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Short Communication

Molecular Genotyping of *Giardia duodenalis* in Humans in the Yazd County, Central of Iran

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Received 15 Oct 2023
Accepted 10 Dec 2023

Keywords:
Giardia duodenalis;
Genotyping;
Assemblages;
Iran

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Abstract

Background: We aimed to investigate the molecular genotyping of *Giardia duodenalis* in Humans in the Yazd County, Central, Iran.

Methods: Total of 35 fecal samples were collected from patients referred to Yazd Central Laboratory, Yazd, Iran from February to July 2022. All the samples were included in this study after microscopic observation of *G. duodenalis*. DNA samples were extracted using related kit and were analyzed by Nano Drop. The molecular assessment was carried out using semi-nested PCR using the target gene of *gdh*. All amplified samples were sequenced using Sanger method. BLAST analyzed the sequences for assemblage identification.

Results: Out of 35 samples, 24 (68.57%) and 11 (31.43%) were male and female, respectively. All included samples were amplified using the specific *gdh* primer pair. The molecular analysis showed 17 isolates (48.57%) as assemblage BIV, 8 isolates (22.86%) as assemblage BIII, 6 isolates (17.14%) as assemblage AII and 4 isolates (11.43%) as assemblage AIII ($P<0.05$).

Conclusion: Assemblages A and B are the most prevalent in Central Iran. The molecular identification of *G. duodenalis* isolates from animals and implementing control programs

Background

Giardiasis is a common intestinal infection caused by *Giardia duodenalis*. This parasite mainly infects the small intestine and causes a range of symptoms, including vomiting and fever (1). Giardiasis, with annually 200 million infected cases and 500,000 deaths, is distributed worldwide (2-4).

G. duodenalis is transmitted through the ingestion of contaminated food or water. In addition, direct contact between individuals or the fecal-oral route can also facilitate the spread of this. Engaging in recreational water activities such as swimming in pools or contaminated lakes can also contribute to this transmission. Factors that increase the risk of giardiasis include drinking untreated or contaminated water, poor personal hygiene, international travel to endemic regions, close contact with infected individuals, and living in crowded or unsanitary conditions (3).

Molecular identification of *G. duodenalis* is necessary to detect prevalent assemblages in each area to disease control. There are eight assemblages of *G. duodenalis* from A to H with different host and geographic distribution. Assemblages A and B are common in humans and responsible for most human infections worldwide. However, other assemblages (C-H) are predominantly found in various animal species, including livestock, wildlife, and domestic pets (4). Each assemblage can be subdivided into multiple sub-assemblages, denoted by alphanumeric characters. However, the sub-assemblage nomenclature is still evolving, and there is no universally accepted naming convention (5). More studies classified the sub-assemblages of *G. duodenalis* based on different target genes such as *β -giardin* (*bg*) or *glutamate dehydrogenase* (*gdh*) genes (7). Understanding the genetic diversity and distribution of assemblages can help improve diagnostic methods, treatment strategies, and preventive measures for this parasitic infection (8).

Considering that there is no genetic data related to human giardiasis in Central Iran, this study was conducted to identify assemblages and sub-assemblages of *G. duodenalis* collected from patients referred to Yazd Central Laboratory in central Iran.

Methods

Ethical statements

All study participants completed a written informed consent before specimen collection. The study was approved by the Ethics Committee of Yazd University of Medical Sciences (IR.SSU.MEDICINE.REC.1399.200).

Sample collection

In this cross-sectional study, we included 35 stool samples from patients referred to Yazd central laboratory who were positive for *G. duodenalis* from February to July 2022. For each participant, a demographic questionnaire including age and sex was completed. The fecal specimens were transferred to the Department of Parasitology and Mycology laboratory, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. All samples were stored at -20 °C till the next steps.

