



Frequency and molecular diagnosis of trichomoniasis in symptomatic women referred to laboratories in urmia north west Iran

Elham Shahnazi¹, Habib Mohammadzadeh¹, Chimam Daneshyar¹, Alireza Chavshin², Shahram Khademvatan¹✉

¹Cellular and Molecular Research Center & Department of Medical Parasitology and Mycology, Urmia University of Medical Sciences, Urmia Iran

²Department of Medical Entomology, School of Public Health, Urmia University of Medical Sciences, Urmia, Iran

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ABSTRACT

Objective: To obtain the frequency of trichomoniasis and molecular diagnosis among women referred to a laboratory in Urmia (north west Iran). **Methods:** In the cross sectional study during the year 2014-2015, women with complaints of abnormal discharge, itching, irritation and unpleasant odor were studied using convenience sampling. These people were referred to one of the laboratories in city by gynecologist. After preparing the checklist of underlying data, vaginal wet smear samples was prepared to direct microscopy and molecular assess. Results were analyzed with statistical tests. **Results:** Totally 39.2% of referred cases were 30-40 years old, and about 54.8% of referred were illiterate and primitive. The frequency of trichomoniasis was 11.1% and statistically there was no significant difference. There was no significant difference among the age, education level, method of contraception, marriage age, location, employment status and the number of delivery with *T. vaginalis*. **Conclusion:** *T. vaginalis* compared to other studies have a moderate frequency among women of Urmia. Because of the concordance of this infection with other sexual transmitted diseases, it is recommended further studies about the prevalence of trichomoniasis in Urmia.

1. Introduction

Vaginitis is one of the common diseases of women that had high health implications degrees and needs health care. Every year about 10 million health center handle to the complaints related to vaginal discharge. Vaginitis is usually caused by the development of a bacterial vaginitis disease, vulvovaginal candidiasis and trichomoniasis. However, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are other causes of vaginitis. Trichomoniasis is caused

by the protozoan parasite *Trichomonas vaginalis* (*T. vaginalis*) and it is one of the most common non-viral disease transmitted through sexual contacts [1-3]. The World Health Organization (WHO) has estimated the prevalence of trichomoniasis to 174 million that is the agent of 10 to 25% of vaginal infections [4]. The prevalence of disease in different parts of the world ranging from zero to 34% have been reported. *T. vaginalis* is a flagellated protozoan which is transmitted through sexual contact. This is also the only type of pathogenic parasites in humans. The parasite grows better in wet environment and PH between 5-6 and temperature 35 to 37 degrees celsius and if this condition is more or less than an optimal level, the organism is destroyed. Women are often involved in the vagina and urethra, but may infect the cervix, bartholin glands, scan or bladder. In men, the organism is often present in the distal urethra, but rarely

✉Corresponding author: Cellular and Molecular Research Center& Department of Medical Parasitology and Mycology, Urmia University Of Medical Sciences,Urmia, Iran
Email: khademvatan@yahoo.com , khademvatan@gmail.com

prostate gland, seminal vesicle and epididymis is also involved [5-7]. Between 50 to 75 percent of infected people are asymptomatic. The most common symptoms include itching of the vulva, increased stinks secretion to the color between green-yellow, redness, vaginitis, urinary irritation and pain in the lower abdomen. The severity of these symptom rises during pregnancy and menstruation [8-9].

Trichomoniasis is associated with premature delivery, infection after hysterectomy, cervical cancer, atypical pelvic inflammatory disease and infertility. It also increases susceptibility to AIDS infections [10]. The carriers cause the spread of disease and from epidemiologic aspects they are extraordinary important. The parasite in women is visible in urinary sediment, vaginal discharge or vaginal chips and in men is remarkable in urine and prostatic secretions. The most common way to diagnose is a direct test, for the detection by vaginal secretions used a swab sample and observe directly with the microscope to the active and ambulant trophozoite. To stain the vaginal prepared smear it can be used Giemsa staining, trichrome, Papa Nicolau and used acridine orange. also many studies have been done to identify the parasite using molecular methods, especially PCR [11]. In these methods parasites have been detected with different genes such as ITS1, ITS2, Actin, rRNA, etc. Also different primers on the subject have been published which they are reported with different sensitivity and specificity in the detection of *T. vaginalis* [11-20].

