GENOTYPING OF Trichomonas vaginalis ISOLATES FROM WOMEN IN SHAHREKORD CITY (SOUTHWESTERN IRAN)

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Trichomonas vaginalis is a causative agent of vaginitis in female and urethritis in men. It is primarily transmitted by sexually route. It is known that each geographical area has its own set of Trichomonas vaginalis strain. Parasite strains in each region have its specific characterizations and different strains of the parasite are able to cause various diseases with the acuity and severity. The aim of this study was to determine the genotyping of Trichomonas vaginalis strains in the Shahrekord city (Chaharmahal Va Bakhtiari province, southwest Iran). A total of 1725 vaginal samples were taken from clinically suspected women for Trichomonas vaginalis infection and 21 specimens were diagnosed as positive by direct smear wet mount and culture repeated
passage of the parasite in the modified TYI-S-33 medium. The genomic DNA was extracted from each sample and the nested polymerase chain reaction was applied using specific oligonucleotide primers for actin gene amplification. Finally, the restriction fragment length polymorphism using Rsal, Msel, and HindII restriction enzymes were done on PCR products for genotyping. PCR-RFLP analysis of 21 positive cases (1.22%) was showing the most frequent genotype was H (8 cases), followed by G (4 cases), E (3 cases), and P (2 cases). N and I genotypes were detected in each 1 case. Also, there was 2 cases mix (E and H) genotype. The findings of the present work were showed 7 different genetic strains in isolated Trichomonas vaginalis from symptomatic and asymptomatic women in Shahrekord city. In this study high level of H genotype in referred women in Shahrekord city was observed and H, G, E, and I genotypes were may be related to burning and itching as well as H, P, and mix genotypes were associated with malodorous discharge with pelvic pain in this region of Iran. For a suggestion, it would be better in further studies the accurate determination of genetic diversity of this parasite done in Chaharmahal Va Bakhtiari province and other parts of the country.

Key words: Trichomonas vaginalis, genotyping, polymerase chain reaction, restriction fragment length polymorphism, Shahrekord

INTRODUCTION

Trichomonas vaginalis (T. vaginalis) is an anaerobic, flagellated protozoan, and it is a causative agent of trichomoniasis in both females and males. The disease is one of the most non-viral sexually transmitted infection (STI) worldwide (PETRIN et al., 1998; CONRAD et al., 2011). Approximately 50% of infected women show symptoms and men usually do not have symptoms of this disease. The incubation period is up to 26 days and trichomoniasis mainly affects on vagina and urethra. In women the symptoms including abnormal vaginal discharge, pain with urination or sexual intercourse, pain in lower abdomen, genital redness, inflammation, and itching. The disease appears to be associated with an increased incidence of post-partum endometritis (LAZENBY et al., 2014; MITCHELL et al., 2014). In men, the signs of trichomoniase not clearly appear and infection has usually short duration but this disease mainly affects on the urethra, although the foreskin, head of the penis and prostate gland (LEWIS et al., 2013; MEITES et al., 2013). The symptoms in men involving pain and slight burning during and after urination or ejaculation, needing to urinate more frequently than usual, soreness, swelling and redness around the head of the penis and foreskin, and white discharge from the penis (MEITES et al., 2013; SEIKE et al., 2013). Infected people by T. vaginalis are high risk for transmission of human immunodeficiency virus (HIV). T. vaginalis has a global spread and it infects 250 to 350 million people worldwide. The infection rate differs in women population (5% in asymptomatic patients, 50–75 % in sex workers, and 0.9 to 39.6 % in STI clinics) (WEINSTOCK et al., 2004; MASHBUM, 2006; PAUL et al., 2007). The infection rate of this pathogen has been reported in Iran are vary (2-25%) but has been yet no reported for the frequency of T. vaginalis in Shahrekord city (southwestern Iran).

In recent decades, molecular techniques such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), PCR-hybridization, PCR-size polymorphism, and random amplification of polymorphic DNA (RAPD) used for detection of virulence genes and variation in T. vaginalis as well as classification of the protozoa (CONRAD et al., 2012; SABAT et
The classification and molecular typing of *T. vaginalis* in Iranian isolates are not clearly investigated and it is important for the taxonomy of this pathogen and identifying the kinds of virulence factors in each strain (MATINI et al., 2012; ESKANDARIAN, 2013; MATINI et al., 2014). The present study aims were to investigate the frequency and genotyping of Iranian *T. vaginalis* isolates from infected women with symptoms of the trichomoniasis in Shahrekord city (southwest Iran) using PCR-RFLP technique.

