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Identifying the Genotype of Giardia Lamblia in HIV+ Patients using the TPI Gene by PCR Method at the Center for Behavioral Diseases in Karaj

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ABSTRACT:

Background and Aim: Giardiasis causes a self-limiting clinical disease characterized by the symptoms such as diarrhea, abdominal cramps, bloating, weight loss, and malabsorption. The present study identifies the genotype of Giardia protozoa in HIV+ patients suffering from giardiasis.

Materials and Methods: Seventy stool samples from HIV+ patients were collected at the center for behavioral diseases in Karaj city and examined microscopically for Giardia cysts. In these samples, DNA purification was performed using the CTAB method and the genotype of Giardia parasites was identified using the Nested PCR method on the TPI gene.

Results: Out of 70 examined samples, 3 samples were positive for Giardia cysts (4.2%). Two samples were male (66%) and one sample was female (34%). After the amplification of the TPI gene, all three samples related to Group B were identified. After identifying the sequence, it was found that genotype BIII is the most common genotype at the center for behavioral diseases in Karaj city.

Conclusion: Group B is the most common genotype infecting Giardia Lamblia in HIV+ people at the Center for Behavioral Diseases in Karaj. Based on the identification of BIII genotypes in this center, there is a possibility of an anthropozoonotic transmission cycle.

Keywords: Giardia, Genotype, TPI, Center for Behavioral Diseases in Karaj

Introduction

Giardia is an intestinal protozoan parasite in humans and a wide range of vertebrate hosts (1). It is one of the 10 primary human parasites and one of the most common non-viral causes of diarrhea in humans and livestock (2). Giardia lamblia is the only species found in humans that have zoonotic significance (3). Based on the estimates, about 200 million people around the world suffer from the symptoms of intestinal giardiasis, and 500,000 new cases are reported annually (4). Economic poverty is directly and indirectly involved in the spread of this parasitic infection. Children are the most vulnerable group, especially in developing countries and deprived areas (5). According to the health significance of this parasite, especially among children in developing countries, Giardia was included among the forgotten diseases with the initiative of the World Health Organization in 2004 (6). Giardia duodenalis includes 8 genotypes with Group A-H, which are genetically different but morphologically similar. Groups A and B can infect humans and other mammals, while Group C to H has host specificity (7). The World Health Organization has classified giardiasis as a possible zoonotic infection since 1979.

Recent studies have evaluated the genetic diversity and geographic distribution of Giardia lamblia with human and animal origin and the potential for zoonosis transmission using different molecular genotyping methods. The transmission ways of Giardia duodenalis (zoonotic or anthroponotic) and potential sources of infection are possible by identifying the infecting genotypes in a geographical area. Multilocus Genotyping (MLG) is proposed as a standard method in the molecular epidemiology of Giardia isolates along with epidemiological data to determine the zoonotic potential, genetic difference, and transmission ways. In a molecular investigation, beta-giardin, glutamate dehydrogenase, and triosephosphate isomerase genes are used to identify the genotype individually or in combination (8).

Using one gene with the capability to identify all Giardia genotypes is cost-effective since molecular studies are expensive and the simultaneous use of three genes in identifying the Giardia genotype is time-consuming. The heterogeneity in the triosephosphate isomerase gene locus sequence has been

very useful in tracing the source of infection, especially if Giardia has a clonal population structure (for example, in a disease outbreak) (9, 10). The results of identifying genotype based on gene single-locus sequence analysis or very high heterogeneity such as beta-giardin or triosephosphate isomerase with similar results as MLG studies are obtained (11). In the present study, the genotypes of Giardia lamblia in HIV+ people were examined using the triosephosphate isomerase gene.

Materials and Methods:

The present study is a descriptive and cross-sectional study. The statistical population of the study included HIV+ patients at the Center for Behavioral Diseases in Karaj. Sampling was done from January 2018 to May 2019 at this center. All people were over 30 years old in this study. Seventy stool samples were prepared using expanded PVA and stained with trichrome dye. Then, they were examined microscopically. Then, DNA was extracted using the CTAB method (12). The polymerase chain reaction in this study was NestedPCR to increase the accuracy in identifying the genotype of human Giardia lamblia isolates. Specific primers of the triosephosphate isomerase gene could amplify 532bp. The list of the used primers is presented below.

Primary PCR:

F:AL3543(AAATIATGCCTGCTCGTCG)

R:AL3546(CAAACCTTITCCGCAAACC)

Secondary PCR:

F:AL3544(CCCTTCATCGGIGGTAACTT)

R:AL3545(GTGGCCACCACICCCGTGCC)

The primary PCR reaction was performed in a final volume of 15 µl, including 5.5 µl of parasite DNA, 7.5 µl of master mix (Ampliqon Company), and 1 µl of each of the 1F and 1R primers. The volume of the secondary PCR reaction was the same as the primary PCR reaction. 2F and 2R primers were used in this stage. The primary PCR product was used as template DNA in this stage. The thermal cycle conditions to perform the primary PCR were as follows. Initial denaturing at 95°C for 5 minutes, 30 cycles with a denaturing temperature of 95°C for 45 seconds, denaturing temperature of 55.5°C for 30 seconds, amplification temperature 72°C for 30 seconds, and finally 72°C for 10 minutes. The thermal cycle conditions in the secondary PCR were the same as the first stage, except for the annealing temperature.

