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Original paper

Screening and molecular characterization of *Trichomonas vaginalis* genotypes isolated from married women in northern Iran

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ABSTRACT. *Trichomonas vaginalis* is an anaerobic protozoan parasite that causes trichomonosis in human. It is one of the most common non-viral sexually transmitted infections. It has been found to be most prevalent in patients referred to sexually transmitted disease clinics. In recent years, molecular methods have been used to identify genotypes of this parasite in different parts of the world and so far 6 types of *T. vaginalis* have identified. The aim of this study was to investigate the prevalence and genotype identification of *T. vaginalis* from married women in northern Iran. A total of 450 vaginal specimens were taken from married women, referring to health centers in northern Iran. Demographic information of women was collected through a questionnaire. The samples were first examined microscopically and then monitored in Dorsch culture medium for up to 10 days. Actin genes of positive samples were amplified by PCR. Finally, PCR products were used to determine the sequence and genotype of the parasite. Overall, 0.7% (3/450) samples were positive for *T. vaginalis*. All of the three infected women were housewives. After sequencing, the genotype of these parasites were type H (66.7%) (Accession no; MW414672-MW414673) and type E (33.3%) (Accession no; MW414671). Low prevalence of *T. vaginalis* in north of Iran indicate high level of hygiene in sexual intercourse and avoiding from high risk sexual behaviors, and also it seems that genotype H is dominant type of the parasite in the study area.

Keywords: *Trichomonas vaginalis*, women, genotype, actin gene, Iran

Introduction

Trichomonas vaginalis (*T. vaginalis*) is an anaerobic flagellate protozoan that causes trichomonosis. It is one of the most common non-viral sexually transmitted disease (STD) [1,2]. According to World Health Organization (WHO) reports, about 170 million people are infected with this infection annually, which varies according to the socio-cultural status of communities. The average prevalence of this infection has estimated at 5–20% [3,4]. The clinical manifestations of this

infection ranges from asymptomatic to severe purulent infection. Clinical symptoms are different in men and women. Clinical manifestations of trichomonosis occur in 50–90% of infected women, including inflammation, burning, itching, profuse foamy, and foul-smelling discharge. In chronic cases, the severity of symptoms is reduced that is important in terms of the spread of infection in communities and these people are considered as carriers of the parasite [1–3]. In most infected women, cervical cells abrasions are seen, which sets the stage for cervical carcinoma [5]. Trichomonosis is also causing low birth weight and premature births. Infection with this protozoan parasite is contributed to the transmission of HIV, HPV, HSV2, hepatitis virus, and *Mycoplasma hominis*, and increases the incidence of cervical cancer and infertility [1,6]. Infection in men is often asymptomatic or mild. Sometimes symptoms such as urethral itching, discharge or burning appear, in which case urination and painful intercourse become painful, as well as complications such as prostatitis, non-gonococcal urethritis, epididymitis, balanoposthitis and sometimes leads to reversible infertility in men [7–10].

Identification of *T. vaginalis* genotypes leads us to a deeper understanding of the biology and epidemiology of this parasite. In recent years, several studies have been performed to identify *T. vaginalis* genotypes in different parts of the world, for example, studies of Spotin et al. in Tabriz [11], Demirag et al. in Turkey [12], Zhang et al. in China [13], Khalili et al. in Shahrekord [14], Moradi et al. in Mahshahr [15], Chety et al. in South Africa [16]. According to studies, using the actin *T. vaginalis* gene can identify at least 6 genotypes, including E, G, H, I, M, and N.

Due to the lack of accurate information about the prevalence of this disease and also with the increasing of HIV in today's society, we decided to study the prevalence and subtype identification of *T. vaginalis* in Rudbar city located in north of Iran.

Materials and Methods

Study area, population, sample size, and ethics

This study was done in Rudbar county located in west of Gilan province. The county has three cities, including, Rudbar, Manjil, and Rostam abad. In total, 450 non-pregnant women who have been referred to the health centers in order to gynecological examinations and treatment or periodic examinations during September 2016 to March 2017 participated in this study and sampling was done randomly. Ethical approval for the present study was duly obtained from and approved by the ethics committee of Qazvin University of Medical Sciences with ethics code IR.QUMS.REC.1397.360.