**MATERIALS AND METHODS**

**Samples collection and culturing**

In this experimental study, a total of 1725 specimens using a sterile cotton swab during the pelvic examination, were collected from the vaginal secretion of symptomatic and asymptomatic women referred to Imam Ali clinic center in Shahrekord city (southwest Iran) and transferred to the Cellular and Molecular Research Center of Shahrekord University of Medical Sciences. The demographic information of women referred to Imam Ali clinic center in Shahrekord city according to their age groups, job, and place of living are shown in Table 1.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Num.</th>
<th>Type of job</th>
<th>Num.</th>
<th>Position</th>
<th>Num.</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-20</td>
<td>88</td>
<td>Housekeeper</td>
<td>1041</td>
<td>Urban</td>
<td>1062</td>
</tr>
<tr>
<td>21-25</td>
<td>305</td>
<td>Employee</td>
<td>211</td>
<td>Rural</td>
<td>486</td>
</tr>
<tr>
<td>26-30</td>
<td>631</td>
<td>Student</td>
<td>473</td>
<td>Nomadic</td>
<td>177</td>
</tr>
<tr>
<td>31-35</td>
<td>403</td>
<td>Total</td>
<td>1725</td>
<td>Total</td>
<td>1725</td>
</tr>
<tr>
<td>36-40</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41-45</td>
<td>102</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45+</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1725</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After the approval of Shahrekord University of Medical Sciences’ Ethics Committee, the individuals’ consents were taken during sampling and the information of each suspected women including age, background disease, the antecedent of antibiotics consuming, and clinical signs were obtained by standard questionnaires. Two swab samples were obtained from each suspected patient and were used for culturing and direct smear wet mount and were observed by 40X lens. The swab samples of each suspected women were cultured in modified TY1-S-33 medium containing inactive bovine serum and incubated at 37˚C under anaerobic conditions. The culture for investigation the presence of *T. vaginalis* was checked on the microscope in days 2 to 7. Genomic DNA was purified from each positive cultured sample using the DNA extraction kit (Fermentas, Germany) according to the manufacturer’s protocol. The DNA quality and quantity were measured by NanoDrop ND- 1000 (Peqlab, Erlangen, Germany) according to the method described by SAMBROOK and RUSSELL (2001).
Actin gene amplification and genotyping analysis

Genotyping of \textit{actin} gene was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The nested-PCR assay was used for amplification of \textit{actin} gene using obtained outer and inner oligonucleotide primers (Table 2) from ESPINOSA \textit{et al.} (2001) study (GenBank accession number: AF237734). Amplification reactions in two rounds of PCR were done in a final volume of 25 µL in 0.5 mL micro-tubes containing 2 mM MgCl$_2$, 200 μM dNTP mix, 2.5 µL of 10X PCR buffer (20mM Tris–HCl pH=8.4, 50 mM KCl), 1 unit of Taq DNA polymerase (all Fermentas, Germany), 1 µg of template DNA, and 1 µM of each primer except in the second round inner primers and 1 µL of PCR product from the first round (instead of template DNA) were used. A 2 µL of sterile ultrapure deionized water was used as a negative control. The mixtures in each round were placed in a Gradient Palm Cycler (Corbett Research, Australia) for gene amplification and thermo-cycling condition including initial denaturation at 94°C for 5 min, following by 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 58°C (for outer primers) and 55°C (for inner primers) for 1 min, elongation at 72°C for 1 min, and a final elongation at 72°C for 5 min was done at the end of the amplification program. After amplification, samples were stored at 4°C (few hours) or at 20°C (days). PCR products in each round were verified on a 2% agarose gel electrophoresis. The electrode buffer was TBE (10.8 g of Tris-base 89 mM, 5.5 g of Boric acid 2 mM, 4 ml of 0.5 M EDTA (pH=8.0), combined all components in sufficient H$_2$O and stir to dissolve). The PCR products (10 µL) were applied to the gel and constant voltage of 80 for 30 min was used for separation of products. The 100 bp DNA ladder (Fermentas, Germany) was used as a molecular weight marker to determine the length of the amplified fragments and finally, the gel was stained with ethidium bromide. The gel images were obtained using UVIdoc gel documentation systems (Uvitec, UK).

