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Double-stranded RNA viral infection of *Trichomonas vaginalis* (TVV1) in Iranian isolates

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ABSTRACT

The Totiviridae family includes a number of viruses that can infect protozoan parasites such as *Leishmania* and *Giardia* and fungi like *Saccharomyces cerevisiae*. Some isolates of *Trichomonas vaginalis* are also infected with one or more double-stranded RNA (dsRNA) viruses. In this study, the frequency of *Trichomonas vaginalis* virus (TVV1) was evaluated in Iranian isolates of *T. vaginalis* in Tehran, Iran. One thousand five hundred vaginal samples were collected from patients attending obstetrics and gynaecology hospitals associated with Iran University of Medical Sciences in Tehran, Iran from October 2015 to September 2016. *Trichomonas vaginalis* isolates were cultured in Diamond's modified medium. Nucleic acids were extracted using a DNA/RNA extraction kit and RT-PCR was performed. Among 1500 collected vaginal samples, 8 (0.53%) cases of *T. vaginalis* infection were found. Half (4/8) of the *T. vaginalis* positive cases were infected with TVV1. Phylogenetic mapping indicated that the Iranian isolates were most closely related to TVV1-OC5, TVV1-UR1. Iranian isolates of *T. vaginalis* were infected with TVV1. The frequency of viral infection (TVV1) in *T. vaginalis* isolates found in this study is higher than previously reported in Iran.

1. Introduction

Trichomonas vaginalis is a protozoan parasite that resides in the genitourinary system and is the causative agent of trichomoniasis [1]. Trichomoniasis is associated with an increased risk of cervical cancer, premature delivery and low birth weight. It may also be associated with increased risk of infection by HIV and papillomavirus [2–4]. The first line of treatment for trichomoniasis is metronidazole, but some strains have shown resistance [5].

Some isolates of *T. vaginalis* are infected with one or more double-stranded RNA (dsRNA) viruses belong to the family Totiviridae [6–10]. Totiviridae is a family that have 5 genera including *Giardia* virus, *Leishmania* virus, Totivirus, Victoria virus and *Trichomonas* virus. The *Trichomonas vaginalis* virus genera have 4 species TVV1,TVV2, TVV3 and TVV4 [11]. The family Totiviridae are viruses with mono-segmented dsRNA genomes and isometric virions that infect parasitic protozoa and fungi and includes *Giardia lamblia* virus (GLV), *Leishmania* virus (LV), *Trichomonas vaginalis* virus (TVV) and *Saccharomyces cerevisiae* virus (ScV), *Trichomonas vaginalis* virus was recently accepted for this family. Its name is based on the genus of its host organism, and phylogenetic analysis based on sequence that differentiate *Trichomonas* virus species from other family members [12–14].

Trichomonas vaginalis viruses (TVVs) are composed of isometric viral particles that have a 4500–5000-bp dsRNA genome [15,16]. The dsRNA genome of TVVs influences gene expression in *T. vaginalis,* as TVVs contain only two genes, which encode structural proteins including a capsid protein (CAP) and a virion-associated CAP-polymerase (POL) fusion protein [17].

TVV infection is associated with the transcriptional upregulation of the host P270 protein, a phenotypically variable surface immunogen [18,19]. This indicates that the virus or its gene products may regulate the transcription and translocation of P270 in *T. vaginalis* [15]. Furthermore, phosphorylation and cytoplasmic expression of P270 occur when there are high levels of iron in the medium of infected *T. vaginalis,* suggesting that iron takes part in

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regulating the surface localization of P270 when TVV is present [6].

The presence of dsRNA viral infection in *T. vaginalis* is also related to the expression of cysteine proteinases that are known virulence factors [20,21]. Cysteine proteinases are linked with *T. vaginalis* cytoadherence to the vaginal epithelium, cytotoxicity of the parasite and degradation of basement membrane components [22]. Thus, dsRNA viruses can induce various phenotypic changes that impact *T. vaginalis* virulence [13,16] and regulate the pathogenicity of *T. vaginalis* infections of the human genital mucosa [1]. This has also been found in the metronidazole sensitive strains of *T. vaginalis* [19].

Today, the near-complete cDNA sequences of 5 TVV strains [5,6,8,23,24] have been reported to GenBank.

Although TVV1 infection rate of 17.39% has been previously reported in Iran [25] but there was no data about phylogenic analysis based on TVV1 sequenced in Iran.

In this study, the frequency of TVV1 was evaluated in women attending obstetrics and gynaecology hospitals associated with Iran University of Medical Sciences, Tehran, Iran and phylogenic analysis based on TVV1 sequenced in Iranian isolates and those corresponding to different TVVs belonging to the genus *Trichomonas* virus of family Totiviridae was performed for the first time.

2. Materials and methods

2.1. Study population

In this cross-sectional study, 1500 vaginal samples were collected from patients who were referred to hospitals associated with Iran University of Medical Sciences, Tehran, Iran from October 2015 to September 2016.