Culture study

After obtaining informed consent, questionnaire data were collected, and then some vaginal secretions were sampled from the posterior fornix of the vagina by using two sterile swabs after placing the speculum. A swab was placed in a tube containing 0.5cc of Ringer and examined immediately under a light microscope. The second swab was placed in the dorsal culture medium and kept in an incubator at 37°C. After 24, 48, 72 hours, and 1 week incubation, the suspected samples were examined microscopically. The positive samples were stored in a –20°C freezer until molecular tests were performed.

Molecular study

In this study, parasite DNA was extracted by two commercial kits, including DNG-plus and QIAGEN based on the instructions of the kits. To determine the molecular genotype of *T. vaginalis*, PCR was used by actin gene amplification (ACTIN) [17]. A part of *T. vaginalis* gene was amplified and analyzed using a pair of TV1R and TV1F specific primers:
TV1R: (5'-CAGACACTCGTTATCG-3');
TV1F: (5'-CGGTGAACGATGGATG-3')

The PCR reaction was performed at a final volume of 20 µl containing 1 µl of template DNA (template cDNA), 2 µl of reciprocating primers, 10 µl of master mix and 7 µl of distilled water. Applied Biosystems thermocycler (USA) was used for PCR. The first stage of the denaturation process started at 95°C for 5 minutes, followed by 35 cycles at 95°C for 30 seconds, the initial annealing process at 60°C for 30 seconds, expansion (extension) was performed at 72°C for 30 seconds and the final elongation step was performed at 72°C for 5 minutes.

After PCR reaction, electrophoresis on agarose gel 1.5% was used to evaluate the amplification of the fragment. Finally, the PCR products were imaged under UV light using a luminaire. In order to determine the sequence of *T. vaginalis* nucleotides isolated from patients' vaginal samples, the PCR products were combined with primers and

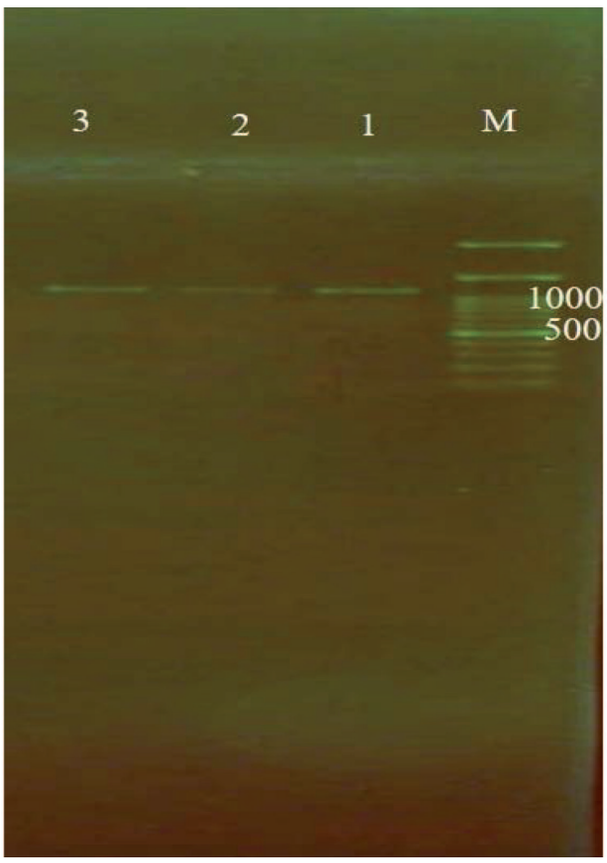

Figure 1. PCR product based on actin gene. Column M: Molecular marker; Column 1–3: Positive examples of *T. vaginalis* with 1100 games

sent to Bioneer, South Korea, for bilateral sequencing by Sanger dideoxy method. The received sequences along with their chromatograms were checked and sorted using Bio edit software. Sequences were evaluated using BLAST software and the parasite species was identified with a percentage of similarity and overlap with the species registered in the gene bank. MEGA6 software with Kimura2-parameter model and Maximum Likelihood algorithm with 1000 replication for actin gene were used to draw the phylogenetic tree.

Data analysis

Data were presented as frequency tables, graphs and numerical indicators. Chi-square test and Fisher's exact test were used to investigate the relationship between qualitative traits. A level of <0.5% was considered as significance. SPSS software version 16 was used for data analysis.

