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Short Communication

Gene Diversity of *Trichomonas vaginalis* Isolates

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ABSTRACT

Background: *Trichomonas vaginalis* is protozoan parasite responsible for trichomoniasis and is more common in high-risk behavior group such as prostitute individuals. Interest in trichomoniasis is due to increase one's susceptibility to viruses such as herpes, human papillomavirus and HIV. The aim of this study was to find genotypic differences between the isolates.

Methods: Forty isolates from prisoners' women in Tehran province were used in this study. The random amplified polymorphic DNA (RAPD) technique was used to determine genetic differences among isolates and was correlated with patient's records. By each primer the banding pattern size of each isolates was scored (bp), genetic differences were studied, and the genealogical tree was constructed by using NTSYS software program and UPGMA method.

Results: The least number of bands were seen by using primer OPD8 and the most by using OPD3. Results showed no significant difference in isolates from different geographical areas in Iran. By using primer OPD1 specific amplified fragment with length 1300 base pair were found in only 8 isolates. All these isolates were belonged to addicted women; however, six belonged to asymptomatic patients and two to symptomatic ones.

Conclusion: There was not much genetic diversity in *T vaginalis* isolates from three different geographical areas.

Keywords: *Trichomonas vaginalis*, Prisoners, RAPD, Gene diversity

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Introduction

Trichomonas vaginalis is one of the protozoan parasites that infect urogenital tracts of both sexes. Sexually transmitted infections (STIs) are worldwide distributed diseases that due to important side effects such as increase one's susceptibility to viruses like herpes, human papillomavirus and HIV, are very important (1). This infection may persist for several months, and mostly be confused with bacterial vaginitis. Culture is considered the reference method in diagnosis of *Trichomonas vaginalis* infection (2).

The random amplified polymorphic DNA (RAPD) technique is popular for comparing the DNA of biological systems that have not had the attention of the scientific community, or in a system in which relatively few DNA sequences are compared. Reports about the strain variation and phylogenetic polymorphisms of *T. vaginalis* are rare.

One study from Philippines demonstrated that sequencing of the 5.8S rDNA gene and the flanking ITS1 and ITS2 regions of *T. vaginalis* isolates show low genetic polymorphism. (3). Hampl et al. (4) used RAPD method to study the genetic relatedness of this parasite strains with respect to its phenotypic similarities such as metronidazole drug resistance. Ribosomal RNA gene sequencing is another method commonly used to find genetic polymorphisms between organisms (5).

In one report on correlation between the genetic relatedness of the isolates and symptomatology, they used analysis of restriction length polymorphism (RFLP) within the intergenic spacer of the ribosomal DNA (IGS) from 60 clinically defined isolates of *T. vaginalis* (6). At present, there are no reports on comparative analysis as well as on the identification of phylogenetic positions of *T. vaginalis* isolates from different geographical areas in Iran. This study, therefore aimed

to determine the aspect of *T. vaginalis* isolated from vaginal and urine samples of prisoners' women from Tehran Province.

Materials and Methods

We evaluated the relationship between 40 strains of *T. vaginalis*, isolated from women attended in gynecology clinics of three prisons in Tehran province, Iran. The isolates collected from 29 symptomatic and 11 asymptomatic women. 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. DNA of strains was extracted by using DNGTM-Plus (CinnaGen Inc.) solution according to the manufacturer's manual.

Random Amplified Polymorphic DNA (RAPD) technique was used based on Jamali et al. (7). In this study, out of ten random primers, six primers [OPD1 (ACCGCGAAGG), OPD2 (GGACCCAACC), OPD3 (GTCGCCGTCA), OPD8 (TCCTCACCGACC), Tv2 (TCG-GCCGCTATC), Tv6 (GGGACCTACTGC)], were found suitable for analyzing these isolates. The DNA amplification was performed at final volume of 25 µl containing 2.5 µl of 10 x PCR reaction buffer, 1 µl of each primer (Cinnagen, Tehran, Iran), 0.5 µl of mixed dNTP, 4 µl of template DNA, 15.35 µl of double distilled water and 0.4 µl of Taq DNA polymerase (5U/µl; Cinnagen, Tehran, Iran). Negative controls, which consisted of PCR mix with primers but no template DNA, were run in experiments. We chose one strain as reference sample and used in all RAPD PCR test for checking the whole steps of experiment and in case of changing the result, the test was repeated. The amplification protocol consisted of an initial denaturation step at 94°C for 5 min followed by 40 cycle's repetitions of 1 min at 94°C, 1

min at 36°C and 2 min at 72°C. The extension was 15 min at 72°C. The PCR products were analyzed by electrophoresis in 1.2% agarose gel in TBE buffer and DNA stain (3µl/100 ml), and visualized under the UV transilluminator. All primers detected DNA polymorphism among isolates of *T. vaginalis*. By each primer the banding pattern size of each isolates was scored (bp) in compare with size marker in DNA ladder (GeneRuler™, Fermentas). The detected DNA bands were analyzed and data were correlated with patient's records.

