Original Article

Double-Stranded RNA Viral Infection in Tehran Trichomonas vaginalis Isolates

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ABSTRACT

Background: Trichomonas vaginalis is a pathogenic protozoan and may be contaminated with dsRNA virus called Trichomonas vaginalis virus (TVV). The viral infection is an important factor for its pathogenesis and sensitivity to metronidazole. The presence of TVV is associated with qualitative and quantitative expression of cysteine proteinases and surface immunogenic; P270. The purpose of this study was to determine TVV frequency in T. vaginalis clinical isolates in Tehran, Iran.

Methods: The 46 T. vaginalis isolates were collected from Tehran Province and cultured in TYI-S-33 culture medium. Viral RNA was extracted and RT-PCR was done.

Results: Of 46 T. vaginalis isolates, 8 isolates (17.39%) were infected with TVV-1. There was not any association between patient age and TVV-infected T. vaginalis. There were 17.39% viral infection in T. vaginalis isolates which was lower than that reported by other researchers.

Conclusion: This is the first report on T. vaginalis isolates infection by TVV-1 in Iran.

Keywords: Trichomonas vaginalis, Viral infection, dsRNA virus

Introduction

Trichomonas vaginalis is a parasitic protozoa that causes trichomoniasis, a disease of global importance (1). Trichomonas vaginalis infection increases risk of HIV (2), premature labor during pregnancy (3), cervical cancer (4), pelvic inflammatory disease (5) and infertility(6). Some
T. vaginalis isolates are infected by a double-stranded RNA virus called T. vaginalis virus (TVV), a divergent group of Totiviridae virus family. It contains three viral genotypes and three satellite dsRNA species. The existence of various dsRNA virus species in some T. vaginalis isolates defines the presence of mixed viral infection. The vertical transmission of TVV occurs in cellular mitotic division time (7).

TVV is described as a heterogeneous population of icosahedral, filamentous, cylindrical and spherical viral particles with a size of 33-200 nm (8, 9). The genome size of the dsRNA is variable as 4.3 to 5.0 kb (10). Electron microscopy studies show that T. vaginalis isolates can be simultaneously infected by several types of virus-like particles (VLPs) (11). This viral infection may be an important aspect for T. vaginalis virulence and pathogenesis; on the other hand, it is also seen in the metronidazole sensitive strains of T. vaginalis. TVV increases the surface expression of the immunogenic protein called P270 (12).

The presence and replication of the viral dsRNA causes the phenotypic changes for the P270 prominent immunogenic among infected trichomonads. There is a relationship between the presence of TVV and upregulation of a major cellular immunogenic (12). Phosphorylation of P270 and cytoplasmic expression occurs in high iron rate. The iron level in medium is associated with the P270 surface expression in dsRNA- infected T. vaginalis. Furthermore, the P270 motion among cytoplasmic and plasma membrane which is iron-regulated is only observed in TVV infected T. vaginalis (13).

The difference in p270 gene expression in multiple virus infected T. vaginalis can make various genetic backgrounds in this parasite. The viral proteins may have important role in p270 gene transcription regulation in T. vaginalis (14).

TVV has a role in cysteine proteinase expression and quantitative and qualitative changes in the composition of total protein in the parasite (15). In addition, the TVV is responsible for different phenotypic changes in T. vaginalis that may have impact on virulence of parasite (16).

It is hypothesised that TVV can be able to translate the reductase enzyme which is responsible for reducing metronidazole and consequently drug sensitivity (8). The aim of this study was to determine TVV frequency in T. vaginalis clinical isolates in Tehran.

Materials and Methods

Collection and culture of Trichomonas vaginalis isolates

Forty-six isolates of T. vaginalis of vaginal discharge and urine samples were collected from Tehran Province, Iran during 2011-2012. The vaginal secretions and urine samples were cultured on the TYI-S-33 Diamond’s medium (17), supplemented with 10% heat-inactivated bovine calf serum at 37 °C. Antibiotics (100 U / mL penicillin, 30 μg / mL streptomycin sulfate) and anti-fungus (40μg/mL amphotericin B) were added to culture medium. Parasites were washed and concentrated in exponential phase at 2×10^6 cells per sample and saved at the – 80 °C for further work.

This study was approved by university ethics committee. An informed consent was written for each participant.

RNA Extraction

Samples were washed by 1x PBS (pH 7.4) for one time using centrifugation for 10 min at 4 °C at 8000×g. The nucleic acids were extracted using GeneJet™ RNA purification kit (K0731, Fermentas) according to manufacturer's instructions.

cDNA Synthesis

Five micrograms of viral dsRNA were heated for 5 min at 95 °C, and used as template for cDNA synthesis. The reaction mixture contained 200 units of RevertAid Reverse Transcriptase (Fermentas), 1x reaction buffer ( 5x reaction buffer contain: 50 mM Tris HCl pH 8.3, 50 mM KCl, 4 mM MgCl2, 10 mM
dithiothreitol), 0.2 mM dNTPs, and 20 pmol each of forward and reverse primer in 20 microliter final volume. Reaction was placed for 60 min at 42 °C. For inactivation of Reverse transcriptase enzyme, reaction was placed at 95°C for 5 min.