Results

Demographic data

Overall, 73.8%, 14.9%, and 11.3% of the participating women were from Rostamabad, Manjil, and Rudbar, respectively. Up to 90.4% of the women were housewives. The lowest and highest number of participants in the project were in age group of 10–20 and 31–40 years, respectively. Education levels of the subjects were 85.1% non-academic and 14.9% had academic education, 42.7% (n=192/450) of patients had one or more symptoms of burning, itching, inflammation, frequent urination and yellow or white discharge. Highest and lowest of contraception methods were naturally prevented pregnancy (44.2%) and ampoule method (2%), respectively. There were 366 women (81.3%) without a history of abortion and 84 women (18.7%) with a history of abortion.

Findings

Only 0.7% of participants (n=3/450) were positive for *T. vaginalis* infection, who were residents of Manjil (2 cases) and Rostamabad (1 case). All of the three positive cases were housewives. Age groups of the cases were 41–50 years (2 cases) and over 51 years (1 case). Education level of them were illiterate (1 case), undergraduate (1 case), and bachelor's degree (1 case). All the three people had symptoms of burning, itching, and discharge, but only one had inflammation and only one had frequent urination. Pregnancy prevention measures were natural preventive (1 case) and tubectomy (1 case). The third patient was postmenopausal women. WBC was observed in discharge samples of 71.6% (n=322) of participants. Only one trichomonosis patient was positive for WBC. RBC was positive in 1.3% (n=6) of participants, and 22.9% (n=103) of them were positive for bacterial infections in discharges. All of the three trichomonosis patients were negative for RBC and bacterial infections.

In PCR test, the fragment size for *T. vaginalis* was 1100 bp. The results of gel electrophoresis of the amplified DNA fragment of the actin gene are shown in figure 1.

After sequencing the PCR products, the genetic diversity of *T. vaginalis* isolates were registered in the gene database. The genotypes of this parasite were identified as H (2 cases) and E (1 case). This isolates were recorded in the NCBI gene database.