To investigate the interspecies molecular differences and polymorphism of these parasite methods is used such as PCR-RFLP, Microsatellite, RAPD-PCR and sequencing and Single Stranded Conformation Polymorphism-PCR (SSCP-PCR). In most studies of genetic diversity, most of two different genetic patterns for *T. vaginalis* is obtained which probably involved in creating or inability to cause clinical manifestations and pathogenesis of the parasite [21-23].

Some studies have investigated the association between the polymorphism and interspecies variation of *T. vaginalis* with clinical symptoms. Also to find the cause of the diversity of different clinical symptoms (from asymptomatic to severe symptoms) and treatment response and resistance to metronidazole in patients with trichomoniasis in addition to the genetic diversity of parasites, cases such the genetic diversity of the people have been investigated [23-25].

Since the detection of Trichomoniasis is a warning to the other sexually transmitted diseases that can subsequently existed and also for those infected have no clinical symptoms who acted as a carrier, and causing the disease to spread, as a result of epidemiologically feature it has a particular importance, so study the genetic diversity of strains and *T. vaginalis* in patients with molecular methods in relation to sexually transmitted diseases is needed [26-27]. The aim of this study, which takes place for the first time among patients in

this region, is assess the frequency of *T. vaginalis* and molecular diagnosis in women with vaginitis symptoms referred to health centers in Urmia in 2014-2015.

2. Materials and methods

2.1. Ethics Statement

All human participants have been obtained in conformity with informed consent, privacy and confidentiality of patients who were sampled and analyzed anonymously during study.

2.2. Sampling and culturing

In this cross-sectional study in year 2014 to year 2015, women with symptoms of genital infection, discharge, itching, irritation and odor in the city of Urmia were referred to a gynecologist were enrolled. The patients following the medical examinations and history taken by a gynecologist if vaginal infection is suspected to order laboratory tests were referred to the laboratory. For all patients a questionnaire includes information on age, location, education, age at marriage, employment, parity, contraception, etc. were completed. The patients were sampled with speculum. For the detection of *T. vaginalis*, the wet smear method was used. Then, positive samples subjected to axenic culture using Diamond's medium (TYI-S-33), previously described by Clark and Diamond [12]. The cultured parasites in TYI-S-33 medium in logarithmic phases were harvested and after washing with PBS (pH 7.4) stored in -20 °C.

2.3. DNA extraction and PCR amplification

Genomic DNA from 42 isolates was extracted using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. The samples were first subjected to three freeze-thaw cycles, and proteinase K digestion. DNA concentration was measured spectrophotometrically at 260 nm. For molecular diagnosis, A pair of oligonucleotide PCR primers were chosen from the conserved ITS1/5.8s/ITS2 region of *T. vaginalis* (GenBank accession No. TVU86613): ITS-S (5' CGG TAG GTG AAC CTG CCG TTG G 3') and ITS-As (5' AGT TCA GCG GGT CTT CCT GCG 3'). The amplification reactions were performed in 25 µL reaction mixtures containing 250 µM of deoxynucleotide, 100 pmol of each primer, 50 mM KCl, 10 mM-Tris-HCl (pH 9), 3 mM MgCl₂, 10% dimethyl sulfoxide (DMSO, Sigma), 2 U of Taq DNA polymerase (Fermentas), and 10-15 ng of template DNA. Amplifications were

performed under the following conditions in MyCycler; Bio-Rad, Hercules, CA: initial denaturation at 94 °C, 5 min, followed by 35 cycles in series of denaturation at 94 °C, 30 sec, annealing at 65 °C, 30 sec, extension at 72 °C, 45 sec, and one final extension step at 72 °C for 10 min. Electrophoresis performed by adding 5 µL of the PCR products to a 2% (w/w) agarose gel and stained with safe stain for 45 min at 100 V. Bands visualized by ultraviolet transillumination (BioSystematica, Devon,UK).