A pair of primers was designed based on TVV1 isolate Changchun capsid protein gene (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´.

**PCR amplification**

The PCR reaction was contained 20 pmol each of forward and reverse primer, 0.2 mM dNTPs, 1.5 unit of Taq DNA polymerase enzyme, 1.5 mM MgCl2, and 1x PCR buffer in 20 micro liters final volume. The reaction was done with following conditions for 40 cycles: denaturation at 94°C for 30 seconds, primer annealing at 51°C for one minute and extension at 72 °C for 30 seconds. PCR products were electrophoresed on 2% agarose gel. The primers were amplified 204bp of TVV Capsid gene.

**Results**

**Agarose gel electrophoresis**

*Trichomonas vaginalis* isolates were investigated using RT-PCR for TVV type 1. The 8 (17.39%) isolates of 46 were infected with TVV-1, and a 204 bp band was demonstrated on agarose gel electrophoresis (Fig. 1). PCR products were purified, sequenced and deposited to GenBank at accession numbers: AB701559, AB701560, AB701561, AB701562, AB701563, AB701564, AB701565, and AB701566.

**Statistical analysis**

All informations were analyzed using SPSS16 software, the Mann-Whitney statistical analysis showed no significant relationship between age and the presence of TVV in *T. vaginalis*.

**Discussion**

Since the presence of TVV in *T. vaginalis* is related to parasite virulence and pathogenesis, moreover, the TVV cause the increase metronidazole sensitivity, thus, the virus frequency in *T. vaginalis* clinical isolates is healthy important

Double-stranded RNA was not seen in vertebrates, but pieces of double-stranded RNA have been observed in phage-infected fungi (18), rat liver cells (19) and Burkitt lymphoma cells (20). Wang et al. reported double-stranded RNA virus in *Giardia* (21) and *T. vaginalis* (8). TVV is described as heterogeneous populations of icosahedral, filamentous, cylindrical, and/or spherical virus particles with a size of 33-200 nm (8, 9). The parts of the dsRNA genome size vary from 4.3 kbp to 5 kbp (10). It is believed that the virus is found in strains sensitive to metronidazole (22). The 17.39% infection rate of *T. vaginalis virus* type 1 reported in this study is lower than that of reported by others. Snipes et al. reported 50% infection rate in 109 *T. vaginalis* isolates in the USA (22). Hampel et al. reported the infection rate of 44% when analyzed 20 isolates from different geographic origins (23) and also Fraga et al. reported 55% infection rate in 40 *T. vaginalis* isolates from Havana, Cuba (24). The high infection rates of 81.9% and 75% have been reported in 72 isolates in South Africa (25).
and 28 isolates in the USA, respectively (26). On the other hand, the 17.39% infection rate reported in this study is more than of 14% infection rate reported in 22 isolates from Korea (27).

It seems that the difference in TVV infection rate in various studies is because of the difference in geographical zones and may be due to the difference in the method for virus detection and sample size. We used RT PCR for virus detection but others used electrophoresis of virus RNA genome.

A significant relationship between patients age and the presence of TVV in *T. vaginalis* has been reported. The patients infected with positive virus isolates are older than ones infected with negative virus isolates (26). However in this study there was no relationship between patients age and TVV presence. Our sample size is more than Wendel et al. (26), but results are different (17.39% and 75% respectively). So it can be said that examination on the more samples due to the better statistical conclusion.

**Conclusion**

This is the first report on *T. vaginalis* isolates infection by TVV-1 in Iran. There was 17.39% infection frequency rate in *T. vaginalis* isolates by dsRNA virus.

