Random amplified polymorphic DNA analysis of *Trichomonas vaginalis* isolates in Tabriz-Iran

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Abstract

*Trichomonas vaginalis* is the causative agent of human trichomoniasis. It is the most common nonviral sexually transmitted disease. The infection may be asymptomatic or may cause severe vaginitis and cervicitis in women. Despite its high prevalence little is known about its genetic variability and factors' leading to asymptomatic infections. The random amplified polymorphic DNA (RAPD) technique is a simple method to detect DNA polymorphism. We performed RAPD method with 4 different Random primers (OPD1, OPD2, OPD3, and OPD5) for typing the 120 isolates of *Trichomonas vaginalis* in Tabriz. Phylogenetic analysis was performed using SPSS program and dendrogram with two distinct clusters was constructed. The asymptomatic isolates tended to form a cluster, separate from symptomatic isolates. Further studies for better understanding the relationship is suggested.

Keywords: *Trichomonas vaginalis*, RAPD, Polymorphism

Introduction:

*Trichomonas vaginalis* is still a prevalent vaginal pathogen, affecting 180 million persons worldwide annually (Wang et al.,2000). Evidence from published studies exists that *T.vaginalis* is independently associated with a variety of adverse health consequences in both women and men, including increased human immunodeficiency virus (HIV) transmission, infertility, cervical intraepithelial neoplasia(CIN) development in women, and nongonococcal urethritis and chronic prostatitis in men (Jane et al.,2003; Soper et al.,2004). Detection of *T.vaginalis* has traditionally relied on wet-mount microscopy or culture. These methods are highly specific but lack sensitivity. Nucleic acid amplification assays are highly desirable alternatives to culturing, having both sensitivity and specificity for detecting *T.vaginalis* DNA(Schee et al.,1999). The random amplified polymorphic DNA (RAPD) technique represents as an efficient tool for the study of genetic polymorphism of DNA. It involves the amplification of random segments of genomic DNA by polymerase chain reaction (PCR) using short single primers of arbitrary sequences (Fraga et al., 2002).

Different studies suggest that RAPD provides powerful markers to analyze the genetic diversity in *T.vaginalis* (Rojas et al., 2004; Kaul et al., 2004; Vanacova et al.,1997). The aim of this study was to genetically characterize by RAPD a collection of *T.vaginalis* isolates from patients with clinical sings and symptoms.

Material and Method: A total of 2630 vaginal discharge specimens from women visiting the health care centers of Tabriz were examined. For each patient vaginal discharge was collected from the posterior vaginal forinx with 2 sterile cotton swabs. First swab were used for wet mount preparation and second one used to inoculate the culture media(Kupferberg Trichomonas Base,QUELAB,CANADA). Culture tubes were incubated at 37°C up to 7 days and examined microscopically on days 2 or 3,5and 7 after inoculation.Subsequently 120 *T.vaginalis* specimens were isolated axenically.

DNA Extraction: *T.vaginalis* cultures in log phase of growth in vitro were washed with PBS (PH=7.4). The pellet was suspended in 400 μl TE (10:1) buffer (PH=8). 60 μl SDS 10%
and 5 μl proteinase K (20 mg/ml) was added and incubated overnight at 55-65°C. 100 μl of Nacl and 80 μl of prewarmed (at 65°C) CTAB /Nacl solution was added and vortexed well. Then incubated at 65°C for 10 min 700 μl of chloroform isoamyl alcohol (24:1) solution was added and vortexed for 20 seconds. The suspension was centrifuged at 11000g for 8 min at 10°C. The supernatant was discarded and the DNA was precipitated with 1ml of cold ethanol (70%) by centrifugation at 12000g for 5 min at 10°C. After air drying, the DNA was dissolved in 50-100 μl T/E buffer(10:1)(PH=8).

RAPD PCR: 4 different 10 base pair primers were used for RAPD Analysis (their sequence is in table 1). The DNA amplification was performed at final volume of 25 μl containing: 2.5 μl of 10X PCR reaction buffer (500 mM KCl and 200 mM Tris-Hcl, PH=8.4), 1.25 μl Mgcl2(50mM), 1 μl of each primer (Cinnagen, Iran), 0.5 μl of mixed dNTP(10 mM), 4 μl of template DNA, 15.35 μl of double distilled water and 0.4 μl of Taq DNA polymerase (5 unit/μl, Cinnagen, Iran). Negative controls for each of 4 primers used contained all component except template DNA.