2.2. Ethical issues

This study was approved by the Ethics Committee of Iran University of Medical Sciences in accordance with the Helsinki Declaration and guidelines and all human participation has been obtained in accordance with informed consent.

2.3. Sample collection and cultivation

Two vaginal swabs were collected from each participant. The first swab was stored in sterile phosphate-buffered saline (PBS; pH 7.3 ± 0.1). At the first wet mount samples were examined by light microscope with 40X magnification. The second swab was inoculated into a culture tube at 37 °C in Diamond's TYM medium [26] with 10% heat-inactivated calf serum, 100 U/mL penicillin and 30 μg/mL streptomycin sulphate. The parasites were harvested in late log phase and centrifuged at 2000 × g for 15 min. The pellets were frozen at -70 °C until use. In the cases that *Trichomonas* isolates showed no growth in Diamond's modified medium, they were cultured in enriched bovine serum.

2.4. RNA detection of TVVs in T. vaginalis cultural samples by RT-PCR

2.4.1. RNA extraction

Trichomonas vaginalis samples were washed with sterile PBS before centrifugation for 10 min at 4 °C at 5000 × g. The nucleic acids were extracted using the Pure Viral Nucleic Acid Kit Roche (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The viral RNA concentration was measured with a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, MA). The A_{260}/A_{280} of all RNA samples was ≥1.90 as previously described [27–29].

2.5. cDNA synthesis

Detection of TVV RNA in *T. vaginalis* samples was performed by reverse-transcription polymerase chain reaction (RT-PCR). Briefly, cDNA synthesis of isolated RNA was carried out at 42 °C for 30 min in a 20 μL reaction mixture containing 0.5 μg of total RNA, 20 pmol of random hexamer, 4 μL of 5x reverse transcriptase reaction buffer, 125 μmol dNTPS, 104 U Moloney Murine Leukemia Virus reverse transcriptase (Fermentas GmbH, St. Leon-Rot, Germany), 19.2 U RNase inhibitor (Fermentas GmbH) and 1 μL of diethylpyrocarbonate treated water, followed by heating at 72 °C for 10 min for inactivation of the reverse transcriptase [27].

2.6. PCR amplification

PCR was performed in a 25 μL mixture containing the template (3 μL of RT reaction), 2.5 U Taq DNA polymerase, 2.5 μL of 10x PCR buffer, 20 pmol of each primer, 100 μmol dNTPs and 0.15 mmol $MgCl_2$. Amplification was performed as follows: initial denaturing for 4 min at 94 °C; 40 cycles of 94 °C for 30 s, 54 °C for 35 s and 72 °C for 40 s; extension at 72 °C for 4 min. PCR products were electrophoresed on a 2% agarose gel. An amplified band of 204 bp corresponded to the TVV Capsid gene.

A pair of primers was designed based on the TVV1-Ch (Changchun) capsid protein gene was used for PCR amplification and sequencing. (Accession DQ528812.1). TVV1 F: 5′- CAC GCA CAT CTC AGA CAG TC -3′ and TVV1 R: 5′- GGG ATG GTT CCT GTA GTT C -3′ [25].

2.7. Sequencing and phylogenetic analysis

To confirm the TVV PCR results, a second round of RT-PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's instructions and were used for direct sequencing using the dye termination method and an ABI 3730xl sequencer. A phylogenetic tree was drawn using MEGA software version 7. Phylogenetic tree was drawn based on the sequences of amplified TVV capsid region with RT-PCR method and those corresponding to different TVV1-TVV4 (dsRNA viruses belonging to the genus *Trichomonas* virus of family Totiviridae) taken from the GenBank database. The optimum model of nucleotide substitution for making the phylogeny tree is JC + G. Bootstrap values lower than 70% achieved after 1000 replicates are not shown. The *Eimeria brunetti* RNA virus 1 (a member of Totiviridae family) is used as the outgroup virus.

The sequences were used in this study:TVV1-OC5(HQ607523.1), TVV1-UR1 (HQ607513.1), TVV1-1 (U08999.1), TVV1- C344 (JF436869.1), TVV1- Changchun (DQ528812.1), TVV1-T5 (U57898.1), TVV1-UH9 (HQ607516.1), TVV1-OC4 (HQ607521.1), TVV1-IH2 (DQ270032.1), TVV1- OC3(HQ607517.1), TVV2-OC3 (HQ607518.1), TVV2-UR1 (HQ607514.1), TVV2-OC5 (HQ607524.1), TVV2-C351 (JF436871.1), TVV2-1 (AF127178.1), TVV2-C76 (JF436870), TVV3-UR1 (HQ607515.1), TVV3-1 (AF325840.1), TVV3-OC3 (HQ607519.1), TVV3-OC5 (HQ607525.1), TVV4-1 (HQ607522.1),TVV4-OC3 (HQ607520.1),TVV4-OC5 (HQ607526.1), *Eimeria brunetti* virus EbV1 (AF356189.1).

2.8. Nucleotide sequence accession numbers

The cDNA sequences of TVVs described in this paper were deposited in GenBank with the accession numbers: KX611794, KX611795, KX611796 and KX611797.