The phylogenetic tree of *T. vaginalis* sequenced

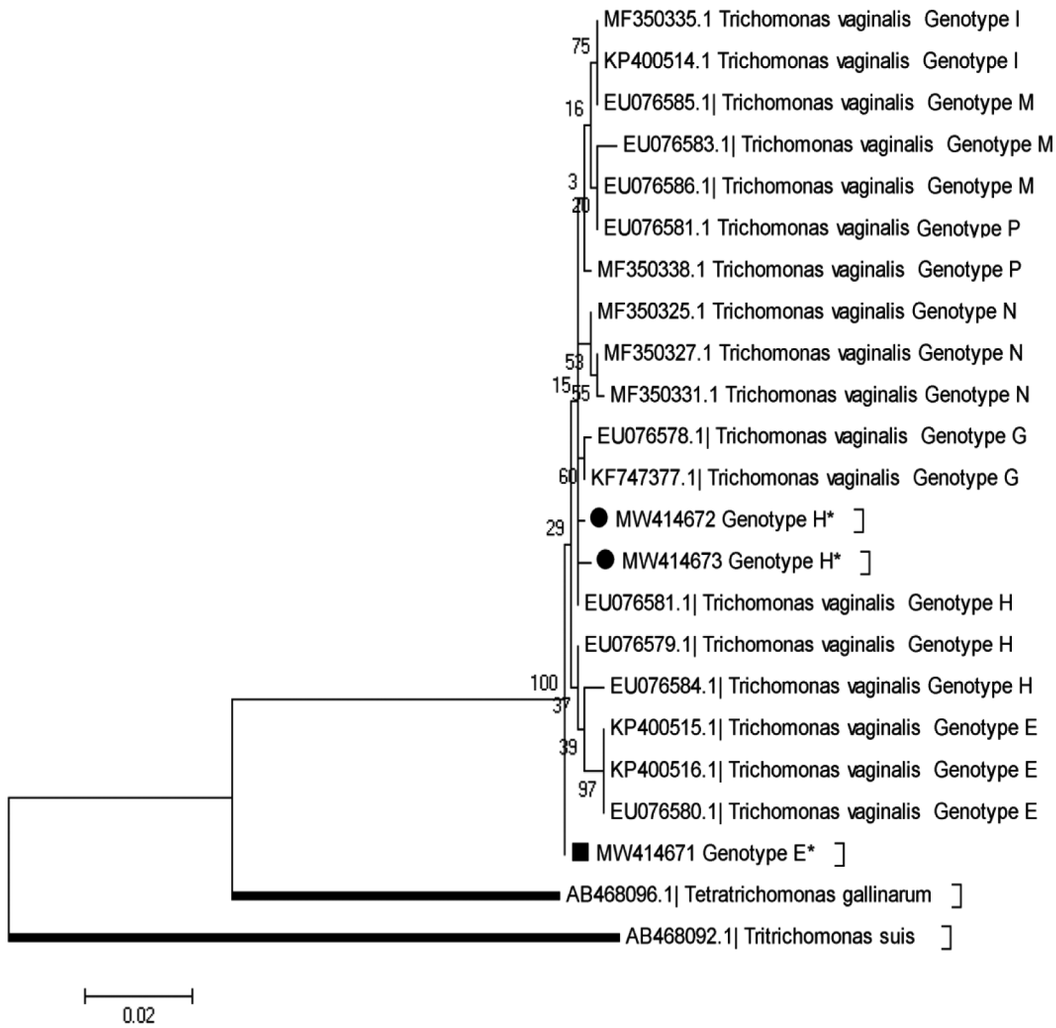

Figure 2. Phylogenetic tree of actin gene for the sequences obtained from *Trichomonas vaginalis* studied in this study and its comparison with the sequences recorded in the gene bank. In this form, *Trichomonas suis* and *Tetratrichomonas gallinarum* were used as an ex-group species for internal control of the tree. The distance scale as an indicator of nucleotide changes was estimated to be 0.02

isolates were demonstrated in figure 2. Based on the phylogenetic tree of the actin gene, two specimens of *T. vaginalis* in this study (identified by the geometric shapes placed next to them) belong to genotype H (Accession no; MW414672-MW414673) and the other isolate also belong to genotype E (Accession no; MW414671), which are located in their specific position next to the reference genes.

Discussion

The present study showed a very low prevalence of *T. vaginalis* among women referred to health centers. This finding is probably related to the socio-religious status in the study area and transmission route of the parasite. The main transmission route of *T. vaginalis* is sexual intercourse, thus, having multiple sexual partners increase the risk for this parasitic infection [18]. This sexual misconduct has religious restrictions among Iranian due to religious beliefs and social considerations. The overwhelming majority of Iranian are Muslims who are religiously allowed to have sexual intercourse only with legal partners, however, men are allowed to have up to four wives at the same time. But, having more than one wife is very rare among Iranian Muslims, especially Shiites, who make up the majority of Iran's population. Kakaire et al. [19] reported being of muslim faith was associated with reduced risk of STI. On the other hand, sexual intercourse with other partners is religiously forbidden in religion of

islam. However, sexual intercourse contrary to Islamic precepts may occur among people who are no or less committed to religious precepts. It seems that such sexual misconduct is not common in the study area.

The epidemiological studies show that the prevalence of *T. vaginalis* has considerably decreased in Iranian women during recent years. In Tehran, 33.8% prevalence in 1990 [20] decreased to 3% in 2002 [21], and 0.4% in 2016 [20]. Also, in Hamedan, the prevalence of the parasite from 3% in 2004, decreased to 2.2% in 2007 [22], 1.7% in 2011 [23] and 0.6% in 2015 [24]. There is no report for prevalence of *T. vaginalis* in Rudbar city, but in comparison with prevalence of 15.3% in Lahijan city located in Gilan province in 2003–2004 [25], it seems that the prevalence of the parasite has remarkably decreased in this province in the recent years.

The decrease of *T. vaginalis* in the area of study and other regions of Iran may be also related to promotion of public health cares (PHC) in Iran. In this country, one of the most basic measures to promote PHC were establishment of health houses in rural areas and health centers in urban areas. Some of the important tasks of these centers are the education of public health, promotion of community participation, and diseases control services [26]. These services have also lead to remarkable decrease of intestinal parasitic infections in Iran, especially Gilan province where has favorable environmental condition for establishing the life cycle of human intestinal parasite [27]. The services provided by these health centers have a probable role in decreasing of *T. vaginalis* in Iran.