The annealing temperature in the secondary PCR was 58°C. In this study, the Giardia parasite sample available in the parasitology department of Tarbiat Modares University was used as a positive control, and sterile distilled water was used as a negative control. Finally, the PCR product was electrophoresed on a 1% agarose gel stained with a safe DNA stain. Finally, 3 obtained isolates were sent to Fazapejooh Company for sequencing. Then, a phylogeny tree was drawn using the Neighbor-Joining algorithm and 500 repetitions. Giardia muris was used as an external group in drawing the phylogeny tree.

Results

In the present study, 70 stool samples of HIV+ patients were examined by staining and microscopic methods. Three samples (4.2 percent) were positive for giardia cysts. Out of these 3 samples, 2 samples (66 percent) were male and one sample (34 percent) was female. Their age range was between 41-60 years. The appearance and color of the samples were natural and consistent. After extracting the DNA of stool samples, PCR was performed on all samples in molecular tests using the triosephosphate isomerase gene, and the PCR product with a 532bp band was observed (Figure 1).

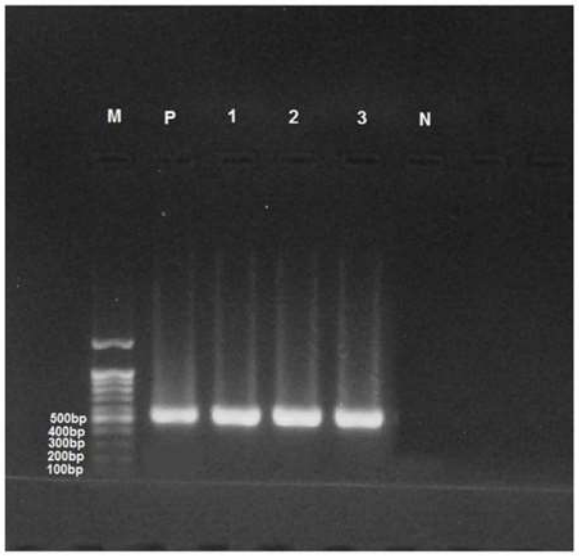

Figure 1: Electrophoresis of the TPI gene PCR product of Giardia lamblia isolates on a 1% agarose gel.

The phylogeny tree was drawn using the Neighbor-Joining Algorithm and 500 sequence repetitions of different genotypes. The drawn phylogeny tree shows that all three isolates belong to the BIII genotype.

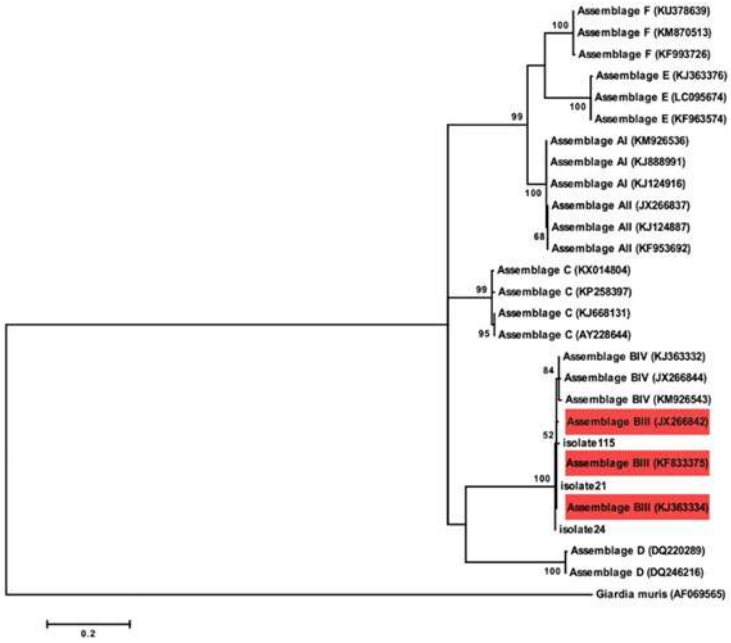

Figure 2: Phylogeny tree of identified Giardia lamblia genotypes and isolates identified based on TPI gene using Neighbor-Joining algorithm and 500 repetitions

Discussion

No study has been conducted so far to examine the prevalence of Giardia infection in this center. To identify the different genotypes of Giardia in stool samples, molecular methods based on PCR increased significantly. Various genes, such as beta-giardin and glutamate (hydrogenases and Triosephosphate isomerase) are used to identify the genotype of Giardia in clinical and environmental samples, especially water. GDH, TPI, and beta-giardin genes have been used in several molecular studies conducted in Iran. Conflicting results have been reported regarding the association between genotype and disease symptoms. In a study in Ethiopia, a strong association was found between genotype B and asymptomatic infection (13). A study by Hakio et al. in Bangladesh revealed that although genotype B is more common, it did not play a role in diarrhea (14). In a study by Manuchehri et al., a statistical correlation was observed between asymptomatic infections and genotype B and diarrhea with genotype A (15).

However, the factors related to the host also play a role in causing the disease. An extensive study is needed to better understand the role of different genotypes in causing clinical symptoms. The presence of genotype BIII was confirmed at this center after identifying the sequence and drawing the phylogeny tree. In a study by Rahimian et al. in Karaj, genotype A was introduced as a dominant genotype. However, genotype B is more common in the world (16). In a study by Nasiri et al. in Baharestan city, results showed that group A was more common in asymptomatic people (17). In a study by Faria et al. on HIV+ patients in Rio de Janeiro, genotype B was introduced as the dominant genotype (18). The present study revealed that genotype B was more common than other genotypes at the health center in Karaj city, like in other parts of the world.

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