Table 2. The sequence of primers used for \textit{actin} gene amplification

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers name</th>
<th>Primer sequence</th>
<th>Accession number</th>
<th>Products length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>actin</td>
<td>Outer</td>
<td>Tv8S  5′-TCTGGAATGGCTGAAGAAGACG-3′</td>
<td>AF237734</td>
<td>1300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tv9R  3′-CAGGGTACATCGTATTGGTC-3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inner</td>
<td>Tv10S 5′-CAGACACTCGTTATCG-3′</td>
<td></td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tv11R 5′-CGGTGAACGATGGATG-3′</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RFLP assay of PCR products

Genotyping of \textit{actin} gene by RFLP was carried out using \textit{Hind}II, \textit{Mse}I, and \textit{Rsa}I restriction enzymes. The amplified PCR products were subjected to digestion by restriction enzymes in a total volume of 20 µL (10 µL of PCR product, 2 µL of enzyme buffer, 1 µL of enzyme, and 7 µL of distilled water) and were placed in the incubator at 37°C (for \textit{Mse}I and \textit{Rsa}I enzymes) and 64°C (for \textit{Hind}II enzyme) for 4 h. The digested products were analyzed on 2% agarose gel. The \textit{actin} gene digestion patterns using \textit{Hind}II, \textit{Mse}I, and \textit{Rsa}I enzymes for genotyping of \textit{T. vaginalis} are shown in Table 3 (CRUCITTI \textit{et al.}, 2008).
Table 3. The digestion patterns of actin gene and size of the fragments for genotyping of T. vaginalis isolates from referred women to Imam Ali clinic center in Shahrekord city

<table>
<thead>
<tr>
<th>Gene Pattern</th>
<th>Restriction with HindII (bp)</th>
<th>Restriction with Msel (bp)</th>
<th>Restriction with Rsal (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>827 426 401 213 66</td>
<td>581 519 333 315 204 186</td>
<td>568 452 236 190 116 106 103 87</td>
</tr>
<tr>
<td>E</td>
<td>+ - + + + + +</td>
<td>+ - + + + + +</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>G</td>
<td>- + + + + + +</td>
<td>- + + + + + +</td>
<td>- + + + + + + +</td>
</tr>
<tr>
<td>H</td>
<td>- + + + + + +</td>
<td>- + + + + + +</td>
<td>- + + + + + + +</td>
</tr>
<tr>
<td>I</td>
<td>- + + + + + +</td>
<td>- + + + + + +</td>
<td>- + + + + + + +</td>
</tr>
<tr>
<td>M</td>
<td>- + + + + + +</td>
<td>- + + + + + +</td>
<td>- + + + + + + +</td>
</tr>
<tr>
<td>P</td>
<td>- + + + + + +</td>
<td>- + + + + + +</td>
<td>- + + + + + + +</td>
</tr>
</tbody>
</table>

Statistical analysis

The data were analyzed by Statistics programs for the Social Sciences (SPSS, Inc., Chicago, IL, USA) software, version 20.

Ethical approval

In the present study, informed consent forms and all protocols were approved by the Regional Research Ethical Committee of Shahrekord University of Medical Sciences (Grant number: 91-7-12).

RESULTS

In the present study, after culturing of 1725 swab samples collected from referred women to Imam Ali clinic center (Shahrekord city) in modified TYI-S-33 medium and also wet mount examination 21 (1.22%) specimens were detected T. vaginalis. Some criteria of infected women are shown in Table 4.

After amplification of actin gene using PCR the products on 2% agarose gel electrophoresis were revealed fragments with the length size of 1300 and 1100 bp using outer and inner oligonucleotide primers, respectively (Figures 1 and 2). The size of the target was 1100 bp, which is only 28 bp shorter than the full length of the open reading frame of the actin gene.

Table 4. Some criteria of infected women by T. vaginalis in Shahrekord city according to their job, place of living, and clinical symptoms

<table>
<thead>
<tr>
<th>Job</th>
<th>Num.</th>
<th>Place of living</th>
<th>Num.</th>
<th>Clinical signs</th>
<th>Num.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Housekeeper</td>
<td>15</td>
<td>Urban</td>
<td>14</td>
<td>Burning and itching</td>
<td>13</td>
</tr>
<tr>
<td>Employee</td>
<td>4</td>
<td>Rural</td>
<td>5</td>
<td>Malodorous discharge with pelvic pain</td>
<td>5</td>
</tr>
<tr>
<td>Student</td>
<td>2</td>
<td>Nomadic</td>
<td>2</td>
<td>Asymptomatic</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>Total</td>
<td>21</td>
<td>Total</td>
<td>21</td>
</tr>
</tbody>
</table>
Figure 1. A 2% agarose gel electrophoresis of amplified actin gene using outer specific oligonucleotide primers (Lane M is a 100 bp DNA ladder (Fermentas, Germany), lanes 1 to 5 and 7 are positive specimens, and lane 6 is negative sample, respectively)