2.4. Statistical analysis

Data were analyzed using *Chi-square* test, and *t*-test. The *P* value less than 0.05 was used as significant. Statistical analyses were carried out using SPSS software, version 16.

3. Results

In this study, 11.1% of patients ($n = 39$) were infected with *T. vaginalis*, most women were in the age group of 30 to 40 years (39.2%), respectively. The average age of the clients was 35.00 ± 8.44 years. The contamination rate highest percent was belongs in the age group 30 to 40 years (43.6%), and then in the age group 20 to 30 years (30.8%), respectively.

Statistical analysis showed that there is a significant relationship between age and prevalence of infection. Demographic characteristics, residence, and marital status, level of education, ethnicity and age) of the patient and control groups are shown in Table 1.

Table 1

Frequency of trichomoniasis in different age groups.

Age (years)	Negative n(%)	Positive n(%)	Total n(%)	<i>P</i> value
≥20	5(1.6)	1(2.6)	6(1.7)	<i>P</i> <0.05, <i>r</i> =0.47
21-30	102(32.6)	12(30.8)	114(32.4)	
31-40	121(38.7)	17(43.6)	138(39.2)	
41-50	77(24.6)	6(15.4)	83(23.6)	
≤50	8(2.6)	3(7.7)	11(3.1)	
Total	313(100.0)	39(100.0)	352(100.0)	

Most women referred in terms of education, 37.1 percent were high school graduates and in terms of the most contaminated by pollution were under diploma (37.8%), respectively. There is no significant relationship between women's education and the *T. vaginalis* infection (*P*>0.05).

Table 2

Education level and *T. vaginalis* infection.

Education	Negative	Positive	Total	<i>P</i> value
	n(%)	n(%)	n(%)	
Illiterate	68(21.6)	12(29.7)	80(22.5)	<i>P</i> >0.05
Primitive	104(33.2)	14(37.8)	118(33.7)	
Diploma	119(38.0)	12(29.7)	131(37.1)	
Bechelor and higher	22(7.2)	1(2.7)	23(6.7)	
Total	313(100.0)	39(100.0)	352(100.0)	

The result showed that among the 352 women participating in the study, 72 patients (20%) had a history of abortion from 1 to 4 times. The rate of infection in people who have had abortions was 27 (37.5%). Between abortion and *T. vaginalis* infection there is a significant relationship (*P*<0.05, *r*=0.43) (Table 3).

Table 3

Abortion history and *T. vaginalis* infection.

Abortion	Negative	Positive	Total	<i>P</i> value
	n(%)	n(%)	n(%)	
0	269(85.9)	12(30.8)	281(79.8)	<i>P</i> <0.05, <i>r</i> =0.43
1	21(6.7)	12(30.8)	33(9.4)	
2	22(7.0)	15(38.5)	37(10.5)	
4	1(0.3)	0(0.0)	1(0.3)	
Total	313(100.0)	39(100.0)	352(100.0)	

Results show that the odds ratio of infection in people who have had abortions was 13 times higher than people who did not have a history of abortion.

In terms of employment of spouse, the highest frequency of the women was whose husbands were self-employed (36.8%) and then drivers (23.7 %), respectively. The results of this study showed a significant relationship between the prevalence of infection in women and their husband's job (Table 4).

Table 4

Frequency of infection in women and their husband's job.