Statistical analysis

The Nei and Li method were used for similarity index between the isolates. The genealogical tree was constructed by using NTSYS software program and the un-weighted pair group methods (UPGMA).

Results

The patients were from following provinces, Tehran, Khorasan, Khoozestan, Qazvin, Golestan, Lorestan, West Azarbaijan, Mazandaran, Kermanshah, Hamedan, and Karaj. Forty axenized isolates from forty-six positive

samples, were amplified, and used in this study. The isolates with similar banding pattern were assigned as a single type. OPD8 indicated least (13 types) while OPD3 indicated highest typing (26 types) ability (Fig. 1). Phylogenetic analysis using RAPD distance software indicated two branches. Upper branch consist of 37 isolates while lower branch consisting of only three isolates (Fig. 2). Two of the three lower branch isolates were belonged to VDRL⁺ patients.

Twenty-one clusters can be distinguished in this dendrogram. The length of amplified fragments by all primers was varied between 200 base pairs (bp) and 4200 bp. By using primer OPD1 one specific amplified fragment with length 1300 bp were found in only eight isolates. All these isolates (six asymptomatic and two symptomatic) were belonged to addicted women. Results showed no significant difference in isolates from different geographical areas in Iran, but the difference between symptomatic and asymptomatic patients were significant ($P=0.009$). Our results confirmed the suitability of the RAPD technique for genealogical studies in *T. vaginalis*.

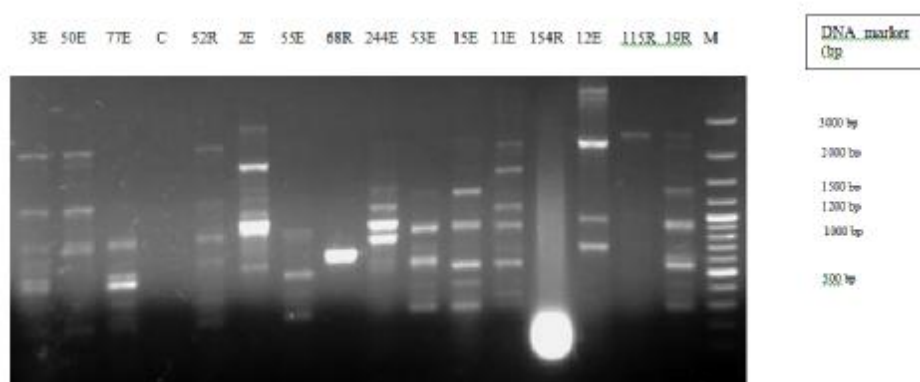


Fig. 1: RAPD banding pattern of *Trichomonas vaginalis* isolates using OPD3 primer. M= DNA marker, 1-20 banding pattern of *T. vaginalis* isolates

E=Evin R=Rajaie shahr V=Varamin M= size Marker bp=base pair C=Control
No. = Number of the patients according to questionnaires