The prevalence of *T. vaginalis* show variations among geographical areas and societies with different living habits, cultures, and religions. For example, 31.3% in women with clinical symptoms and 13.3% in asymptomatic women in Vietnam [28], 1.3% in the United States [29]. In Iran, prevalence of the parasite has reported between 0.5 and up to 30 percent [26], including, more than 30% among the population of women prisoners and people with high-risk socio-health behaviors [30], 2.67% by direct smears and 11.3% by cultivation in women referred to Qom health centers [31], 0.3% by direct smears and 0.6% by culture method in women referred to Hamedan Health Centers [24], and 0.4% in women referred to Clinical Centers in South of Tehran [20]. Probably, differences in sample size of the populations studied, social behaviors, the level of health, and living habits are effective in the prevalence of this protozoan parasite.

In the present study, in terms of occupation, up to 90% of women were housewives and all three positive cases were observed in these individuals. Also, in report of Nazari et al. [32] 12 out of 13 positive cases in Kermanshah province, west of Iran were housewives.

In our study, 2 out of 3 positive cases for *T. vaginalis* were in the age range of 41–50 years. In study of Pellrud et al. [33] in Sweden, an average age of trichomonosis infections was 38 years. The most cases of the infection were observed in Iranian aged 41–50 years [34] which is agreed with the present study.

Education plays a significant role in the prevalence of *T. vaginalis*. In the present study, only one patient had a university education. In the report of Kakaire et al. [19], less education was associated with increased risk of sexually transmitted infections (STIs). The highest rate of infection was related to illiterate people that is similar to the results of studies conducted in Hamedan, Tehran, and Sirjan [20,35].

A study in Vietnam demonstrated that the prevalence of trichomonosis was higher in women with clinical symptoms than women without clinical symptoms [28]. In our study, all positive cases of *T. vaginalis* were observed in symptomatic women

Kakaire et al. [19] reported that condom can be effective in preventing trichomonosis in at-risk women, so that 13 out of 62 condom users and 22 out of 50 people who did not use it were positive *T. vaginalis*. In our study, 68 out of 450 people were condom users and all of them were negative for this protozoan parasite. These findings consistent with results of previous studies [35,36]. In our study, none of 32 intrauterine devices (IUD) users were infected with *T. vaginalis*. In the Cheraghi study, 3.2% had used from this device and the infection was not observed in them [34]. Kakaire et al. [19] reported prevalence of 1.5% and 4.9% in high-risk women using and non-using IUD, respectively. It can be concluded that the use of the IUD is likely to prevent the infection.

According to studies conducted in Iran and other countries, at least 6 genotypes (E, G, H, I, M, N) have identified using the actin gene in *T. vaginalis*. However, in some studies, genotypes P and A have mentioned. In the present study, only H and E genotypes of *T. vaginalis* was identified that are

probably predominant genotypes in the study area. Predominant genotype of this parasite was type E (55.7%) in Congo and type G in Zambia (46.7%) [37]. I, P, G, N, and E genotypes with dominancy of E genotype of *T. vaginalis* has reported from Canada [38]. In Turkey, H, E, N, H, G, E genotypes reported and type E was demonstrated as dominant genotype [12]. In China, sequencing of 267 isolates of *T. vaginalis* showed genotypes of H, E with dominancy of E [13]. Chatty et al. [16] reported G, H, I genotypes in South Africa, and the predominant genotype was G.

So far, a few genotyping studies of *T. vaginalis* have performed in Iran that demonstrate genetic diversity of the parasite in this country. Genotypes of H, E, G, M, N, I with dominancy of H and M have reported from Kerman and Shiraz, respectively [39]. In another study conducted in Karaj on 45 positive samples of *T. vaginalis*, the genotypes of G, E, I, and H were reported so that genotype G was the predominant genotype in this region [17]. Matini et al. [40] reported A, I, N, M, H, G, and E genotypes with dominancy of type E in Hamedan. Based on a study conducted by Spotin et al. [11] in Tabriz, genotypes of E, G were predominant genotypes. In Tehran, identified genotypes of *T. vaginalis* were E, G, and I so type E was the predominant type [41]. The reported genotypes from Shahrekord were H, G, E, P, N, and I types with dominancy of H type [14]. Low diversity of genotypes of the parasite in our study is probably related to the low prevalence of this parasite in the study area. It seems that studying more sample sizes will demonstrate a clear pattern of genotypes of *T. vaginalis* in the region. From results of the present study concludes that prevalence of *T. vaginalis* is very low in the study area that can be indicated the role of religious orders and high level of hygiene in sexual intercourse and avoiding from high risk sexual behaviors. Also, it seems that genotype H is the predominant type of the parasite in the study area. Further studies using more sample size was recommended in order to clear of geographical distribution of the parasite in Iran.