Groups	Negative	Positive	Total	<i>P</i> value
	n(%)	n(%)	n(%)	
Working	67 (21.4)	7(18.4)	74(21.0)	<i>P</i> >0.05
Employee	69(22.1)	3(7.9)	72(20.4)	
Driver	15(4.8)	9(23.7)	24(7.0)	
Self-employed (azad)	130(41.4)	14(36.8)	144(40.9)	
Farmer	18(5.9)	3(7.9)	21(6.1)	
Unemployed	14(4.5)	2(5.3)	16(4.6)	
Total	313(100.0)	39(100.0)	352(100.0)	

Most women referred (71.2%) had no history of previous symptoms and the prevalence of infection in women who have had a history of symptoms of more than one year (44.7%), respectively. The results of this study showed a significant relationship between women and the prevalence of a history of previous symptoms (*P*<0.05, *r*=0.60) (Table 5).

Most of the complaints referred to the women's section were burning and itching (44.6%), and the highest of abundance

contamination was in the group with abnormal discharge (89.7%). The results of this study showed a significant relationship between frequency of disease and symptoms ($P < 0.05$; $r = 0.52$) (Table 6).

In the term of symptom intensity in patients with test results showed that 84.6% of patients complained of severe symptoms. The results of this study showed a significant relationship between women and the frequency of the severity of symptoms ($P < 0.05$; $r = 0.53$).

The results showed there was no significant difference between the frequency of the disease and number of children, marital status and length of marriage.

With statistical considerations, significant differences in level of education, location, parity, employment, marriage time and infection were significant (Table 7).

Forty samples isolated from infected persons were examined by PCR analysis of the conserved ITS1/5.8s/ITS2 region of *T. vaginalis* gene. The fragment size of PCR amplicons was 300 bp.

Table 5

Relationship between *T. vaginalis* infection and history of previous symptoms.

Group	Negative n(%)	Positive n(%)	Total n(%)	P value
No	248(79.3)	3(7.9)	251(71.2)	$P < 0.05$, $r = 0.60$
Less than a year	35(11.0)	6(15.8)	41(11.6)	
One year	20(6.4)	12(31.6)	32(9.2)	
More than a year	10(3.3)	18(44.7)	28(8.0)	
Total	313(100.0)	39(100.0)	352(100.0)	

Table 7

Relationship between *T. vaginalis* infection, marriage status and number of children.

Parameters	Negative n(%)	Positive n(%)	Total n(%)	Value
No. of children	0	5(1.7)	1(2.6)	Value = 7.167 Sig. = 0.306
	1	60(19.3)	5(13.2)	
	2	118(37.5)	18(44.7)	
	3	71(22.6)	7(18.4)	
	4	33(10.6)	2(5.3)	
	5	26(8.3)	6(15.8)	
Marriage	Married	312(99.7)	38(97.4)	Value = 3.093 Sig. = 0.079
	Divorced	1(0.3)	1(2.6)	
Age of marriage	10-20 years	252(80.9)	30(79.4)	Value = 13.889 Sig. = 0.458
	20-30 years	60(19.1)	8(20.6)	
	Illiterate	68(21.6)	12(29.7)	
	Primitive	104(33.2)	14(37.8)	
	Diploma	119(38.0)	12(29.7)	
	Bachelor and higher	22(7.2)	1(2.7)	
	Total	313(100.0%)	39(100.0)	

Table 6

Relationship between *T. vaginalis* infection and symptoms.

Symptoms	Negative n(%)	Positive n(%)	Total n(%)	P value
Irritation	11(3.5)	0(0)	11(3.5)	$P < 0.05$, $r = 0.53$
Itching	93(29.7)	0(0)	93(29.7)	
Discharge	91(17.9)	35(89.7)	91(25.9)	
Irritation and itching	153(48.9)	4(10.3)	157(44.6)	
Total	39(100.0)	313(100.0)	352(100.0)	

4. Discussion

More than one hundred different types of Trichomonas species has been reported that *T. vaginalis* is the most common and the only known pathogenic species in humans. Trichomoniasis, is a protozoan infection of the urogenital tract in men and women usually occurs during their reproductive years. The disease categories as an emerging sexual disease which its association with HIV infection.