Molecular identification

Based on the manufacturer's instructions, DNA was extracted using QIAamp DNA Stool Mini Kit (QIAgen Company, Germany). The extracted DNA was analyzed using Nano Drop and agarose gel electrophoresis for quantity and quality assessment. The samples were stored at -20 °C until the next experiments. Amplification was carried out by semi-nested PCR method using the specific primer pair of *gdh* gene in two rounds; the first round with 5'- GACGCATCAACGTCAACC-3' and 5'- GAGCTTCTCGCAAGCAAAC-3' at 58 °C for annealing step and the second round with 5'-CAGTACAACTCTGCTCTCGG-3'5'-GTTGTCCTTGCACATCTCC-3' at 61 °C

for annealing the primers. The amplicon sizes were 539 and 432, respectively (10). Amplification in both rounds was done using *Taq* DNA Polymerase Master Mix Red (2X; Amplicon, Denmark) and 0.5 mM of each primer. PCR products were visualized using 1% agarose gel electrophoresis. All sequences were assessed using BLAST for assemblage identification and submitted in GenBank, NCBI. The phylogenetic tree was drawn by MEGA 5.05.

Statistical analysis

The collected data were analyzed by SPSS (version 21, SPSS IBM corp., Armonk, NY, USA) using the Chi-square (X2) test. The *P*-value of < 0.05 was considered significantly different.

Results

Of the 35 samples collected, 24 (68.57%) were male and 11 (31.43%) were female. The age of the statistical population was 2-46 years, 25 (71%) were over 15 years old, while 10 (29%) were under 15 years old.

All 35 samples were amplified using semi-nested PCR with an amplicon size of 432 bp (Fig. 1).

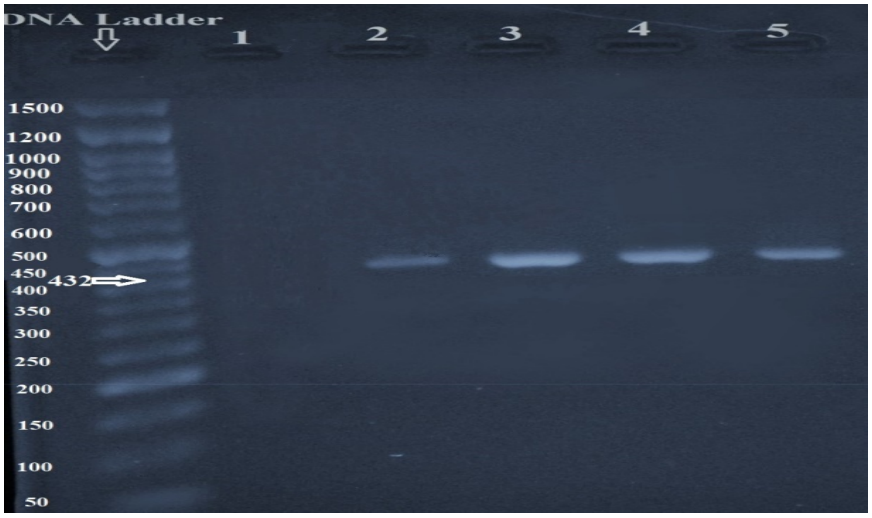

Fig. 1: Agarose gel electrophoresis for analyzing the amplification of the target gene of gdh in Giardia duodenalis. Lane 1: negative control; lanes 2-5: positive samples; Lane Ladder: 50 bp DNA ladder
The expected PCR product size was 432 bp

The genotyping results indicated 17 isolates (48.57%) as assemblage BIV, 8 isolates (22.86%) as assemblage BIII, 6 isolates (17.14%) as assemblage AII and 4 isolates (11.43%) as assemblage AIII based on the DNA sequence analysis of the glutamate dehydrogenase locus of *G. duodenalis*.

Assemblage BIV was found to be commonly present in both male and female patients. Interestingly, it was frequently observed in patients over 15 years old. On the other hand, in patients below the age of 15, both Assemblage BIV and AII had similar frequencies.

Fig. 2 displays the phylogenetic tree constructed using the maximum likelihood method to compare the obtained sequences at the *gdh* locus with representative reference sequences from GenBank. The accession numbers for the representative sequences in GenBank are ON646696, ON653213 to ON653217, OP265168 to OP265172, OP326605 to OP326609, OP576278 to OP576286 and OP641815 to OP641824.