The amplification profile consist of an initial denaturation step at 94°C for 5 min followed by 40 cycles repetitions of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. The final cycle “the extension step” was 15 min at 72°C. PCR products were analyzed by electrophoresis in 1.2% agarose gel in TBE buffer. The gels were then stained with ethidium bromide (0.5 μg/ml) and viewed under the U.V transiluminator.

Result: DNA extracted from the 120 isolates (74 symptomatic patient and 46 asymptomatic patients) was subjected to RAPD Analysis and amplified with 4 different Random primers. (Figure 1a & 1b shows the RAPD patterns obtained with primers used). The isolates with similar banding pattern were considered as a single type. OPD1 had the least typing ability (32 types) and OPD5 had the highest typing ability (58 types). Bands scored as present (1) or absent (0) for each isolate, and matrix table was constructed. Dendrogram based on Ward method was built using SPSS 11.0 program (Fig.2). According to phylogenic tree the isolates fell into two major groups the upper branch consisted of 65 isolates which 24 of them were from symptomatic patients and 41 isolates belong to asymptomatic patients. The asymptomatic isolates tended to form a cluster separate from symptomatic isolates. The lower major branch of tree, consist of 55 isolates, was more related to the isolates from symptomatic patients (50 symptomatic isolates).

Discussion: Recent studies have shown the ability of DNA fingerprinting techniques in differentiating strains of various organisms (Tibayrenc, 1998). Vanacova et al., (1997) for the first time used RAPD technique for phylogenetic analysis of T. vaginalis and find it a useful method in epidemiological analysis. Their result suggested a concordance between the genetic markers with resistance to Metronidazole and clinical findings, but they found no concordance with the presence of Trichomonas vaginalis virus (TVV) and the virulence of strains.

Hample et al., (2001) assayed the relationship between 20 strains of T. vaginalis from 8 countries using RAPD analysis and they found that the phylogenetic tree reflects the pattern of virulence, geographic origin or infection by TVV. Rojas et al.,(2004) used RAPD technique in 40 isolates of T. vaginalis to find the association between genetic polymorphism of organism with clinical characterization. Their results emphasize that the severity of infection depends on the genetic type of T. vaginalis involved.

In present study we used RAPD method for genetic analysis of T. vaginalis of 120 clinical isolates and we investigated the association of T. vaginalis genetic polymorphism with clinical
classification as symptomatic or asymptomatic. In conclusion our results show that the isolates from asymptomatic patients tend to form a distinct cluster separate from symptomatic isolates, and shows that *T. vaginalis* isolates from patients with or without symptoms are genetically different. Further studies are necessary to better understand the relationship between genetic markers and the pathogenesis of organism.

**References**


Fig. 1.a - RAPD banding pattern obtained with OPD3 primer:
Lane 1: 100bpDNA ladder, Lane 3: Negative control, Lane 2 and 4-29 *T. vaginalis* isolates,
Lane 30: lambda DNA/Eco R I + Hind III Marker, 3

Fig. 1.b - RAPD banding pattern obtained with OPD1 primer:
Lane 1: 100bpDNA ladder, Lane 2-24 *T. vaginalis* isolates, lane 30: lambda DNA/Eco R I + Hind III Marker, 3
Table 1- The sequence of 4 primers used for RAPD analysis

<table>
<thead>
<tr>
<th>Primer</th>
<th>Size (mer)</th>
<th>Sequence (5’ to 3’)</th>
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<tbody>
<tr>
<td>OPD1</td>
<td>10</td>
<td>ACCgCgAAgg</td>
</tr>
<tr>
<td>OPD2</td>
<td>10</td>
<td>ggACCCAACC</td>
</tr>
<tr>
<td>OPD3</td>
<td>10</td>
<td>gTCgCCgTCA</td>
</tr>
<tr>
<td>OPD5</td>
<td>10</td>
<td>TgAgCggACA</td>
</tr>
</tbody>
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Fig. 2: Dendrogram for 120 isolates of Trichomonas vaginalis based on RAPD-PCR data.