Figure 2. Amplification of PCR products on 2% agarose gel electrophoresis using inner primers (Lane M is a 100 bp DNA marker (Fermentas, Germany), and lanes 1 to 5 are amplified fragments, respectively)

Genotyping of T. vaginalis

After digestion of amplified actin gene in 21 infected patients by HindII, MseI, and Rsal restriction endonuclease enzymes (Figures 3 and 4) the restriction patterns on 2% agarose gel were investigated and H, G, E, P, I, N and mix (E and H) genotypes were identified in 8 (38.1%), 4 (19.05%), 3 (14.28%), 2 (9.52%), 1 (4.76%), 1 (4.76%), and 2 (9.52%) of samples, respectively.
Figure 3. Gel electrophoresis of amplified actin gene after digestion by HindIII, MseI, and RsaI restriction enzymes (100 bp DNA marker (Fermentas, Germany) and the restriction patterns including 5 (568 bp, 236 bp, 106 bp, 103 bp, and 87 bp), 4 (426 bp, 401 bp, 213 bp, and 60 bp), and 2 (581 bp, 519 bp, 333 bp) restriction pattern after digestion by RsaI, HindIII, and MseI restriction enzymes, respectively)

Figure 4. Separate digestion of amplified actin gene by HindIII, MseI, and RsaI restriction enzymes, respectively (100 bp DNA marker (Fermentas, Germany) and the restriction patterns)

The relationship between each genotype and the clinical symptoms of infected women by T. vaginalis is shown in Table 5.
Table 5. Distribution of clinical symptoms associated with T. vaginalis in each genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>H</th>
<th>G</th>
<th>E</th>
<th>I</th>
<th>P</th>
<th>N</th>
<th>Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burning and itching</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Malodorous discharge with pelvic pain</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In general, identification of the genetic profile of *T. vaginalis* is important in the study of genetic diversity of organisms and could be more helpful for preparing genetic information about the nature and extent of this organism in specific populations. The prevalence of *T. vaginalis* in Iran is varied (0.5% to about 39%) in different age groups of women and related to each area (HEZARJARIBI et al., 2015). In this work, after culturing and direct smear wet mount of 1725 collected swab samples obtained from symptomatic and asymptomatic women in Shahrekord city (southwestern Iran) *T. vaginalis* infection were detected in 21 (1.22%) of cases. During 2012 to 2013 years 2% had a trichomonal infection in Kashan city (ARBABI et al., 2014), in Kermanshah district (33%), and in Hamadan city (western Iran) 2.1% were infected by this protozoan (CHALECHALE and KARIMI, 2010; MATINI et al., 2012). Moreover, in unmarried male referred to a clinic in Tehran city (Iran) 7% of urine samples were positive for *T. vaginalis* by direct smear cultured in TYI-S-33 medium (VALADKHANI et al., 2013). The prevalence of *T. vaginalis* in pregnant women in Zanjan (Northwestern Iran) was 1.83% (NOURIAN et al., 2013) and the frequency of this parasite in Chaharmahal Va Bakhtiari (1996) and Ardabil city (2013-2014) were 3.3 and 2.4%, respectively (SADEGHI and MANOUCHEHRI NAEINI, 2001; MOHAMMADI-GHALEHVIN et al., 2015). The difference among the infection rates in this research with other studies from different parts of Iran may be related to study design or vary studied population.