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**References**

1. World Health Organization. Global prevalence and incidence of selected curable sexually transmitted infections: overview and estimates. HIV/AIDS Publications. WHO, Geneva, Switzerland. 2001.
2. Sorvillo F, Kerndt P. *Trichomonas vaginalis* and amplification of HIV-1 transmission. Lancet. 1998;351:213-14.
3. Minkoff H, Grunebaum AN, Schwarz RH, Feldman J, Cummings M, Crombleholme W, et al. Risk factors for prematurity and premature rupture of membranes: a prospective study of the vaginal flora in pregnancy. Am J Obstet Gynecol. 1984;150:963-72.
4. Zhang ZF, Begg CB. Is *Trichomonas vaginalis* a cause of cervical neoplasia? Results from a combined analysis of 24 studies. Int J Epidemiol. 1994;23:682-90.
5. Moodley P, Wilkinson D, Connolly C, Moodley J, Sturm AW. *Trichomonas vaginalis* is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. Clin Infect Dis. 2002;34(4):519-22.
6. Grodstein F, Goldman MB, Cramer DW. Relation of tubal infertility to a history of sexually transmitted diseases. Am J Epidemiol. 1993;137:577-84.
7. Goodman RP, Ghabrial SA, Fichorova RN, Nibert ML. Trichomonasviruses: a new genus of protozoan viruses in the family Totiviridae. Arch Virol. 2011;156(1):171-179.
8. Wang AL, Wang CC. A linear double-stranded RNA in *Trichomonas vaginalis*. J Biol Chem. 1985;260:3697-702.
9. Benchimol M, Monteiro SP, Chang TH, Alderete JF. Virus in trichomonads—an ultrastructural study. Parasitol Int. 2002;51:293-8.
10. Khoshnan A, Alderete JF. Multiple double-stranded RNA segments are associated with virus particles infecting *Trichomonas vaginalis*. J Virol. 1993;67(12):6950-5.
11. Benchimol M, Chang TH, Alderete JF. *Trichomonas vaginalis* observation of coexistence of multiple viruses in the same isolate. FEMS Microbiol Lett. 2002;215(2):197-201.
12. Khoshnan A, Alderete JF. *Trichomonas vaginalis* with a double-stranded RNA virus has upregulated levels of phenotypically variable
immunogen mRNA. J Virol. 1994;68(6):4035-8.

13. Alderete JF. Iron modulates phenotypic variation and phosphorylation of P270 in double-stranded RNA virus-infected *Trichomonas vaginalis*. Infect Immun. 1999; 67(8): 4298-302.

14. Bessarab IN, Nakajima R, Liu HW, Tai JH. Identification and characterization of a type III *Trichomonas vaginalis* virus in the protozoan pathogen *Trichomonas vaginalis*. Arch Virol. 2011; 156(2):285-94.

15. Liu HW, Chu YD, Tai JH. Characterization of *Trichomonas vaginalis* virus proteins in the pathogenic protozoan *T. vaginalis*. Arch Virol. 1999;143(5):963-70.

16. Wang AL, Wang CC, Alderete JF. *Trichomonas vaginalis* phenotypic variation occurs only among trichomonads infected with the double-stranded RNA virus. J Exp Med. 1987;166(1):142-50.

17. Diamond LS, Harlow DR, Cunnick CC. A new medium for the axenic culture of *Entamoeba histolytica* and other Entamoeba. Trans Roy Soc Trop Med Hyg. 1978;72:431-2.

18. Lemke PA, Ness TM. Isolation and characterization of a double-stranded ribonucleic acid from *Penicillium chrysogenum*. J Virol. 1970;6(6):813-9.

19. Harel L, Montagnier L. Homology of double stranded RNA from rat liver cells with cellular genome. Nat New Biol. 1971;229(4):106-8.

20. Stern R, Friedman RM. Ribonucleic acid synthesis in animal cells in the presence of actinomycin. Biochemistry. 1971;10(20): 3635-45.

21. Wang AL, Wang CC. Discovery of a specific double-stranded RNA virus in *Giardia lamblia*. Mol Biochem Parasitol. 1986;21(3):269-76.

22. Snipes LG, Gamard PM, Narcisi EM, Beard CB, Lehmann T, Secor WE. Molecular epidemiology of metronidazole resistance in a population of *Trichomonas vaginalis* clinical isolates. J Clin Microbiol. 2000;38(8):3004-9.

23. Hampl V, Vanacova S, Kulda J, Flegr J. Concordance between genetic relatedness and phenotypic similarities of *Trichomonas vaginalis*. BMC Evol Biol. 2001;48:1-11.

24. Fraga J, Rojas I, Sariego I, Fernández-Calienes A. Double-stranded RNA viral infection in Cuban *Trichomonas vaginalis* isolates. Braz J Infect Dis. 2005;9(6):521-4.

25. Weber B, Mapeka TM, Maahlo MA, Hoosen AA. Double stranded RNA virus in South African *Trichomonas vaginalis* isolates. J Clin Pathol. 2003;56(7):542-3.

26. Wendel KA, Rompalo AM, Erbelding EJ, Chang TH, Alderete JF. Double-stranded RNA viral infection of *Trichomonas vaginalis* infecting patients attending a sexually transmitted diseases clinic. J Infect Dis. 2002;186(4):558-61.

27. Kim JW, Chung PR, Hwang MK, Choi EY. Double-stranded RNA virus in Korean isolate IH-2 of *Trichomonas vaginalis*. Korean J Parasitol. 2007;45(2):87-94.

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