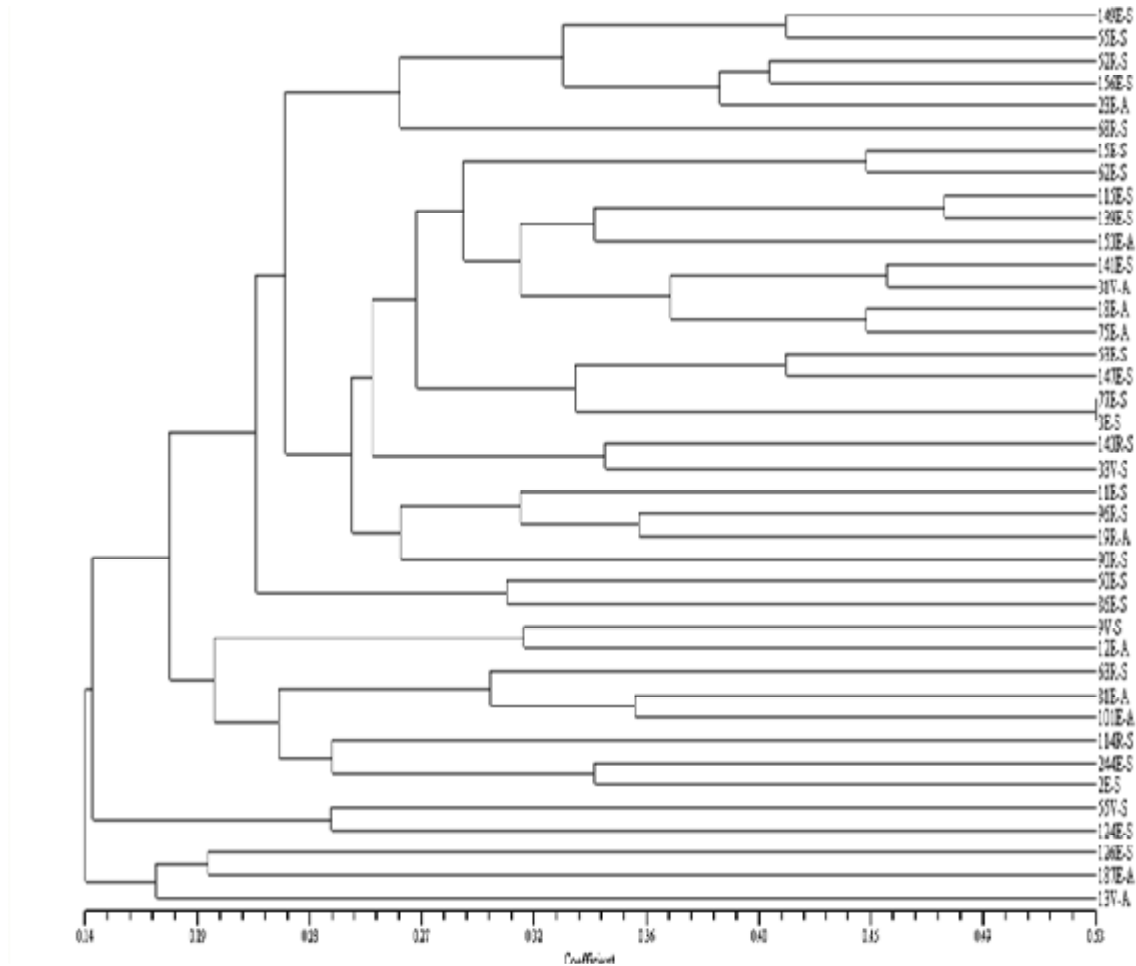


Fig. 2: Constructed dendrogram by using UPGMA method and NTSYS software based on data resulted from 40 isolates by RAPD method: S=symptomatic, A=asymptomatic, E=Evin, R=Rajae shahr, V=Varamin

Discussion

STIs are more common in high-risk behavior group in prisons such as addicted and prostitute individuals (8). Trichomoniasis also considered as a biological marker trigger and evaluation for other STD pathogens. The infection may cause discomfort during intercourse and urination, as well as irritation and itching of the female genital area, but many also are asymptomatic and remain as carriers

(9). It can increase one's susceptibility to viruses such as herpes, human papillomavirus and HIV, and thus to dead. Nucleic acid amplification assays are considered a new gold standard for *T. vaginalis* detection, although culture is still the most specific technique (10). The sequencing of the 5.8S rDNA gene and flanking ITS1 and ITS2 regions of *T. vaginalis* isolates from Philippines, reported

low genetic polymorphism (3), as the results of current study also showed. The report from India by Kaul et al. (11) indicated that OPD 3 had least (nine types) while OPD 4 had highest typing (18 types) ability, however in this study OPD8 indicated least (13 types) while OPD3 indicated highest typing (26 types) ability.

Phylogenetic analysis using RAPD distance software might be helpful to delineate the pathogenic mechanism(s) for its virulence. Geographic origins of *T. vaginalis* strains are not reflected in a phylogenetic tree (4). Rojas et al. (12) also used RAPD technique to determine genetic differences and correlate with patient's records among 40 *T. vaginalis* isolates. They reported four main different patients' categories (asymptomatic, symptomatic patients consist of light, moderate, and severe infection), however they did not have any concomitant vaginal infection. Simoes-Barbosa et al. did not find any correlation between the genetic relatedness of *T. vaginalis* isolates and clinical phenotype (6). There are a close genetic relationship and certain gene polymorphism among the *T. vaginalis* isolates, thus geographical origin plays little role to the genetic characteristics (13), as our results showed.

Although the positive patients were from different geographical areas in Iran, and the number of samples in two provinces were more than the others were, but results showed that there is not genetic diversity in isolated *T. vaginalis*. Thus, emphasis should be taken on clinical manifestation of patients, and asymptomatic patients should be diagnosed and cured. This is the first description of a 1300 bp possible marker for *T. vaginalis* in asymptomatic patients (six asymptomatic out of eight samples). Thus more study is needed to correlates this marker to asymptomatic patients, however to control the STDs public health program have to target asymptomatic infections.

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