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References

[1] Vatanshenassan M., Rezaie S., Mohebali M., Niromand N., Kazemi B., Babaei Z. 2010. *Trichomonas vaginalis*: investigation of a novel diagnostic method in urine samples using cysteine proteinase 4 gene and PCR technique. *Experimental Parasitology* 126(2): 187–190. doi:10.1016/j.exppara.2010.04.021

[2] Matini M., Rezaie S., Mohebali M., Maghsood A.H., Rabiee S., Fallah M. 2014. Genetic identification of *Trichomonas vaginalis* by using the actin gene and molecular based methods. *Iran Journal of Parasitology* 9(3): 329–335.

[3] Cudmore S.L., Delgaty K.L., Hayward-McClelland S.F., Petrin D.P., Garber G.E. 2004. Treatment of infections caused by metronidazole-resistant *Trichomonas vaginalis*. *Clinical Microbiology Review* 17(4): 783–793. doi:10.1128/cmr.17.4.783-793.2004

[4] Stark J.R., Judson G., Alderete J.F., Mundodi V., Kucknoor A.S., Giovannucci E.L. 2009. Prospective study of *Trichomonas vaginalis* infection and prostate cancer incidence and mortality: physicians' health study. *Journal of the National Cancer Institute* 101(20): 1406–1411. doi:10.1093/jnci/djp306

[5] Yang S., Zhao W., Wang H., Wang Y., Li J.. Wu X. 2018. *Trichomonas vaginalis* infection-associated risk of cervical cancer: a meta-analysis. *European Journal of Obstetrics, Gynecology and Reproductive Biology* 228: 166–173. doi:10.1016/j.ejogrb.2018.06.031

[6] Donders G.G., Depuydt C.E., Bogers J.P.. Vereecken A.J. 2013. Association of *Trichomonas vaginalis* and cytological abnormalities of the cervix in low risk women. *PLoS One* 8: e86266. doi:10.1371/journal.pone.0086266

[7] Twu O., Dessí D., Vu A., Mercer F., Stevens G.C., de Miguel N. 2014. *Trichomonas vaginalis* homolog of macrophage migration inhibitory factor induces prostate cell growth, invasiveness, and inflammatory responses. *Proceedings of the National Academy of Sciences of the U S A* 111(22): 8179–8184. doi:10.1073/pnas.1321884111

[8] Crucitti T., Jespers V., Mulenga C., Khondowe S., Vandepitte J.. Buvé A. 2010. *Trichomonas vaginalis* is highly prevalent in adolescent girls, pregnant women, and commercial sex workers in Ndola, Zambia. *Sexually Transmitted Diseases* 37(4):