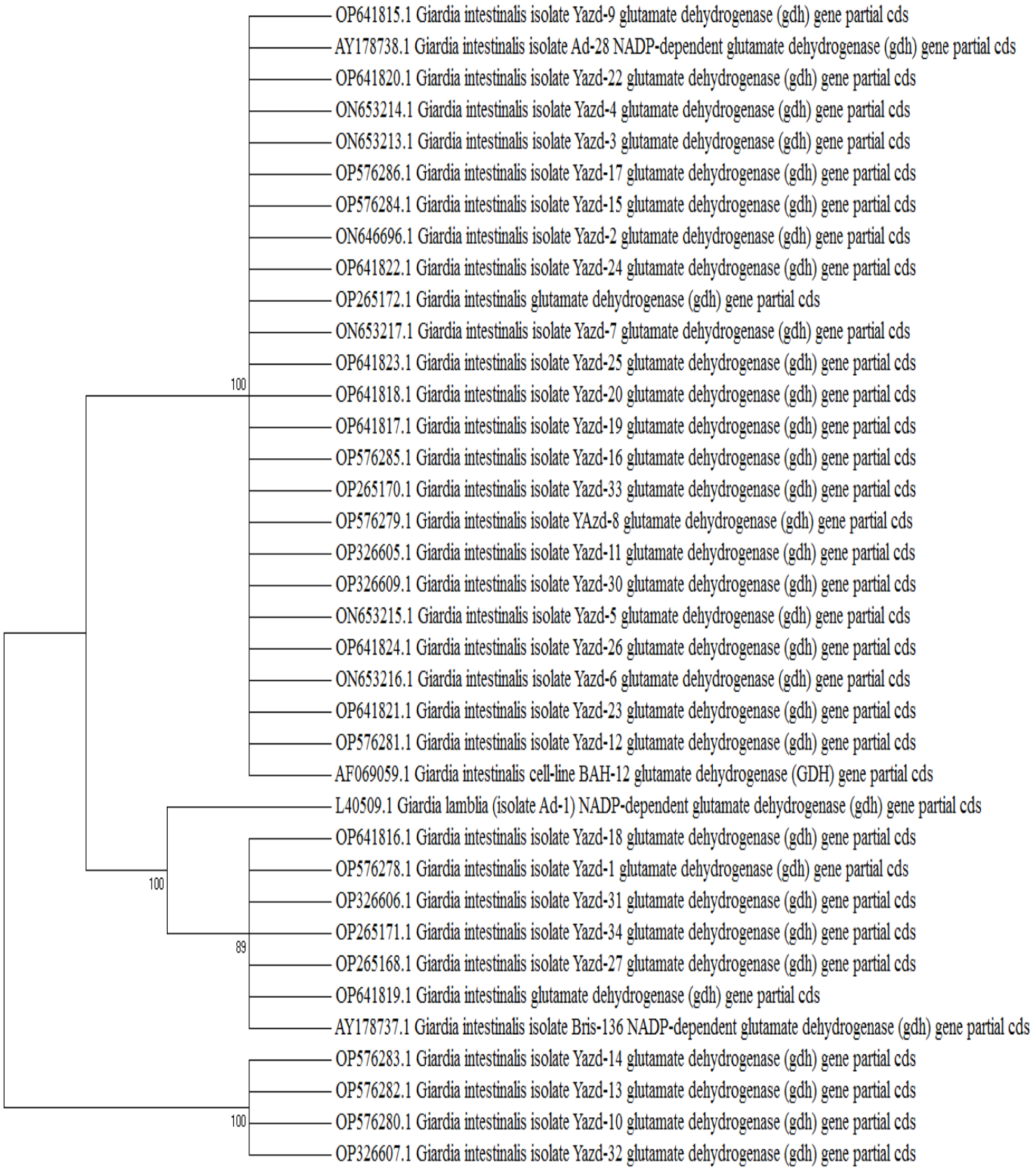

Fig. 2: The evolutionary relationships among *Giardia duodenalis* sequences at the gdh locus

Discussion

The age range of the patients in the study varied from 2 to 46 years old. Notably, 71% of the patients were over the age of 15, which aligns with similar findings from previous studies (9, 10). Additionally, data collected from one study indicate a higher prevalence of positive cases among male patients. This finding is consistent with Al-Mekhlafi et al (11). One possible explanation for this discrepancy could be that males are more exposed to the disease-causing agent than females. However, it is crucial to highlight that further research is necessary to understand the underlying factors contributing to this observation comprehensively. Moreover, additional research may be warranted to investigate the reasons behind the observed gender and age differences in *G. duodenalis*.

