of 3 and 7.7% for this parasite in the United States [25] and among parturient women aged 15–24 years in Brazil [26], respectively. The prevalence of this infection has been reported to be 9.5% in people at high risk of HIV in Zimbabwe [27], 10.7% in women and 6.3% in men in Moshi urban district, northern Tanzania [28], 42.6% in rural women aged 15 to 45 years in the Eastern Highlands Province of Papua New Guinea [29], and 11.1% in pregnant women in Papua New Guinea [30]. In a study conducted by Miller et al. (2005) in the United States, the overall prevalence of this parasite was reported to be 2.3% in U.S. young adults aged 18-24 years, which was slightly higher among women than men [31]. Spinillo et al. (1997) showed a remarkable prevalence of trichomoniasis infection among postmenopausal women compared to women of childbearing age. The prevalence of *T. vaginalis* infection was 1.92% in women of childbearing age and

10.8% in postmenopausal women [32]. In a study by Sutton et al. (2007), the prevalence of *T. vaginalis* infection among reproductive-age women in the United States was reported to be 3.1% in non-Hispanic white women, 1.3% in Mexican American women, and 13.3% in non-Hispanic black women [33]. On the other hand, the rate of infection is very different among women in different cities of Iran. Rezaeian et al. (2009) reported a prevalence of 2 to 8% for trichomoniasis in Iran [19]. Hezarjaribi et al. (2015) reported a prevalence of 0.8-0.9% in different Iranian populations during 1992 to 2012 [20]. Bafghi et al. (2009) reported the infection rate in Ardakan, Meybod, and Yazd as 5.9, 5, and 2%, respectively [34]. Valdekhani et al. (2010) reported a prevalence of 10.2% for trichomoniasis in Tehran [35]. Hezarjaribi et al. (2012) reported an infection rate of 7.3% in Pap smear samples in Sari city [36]. In other countries, such as the Netherlands, France, the United States, and Brazil, the prevalence of *T. vaginalis* has been reported to be 1.4, 1.7, 0.03, and 7.7%, respectively [37-40]. The difference in the prevalence of this infection may be highly due to cultural and national customs and official religion of Islam in our country [26]. A summary of the prevalence of trichomoniasis among pregnant women in different regions in the world is shown in Table 1 [41].

Table 1) Mean prevalence of *T. vaginalis* among pregnant women in different regions during 2010–2015

Regions	Tested (No.)	Mean Prevalence% (95% CI)
Asia	85	13.6 (6.8–20.4)
Central and South America	2201	3.9 (2.2–5.6)
Southern Africa	4,861	24.6 (17.9–31.4)
Eastern Africa	3,688	6.8 (4.6–9.0)

Although some researchers have attempted to link the infection to the black race [42], this infection, like other parasitic diseases, is directly associated with the poverty of the community, and race has no direct effect on the prevalence of this parasite. The most important predisposing factors for *T. vaginalis* infection are poverty, social exclusion, age, imprisonment, injecting drug use, prostitution, misdemeanor [43], as well as vaginal microbial infection [44]. The infection with this parasite is completely related to age and sexual contact. This parasite is mainly transmitted by adults with sexually transmitted diseases, although some non-adult or non-sexually transmitted cases have been reported by some investigators [45]. The most important clinical symptoms reported in patients with *T. vaginalis* infection are abdominal pain, vaginal discharge with an unpleasant smell, vaginal itching and burning, dyspareunia during sexual intercourse, and dysuria during urination [45, 46]. The cervicitis may be asymptomatic or in the form of purulent or bloody secretions [47]. In the present study, the most commonly observed symptoms were vaginal itching, irritation, and discharge. In general, clinical symptoms were observed in 64.70% of infected women, of whom 64.6% had only vaginal itching and burning, 26.47% had only unusual discharge, 26.47% had unusual vaginal itching, burning, and discharge, and 35.29% did not have the aforementioned symptoms.

Conclusion

The prevalence rate of *Trichomonas* infection was relatively low in women living in Mahshahr, and about 35.29% of infected women were clinically asymptomatic.

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Ethical Permissions: This study was authorized by the Ethics Committee of Tarbiat Modares University of Medical Sciences, Tehran, Iran with the code No. IR.TMU.REC.1394.201. This study was conducted in accordance with the guidelines proposed in the Helsinki Declaration.

Conflicts of interest: The authors declare no potential conflicts of interests with respect to the research, authorship, and/or publication of this paper.

Authors Contribution: Conceptualization: DA; data curation: DA, ME; formal analysis: DA; funding acquisition: DA; Investigation: ME; methodology: DA, ME ; project administration: DA; resources: DA; supervision: DA, PM; writing of the original draft: DA; writing-review and editing: DA, SJ and PM.

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