223–227. doi:10.1097/OLQ.0b013e3181c21f93
[9] Miranda A.E., Pinto V.M.. Gaydos C.A. 2014. *Trichomonas vaginalis* infection among young pregnant women in Brazil. *Brazilian Journal of Infectious Diseases* 18(6): 669–671. doi:10.1016/j.bjid.2014.07.002
[10] Sutcliffe S., Giovannucci E., Alderete J.F., Chang T.H., Gaydos C.A., Zenilman J.M. 2006. Plasma antibodies against *Trichomonas vaginalis* and subsequent risk of prostate cancer. *Cancer Epidemiology Biomarkers and Prevention* 15(5): 939–945. doi:10.1158/1055-9965.epi-05-0781
[11] Spotin A., Eghtedar S.T., Shahbazi A., Salehpour A., Sarafraz S., Shariatzadeh S.A. 2016. Molecular characterization of *Trichomonas vaginalis* strains based on identifying their probable variations in asymptomatic patients. *Iranian Journal of Parasitology* 11(4): 507–514.
[12] Demirağ S., Malatyalı E., Ertuğ S.. Ertabaklar H. 2017. Determination of *Trichomonas vaginalis* genotypes using PCR-restriction fragment length polymorphism (RFLP). *Turkiye Parazitolojii Dergisi* 41(4): 188–191. doi:10.5152/tpd.2017.5496
[13] Zhang Z., Kang L., Wang W., Zhao X., Li Y., Xie Q. 2018. Prevalence and genetic diversity of *Trichomonas vaginalis* clinical isolates in a targeted population in Xinxiang City, Henan Province, China. *Parasitology Vectors* 11: article number 124. doi:10.1186/s13071-018-2753-4
[14] Khalili B., Ghasemi-Dehkordi P., Pourshahbazi G., Yousofi-Darani H., Hashemzadeh-Chaleshtori M.. Doosti A. 2017. Genotyping of *Trichomonas vaginalis* isolates from women in Shahrekord city (Southwestern Iran). *Genetika* 49(3): 1059–1070. doi:10.2298/GENSR1703059K
[15] Moradi E., Dalimi A.. Sadraei J. 2017. *Trichomonas vaginalis* genotyping isolated from women of Mahshahr, Iran by nested-PCR. *Pathobiology Research* 20(3): 53–61.
[16] Chetty R., Mabaso N.. Abbai N. 2020. Genotypic variation in *Trichomonas vaginalis d*etected in South African pregnant women. *Infectious Diseases in Obstetrics and Gynecology* 2020: article number 1687427. doi:10.1155/2020/1687427
[17] Momeni Z., Sadraei J., Kazemi B.. Dalimi A. 2015. Molecular typing of the actin gene of *Trichomonas vaginalis* isolates by PCR-RFLP in Iran. *Experimental Parasitology* 159: 259–263. doi:10.1016/j.exppara.2015.10.011
[18] Scott-Sheldon L.A., Senn T.E., Carey K.B., Urban M.A.. Carey M.P. 2013. Quantity, not frequency, of alcohol use moderates the association between multiple sexual partners and *Trichomonas vaginalis* among women attending an urban STD clinic. *Sexually Transmitted Infections* 89(6): 498–503. doi:10.1136/sextrans-2012-050983
[19] Kakaire O., Byamugisha J.K., Tumwesigye N.M.. Gamzell-Danielsson K. 2015. Prevalence and factors associated with sexually transmitted infections among HIV positive women opting for intrauterine contraception. *PLoS One* 10(4): e0122400. doi:10.1371/journal.pone.0122400
[20] Azambakhtiar A., Nikmanesh B., Rezaeian M., Dashti N., Safari F.. Zarebavani M. 2018. The prevalence of trichomoniasis in women referred to clinical centers in South of Tehran, Iran during 2015–2016. *Iranian Journal of Parasitology* 13(1): 108–113.
[21] Akbarian A., Akhlaghi L., Ourmazdi H., Foroohesh H., Falahati M.. Farokhnejad R. 2005. An investigation on coincidence of trichomoniasis and bacterial vaginosis and their effects on pregnant women referred to Shahid Akbarabadi Maternity Hospital in Tehran during 2002–2003. *Razi Journal of Medical Sciences* 12(46): 227–234.
[22] Rabiee S., Fallah M.. Zahabi F. 2010. Frequency of trichomoniasis in patients admitted to outpatient clinics in Hamadan (2007) and relationship between clinical diagnosis and laboratory findings. *Journal of Research in Health Sciences* 10(1): 31–35.
[23] Matini M., Rezaie S., Mohebali M., Maghsood A., Rabiee S., Fallah M. 2012. Prevalence of *Trichomonas vaginalis* infection in Hamadan City, Western Iran. *Iranian Journal of Parasitology* 7(2): 67–72.
[24] Akbari Z., Matini M. 2017. The study of trichomoniasis in pregnant women attending hamadan city health centers in 2015. *Avicenna Journal of Clinical Microbiology and Infectious* 4: 41533. doi:10.5812/ajcmi.41533
[25] Masiha A., Asmar M. 2007. A survey of the prevalence of vaginitis and vahinosis comon microbial factors in outpatients and bedridden patients in Lahijan hospitals and health denters during 2003–2004. *Journal of Biology Science* 1(2): 49–58.
[26] Arbabi M., Delavari M., Fakhrieh-Kashan Z., Hooshyar H. 2018. Review of *Trichomonas vaginalis* in Iran, based on epidemiological situation. *Journal of Reproduction and Infertility* 19(2): 82–88.
[27] Sharifdini M., Ghanbarzadeh L., Barikani A.. Saraei M. 2020. Prevalence of intestinal parasites among rural inhabitants of Fouman, Guilan Province, Northern Iran with emphasis on *Strongyloides stercoralis*. *Iranian Journal of Parasitology* 15(1): 91–100.
[28] Ton Nu P.A., Nguyen V.Q., Cao N.T., Dessì D., Rappelli P., Fiori P.L. 2015. Prevalence of *Trichomonas vaginalis* infection in symptomatic and asymptomatic women in Central Vietnam. *Journal of Infection in Developing Countries* 9(6): 655–660. doi:10.3855/jidc.7190
[29] Flagg E.W., Meites E., Phillips C., Papp J.. Torrone E.A. 2019. Prevalence of *Trichomonas vaginalis* among civilian, noninstitutionalized male and female population aged 14 to 59 years: United States, 2013 to