One molecular study on *G. intestinalis* have predominantly utilized the analysis of various genes, including the small subunit ribosomal RNA (SSU-rRNA), β-giardin (*BG*), glutamate dehydrogenase (*gdh)*, elongation factor 1-alpha (EF-1), triose phosphate isomerize (*tpi*), and variant surface protein (VSP) genes (13). The *gdh* locus variable has been effectively utilized to genotype and subtype *G. duodenalis* isolates from various vertebrate hosts (14). In this study, all samples tested positive for the *gdh* gene in the semi-nested PCR. In multiple regions, worldwide, diverse studies rely on the *G.* gene for molecular diagnosis and genotyping of *G. duodenalis*. Various studies (15-18) also used *gdh* gene for molecular diagnosis and even genotyping of *G. duodenalis*. Genotyping techniques, such as Nested-PCR and sequencing, are widely employed as sensitive and powerful analytical tools in various fields. One beneficial gene for genotyping *G. duodenalis* is the *gdh* gene.

In this study, assemblage B was more prevalent than assemblage A. The discovery was comparable to the results of research conducted in Malaysia (19), India (20), Australia (21), UK (22), Bangladesh (23), as well as studies conducted in the southern (15) and northwestern regions (24) of Iran. However, the findings of studies undertaken in Shushtar (25), Andimeshk (14), and Shiraz (26) in Iran, as well as Brazil (17) and Egypt (27), do not support this conclusion. These studies have found that assemblage A was the dominant one, contradicting the statement above. The prevalence of each assemblage can vary from country to country and sometimes even within the same country. These variations could be associated with differences in the total number of samples that have been genetically tested in each region and the specific socioeconomic and epidemiological factors of the population under study.

Our study reveals a seasonal trend, indicating that infection rates reach their peak during the summer. This finding aligns with other similar studies (28-30). The significant increase in infection cases during this season is likely attributed to the higher consumption of contaminated vegetables and fruits. Furthermore, the warm climate of Yazd City during the summer creates favorable conditions for the cyst's survival.

By analyzing the comparative phylogeny of 35 samples sequenced for the *G. duodenalis* gdh gene at the Yazd Central Laboratory, Iran, and comparing them with one another, it can be concluded that these samples can be grouped into two clusters. It was found that 25 samples belong to assemblage B, with 17 samples falling under sub-assemblage BIV and 8 samples under sub-assemblage BIII, forming cluster I. The remaining 10 samples are associated with assemblage A, with 6 samples falling under sub-assemblage AII and 4 samples under sub-assemblage AIII, creating cluster II. This analysis demonstrates the genetic distance between different assemblages of *G. duodenalis*.

The limitations of this study included the lack of sample size, geographical representation, confounding factors, and resource constraints.

Conclusion

Various essential factors, including socioeconomic, geographical, sanitary and hygienic, cultural, and nutritional factors, play a significant role in determining the prevalence of the parasite. The molecular analysis conducted in this study showed that assemblages A and B are the most prevalent types of human giardiasis in Yazd city. Furthermore, giardiasis could potentially be a zoonosis. To prevent the transmission of giardiasis to humans, it is recommended to carry out molecular identification of *G. duodenalis* isolates found in animals and implement control programs.

Acknowledgements

The Present Article results from an MSc thesis under the code number 9038, financially supported by the Deputy Research of the School of Medicine, Yazd University of Medical Sciences. We want to express our sincere gratitude and special thanks to all the personnel, Especially Mrs. Raziyeh Asadi, an expert in laboratory sciences of Yazd Central Laboratory, Iran, for their cooperation.

Conflicts of Interest

The author declares that they have no conflict of interest.

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