The clinical signs of *T. vaginalis* have a particular intensity and related to the species that isolated from each region. In the present study, genotyping of *T. vaginalis* isolated from symptomatic and asymptomatic women in Shahrekord city (southwestern Iran) was investigated. Genotyping was done by PCR-RFLP technique on amplified *actin* gene using Rsal, Msel, HincII restriction enzymes. In this work, 7 different genotypes including H, G, E, P, I, N, and mix (E and H) were identified in 8, 4, 3, 2, 1, 1, and 2 samples, respectively by analysis of restriction patterns. The most frequent were H genotype (38.1%) in isolated parasites from referred women in Shahrekord city. Furthermore, in cases with H, G, E, and I genotypes burning and itching was observed as well as in patients had H, P, and mix genotypes malodorous discharge with pelvic pain were found. VANACOVA et al., 1997 using RAPD technique has investigated the genotype of *T. vaginalis*. In addition, Stiles and colleagues using the PCR-RFLP method and *EcoRI* restriction enzyme were investigated the genetic variations of hsp70 gene of *T. vaginalis* (STILES et al., 2000). Also, SNIPES et al. (2000) using this technique were investigated the genetic
variations of rDNA, ITS1, and ITS2 genes area of this parasite. There is no study about
genotyping of T. vaginalis using actin gene in Iran and other parts of the world except CRUCITTI
et al. (2008) research. They demonstrated that type E was more common (57% of isolates) in the
Democratic Republic of Congo, whereas type G was the commonest type (47% of isolates) in
Zambia. The lowest genetic diversity was M and P types that observed in Kinshasa and Zambia,
respectively (CRUCITTI et al., 2008). While H genotype was frequent (38.1%) in the present study
and I and N genotypes were lowest (4.76%) in T. vaginalis isolated from symptomatic and
asymptomatic women in Shahrekord city. In addition, mix genotype (E and H) were determined
in 2 specimens and these cases had a history of resistance to metronidazole. Therefore, it would
be better in further researches metronidazole resistant strains and its activities against this
parasite investigate.

CONCLUSIONS

According to the findings of this study, H genotype in 38.1% of isolated parasites in
referred women in Shahrekord city was detected and H, G, E, and I genotypes are may be related
to burning and itching as well as H, P, and mix genotypes are related to patients with malodorous
discharge with pelvic pain in this region of Iran. The present study showed that 2 cases with mix
genotypes had a history of resistance to metronidazole and it seems that more precise studies
about genetic of drug resistance in T. vaginalis strains, particularly mix (E and H) genotype is
necessary. This work and previous researches are indicated that each region has a specific
genotype of the T. vaginalis. Moreover, there is a little information about the genetic diversity of
T. vaginalis in Iran, therefore further studies in other parts of the country should prepare more
precise and accurate view in the genetic variations of the parasite. In addition, more detailed
research in this area could be defining a possible relation between the different strains of parasite
and risk of HIV infection.

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REFERENCES


CHALECHALE, A., I. KARIMI (2010): The prevalence of Trichomonas vaginalis infection among patients that presented to

CONRAD, M.D., A.W. GORMAN, J.A. SCHILLINGER, P.L. FIORI, R. ARROYO, N. MALLA, M.L. DUBEY, J. GONZALEZ, S. BLANK,

polymorphism in the sexually transmitted human pathogen Trichomonas vaginalis indicates a genetically


B. KHALILI et al.: GENOTYPING OF *Trichomonas vaginalis*
GENOTIPIZACIJA IZOLATA Trichomonas vaginalis KOD ŽENA U GRADU ŠAHREKORD (JUGOZAPADNI IRAN)

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Izvod

Trichomonas vaginalis je uzročnik vaginitisa kod žena i uretritisa kod muškaraca. Primarno se prenosi seksualnim putem. Poznato je da svaka geografska oblast ima sopstveni set Trichomonas vaginalis sojeva, koji imaju specifične karakteristike, a različiti sojevi parazita mogu izazvati bolesti različitog intenziteta i ozbiljnosti. Cilj ovog rada je bio genotipizacija soja Trichomonas vaginalis u gradu Šahrekord. Uzeto je ukupno 1725 uzoraka kod žena kod kojih se sumnjalo na infekciju Trichomonas vaginalis-om, a dijagnostikovan je 21 primerak kao pozitivan. Genomska DNK je izolovana iz svakog uzorka i urađen je PCR korišćenjem specifičnih oligonukleotidnih prajmera za amplifikaciju gena actin. RFLP je urađen korišćenjem Rsal, Msel i HindII restrikcionih enzima na PCR produktima za genotipizaciju. PCR-RFLP analiza 21 pozitivnog uzorka (1.22%) pokazala je da je najčešći bio genotip H (8 uzorka), zatim G (4 uzorka), E (3 uzorka), i P (2 uzorka). N i I genotipovi su potvrđeni u svakom uzorku. Takođe su utvrđena i dva uzorka sa pomešanim genotipovima (E and H). Rezultati ovog rada ukazuju na prisustvo 7 različitih genetičkih sojeva u izolatima Trichomonas vaginalis. Utvrđen je visok nivo H genotipa kod žena u gradu Šahrekord, a genotipovi H, G, E i I, mogu biti povezani sa upalom i svrabom. Za budući rad, preporučuje se preciznije određivanje genetičkog diverziteta ove parazitske vrste u ispitivanoj provinciji, ali i u ostalim delovima Iran.

Odobreno 15. VII. 2017.