2016. *Sexually Transmitted Diseases* 46(10): e93–e6. doi:10.1097/olq.0000000000001013

[30] Haynie D.L., Whichard C., Kreager D.A., Schaefer D.R.. Wakefield S. 2018. Social networks and health in a prison unit. *Journal of Health and Social Behavior* 59(3): 318–334. doi:10.1177/0022146518790935

[31] Habibi A., Nateghi Rostami M., Douraghi M., Dolati M., Rashidi B., Ahangari R. 2016. [Frequency of genital infection with *Trichomonas vaginalis* in women referred to gynecology hospital of the city of Qom]. *Journal of Dermatology and Cosmetic* 6(4): 190–199 (in Persian).

[32] Nazari N., Zangeneh M., Moradi F.. Bozorgomid A. 2015. Prevalence of trichomoniasis among women in kermanshah, iran. *Iran Red Crescent Medical Journal* 17(3): e23617. doi:10.5812/ircmj.23617

[33] Pellrud H., Golparian D., Nilsson C.S., Falk M., Fredlund H.. Unemo M. 2015. *Trichomonas vaginalis* infections are rare among young patients attending an STI clinic in Sweden. *Acta Dermato-Venereologica* 95(3): 343–344. doi:10.2340/00015555-1946

[34] Maria C., Zahra R.. Sara P. 2013. Prevalence of cervical-vaginal infections in the pap-smear samples in Iran. *Global Journal of Health Science* 6(1): 201–206. doi:10.5539/gjhs.v6n1p201

[35] Sharifi I., Khatami M.. Tahmors-Kermani E. 1994. Prevalence of *Trichomonas vaginalis* in women referred to Vali-Asr polyclinic and the health center number 3 in Sirjan city. *Journal of Kerman University of Medical Sciences* 1: 125–134.

[36] Crosby R.A., Charnigo R.A., Weathers C., Caliendo A.M.. Shrier L.A. 2012. Condom effectiveness against non-viral sexually transmitted infections: a prospective study using electronic daily diaries. *Sexually Transmitted Infections* 88(7): 484–489. doi:10.1136/sextrans-2012-050618

[37] Crucitti T., Abdellati S., Van Dyck E.. Buvé A. 2008. Molecular typing of the actin gene of *Trichomonas vaginalis* isolates by PCR-restriction fragment length polymorphism. *Clinical Microbiology and Infection* 14(9): 844–852. doi:10.1111/j.1469-0691.2008.02034.x

[38] Masha S.C., Cools P., Crucitti T., Sanders E.J.. Vaneechoutte M. 2017. Molecular typing of *Trichomonas vaginalis* isolates by actin gene sequence analysis and carriage of *T. vaginalis* viruses. *Parasite and Vectors* 10(1): article number 537. doi:10.1186/s13071-017-2496-7

[39] Tavakoli Oliaee R., Babaei Z., Hatam G.R., Tavakoli Kareshk A., Mahmoudvand H., Vafafar A. 2017. Considerable genetic diversity of *Trichomonas vaginalis* clinical isolates in a targeted population in South of Iran. *Iranian Journal of Parasitology* 12(2): 251–259.

[40] Matini M., Rezaei H., Fallah M., Maghsood A.H., Saidijam M.. Shamsi-Ehsan T. 2017. Genotyping, drug susceptibility and prevalence survey of *Trichomonas vaginalis* among women attending gynecology clinics in Hamadan, Western Iran, in 2014–2015. *Iranian Journal of Parasitology* 12(1): 29–37.

[41] Orujzadeh F., Tabatabaie F., Khanaliha K., Akhlaghi L., Bokharaei-Salim F., Fallah S. 2019. Prevalence and genotyping of *Trichomonas vaginalis* infected to dsRNA virus by PCR-restriction fragment length polymorphism (RFLP). *Iranian Journal of Parasitology* 14(2): 250–257. doi:10.18502/ijpa.v14i2.1137

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