Depending on various factors, prevalence of trichomoniasis is highly variable in research conducted in the parts of Iran. In the present study, the prevalence of *T. vaginalis* infection was found higher than the previous studies in Iran (11.1 %), such as in nonpregnant women referred to health centers 9.2% in Tabriz (28), 4.8% in Zanjan [29] and in Tehran 3.2% [30].

Compare to another part of world, the prevalence of trichomoniasis in Iran is low i.e. in southern Mozambique [31], Palestine [32], and USA [33] were reported 31%, 28.1%, and 13%, respectively

which may be due to different factors, such as hygiene behaviors, socio-demographic characteristics, religious beliefs that prohibit nonmarital sex and universal male circumcision in Iran.

In Gaza City, on any study of 430 pregnant women between the years 2000-2006 was evaluated and results showed that the frequency of trichomoniasis and level of education, age, working front of a housewife, location, number of delivery age at marriage has no significant relationship.

The study found that the trichomoniasis and level of education, age, location, parity, age at marriage, there is no significant difference and results are in concordance with similar studies [11,18-20].

In relation to age and level of infection current study suggests that despite the lack of significant findings in terms of the prevalence of different ages in the age range 25-34 years age group is more than the infection. This probably due to exposure to this group as a sexually active age and PH changes there is conducive growing conditions for *T. vaginalis*.

In terms of jobs, although the difference between being an employed and being a homemaker was not found with the infection, but percentage of contamination among employed women were more than homemakers, which is probably due to employed person who have less opportunity to handle the health conditions the chances of contracting the disease increases. Among women literate and illiterate despite absence of significant difference in infection rate, the percentage of illiterate people is nearly twice than elementary and middle and it was 5 times more than cases high schools or higher.

This finding is consistent with results by Mazlumi in Tabriz, which was done on 100 non-pregnant women is Our results are contrary to the Mazlumii et al. in Tabriz 2008, which was done on 100 non-pregnant women.[21]. Differences in sample size could be possible reasons for this difference. Due to the use of contraception methods, most commonly used method of using was IUD (32.9 percent) among people that Mazlumi studying. However, between the use of different techniques pregnancy contraception and Infection there was no statistic correlation. In the study done by Sehhati in Tabriz, is also most widely method of contraception related to IUD (33.3 percent).

According to the results of this study, there was no significant difference so it seems that the use of condoms as the safest method of infection is trichomoniasis. Research conducted in Colorado had shown that in women whose partners use condoms to avoid pregnancy, compared to other women does not use condoms are less than 20 percent infected with trichomoniasis [22].

5. Conclusion

Overall by taking frequency of the disease in the city of Urmia (Northwest Iran) and according to individual and social consequences and accompanied by infection with other sexually transmitted disease(STD) seems essential that further studies will done on the prevalence of microorganisms that cause vaginitis. Also in the planning of the health sector its need a special attention to women's health and particularly in terms of the reproductive system diseases, to increase health services and individual health education among them and be able have seen a reduction to such diseases. Some studies have examined the association between the polymorphism and variation within species of *T. vaginalis* with clinical symptoms.

Also for the cause of a variety of different clinical symptoms (from asymptomatic to severe symptomatic) Also there were examined treatment response and resistance to metronidazole in patients with trichomoniasis in addition to the genetic diversity of parasites such as genetic diversity [23-25].

Since the detection of trichomoniasis is a warning to other sexually transmitted diseases, there can be subsequently and for those infected have no clinical signs who acted as a carrier and the spread of their disease, epidemiologically have a particular importance. So study the genetic diversity of strains and *T. vaginalis* in patients with molecular methods in relation to sexually transmitted diseases needed.

Declare of interest statement

We declare that we have no conflict of interest

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