Predominance of Giardia intestinalis assemblage B in diarrhoeic children in Sharkia, Egypt

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ABSTRACT

Background: Giardia intestinalis (G. intestinalis) is one of the most common human enteric protozoa that infect Egyptian children, causing most of the diarrhoeic outbreaks worldwide. Identification of G. intestinalis assemblage is important because of its role in determining sources of infection, in understanding the ecological and clinical impact of giardiasis, and hence its management and control.

Objective: Study of this cross-sectional sample aimed to determine the genotypes of G. intestinalis isolated from the feces of diarrhoeic children attending outpatient clinic of Zagazig University hospital.

Subjects and Methods: A single fecal sample was collected from each child (n=126). All samples were subjected to microscopic examination by direct wet mount before and after formal-ethyl acetate concentration. Positive samples were amplified by nested PCR (nPCR) and sequenced for intra and inter assemblage identification targeting tpi gene.

Results: Microscopic examination revealed detection of G. intestinalis in 38 samples (30.2%). In 36 (28.6%) samples there was assemblage B predominance in 34 (94.4%) isolates, among which 24 (70.6%) were subgenotype BIV and 10 (29.4%) were sub-genotype BIII. Only 2 (5.6%) samples had assemblage A; all of them were AII subgenotype. All studied patient’s demographic and clinical data showed no significant association with Giardia infection or prevailing Giardia assemblages.

Conclusion: G. intestinalis is the prevailing intestinal pathogen in diarrhoeic Egyptian children, with Giardia assemblage B predominance. These findings necessitate physicians’ attention and further genetic studies in Egypt and other endemic areas targeting different genetic loci, with the inclusion of larger population samples. This will lead to a better understanding of the ecological and clinical impact of giardiasis, its management and control.

INTRODUCTION

Giardiasis, an infection with G. intestinalis (synonyms: G. duodenalis, G. lamblia), is one of the most common intestinal protozoan present in humans, both in Egypt and worldwide¹². Clinical presentations of giardiasis vary, ranging from asymptomatic infection to chronic symptomatic infection. Infected patients present with diarrhea, steatorrhea, flatulence, and malabsorption and/or weight loss³⁴. Giardiasis can affect all age groups; however, children are the most affected age group, for whom it can cause severe acute diarrhea that may in turn lead to malabsorption, malnutrition with growth impairment⁶⁷⁻⁸.

The prevalence of human giardiasis varies from 2% in developed countries to 70% in developing countries⁹. In Egypt, prevalence of giardiasis is up to 48%¹⁰, a fact that makes Egypt a hyperendemic region according to the World Health Organization (WHO) criteria¹⁰. However, its true prevalence is unknown.

G. intestinalis is classified into eight assemblages from A to H¹⁰⁻¹³. Determination of Giardia assemblages is needed to understand routes of transmission, epidemiology, and range of the host, in addition to study of outbreaks¹⁴. In a report from Egypt, El Tantawy and Taman¹⁵, reported the predominance of assemblage B over assemblage A, and few mixed A and B. The aim of the present study was to update the understanding of the epidemiology of Giardia assemblages prevailing in children attending outpatient clinics of Zagazig University Hospital.

MATERIALS AND METHODS

Study type and populations: This cross-sectional study sample included 126 children (5 to 12 years old),
suffering from diarrhea and/or other gastrointestinal symptoms, attending outpatient clinics of Zagazig University Hospital, Egypt.

**Samples collection and processing:** A single stool sample was collected from each diarrheic child attending the outpatient clinic. All collected samples were examined microscopically with iodine wet mount, after formal-ethyl acetate concentration at Parasitology Department Laboratory, Faculty of Medicine, Zagazig University to detect *G. intestinalis* and other parasites. Part from each stool sample was freshly frozen at -20°C for copro immuno-molecular assays at the Laboratory of Molecular Medical Parasitology (LMMP), Kasr Al-Ainy Faculty of Medicine, Cairo University.

**Copro-PCR assay:** All samples microscopically positive for *G. intestinalis* trophozoites and/or cysts were subjected to genomic DNA extraction using Favor Prep stool DNA isolation Kit (Favorgen Biotech corporation ping-Tung 908, Taiwan), according to the manufacturer’s instructions. The extracted DNA was submitted to a nPCR procedure for amplification of a 530-bp region targeting the triosephosphate isomerase (*tpi*) gene, using AL3543 (19 bp): 5’-AAA TIA TGC CTG CTC GTC G-3’ and AL3546 (19 bp): 5’-CAA ACC TTI TCC GCA AAC T-3 for first PCR, AL3544 (20 bp): 5’-C C C T T C A T C G G I G G T A A C T T C-3′ and AL3545 (20 bp): 5’- GTG GCC ACC ACI CCC GTG CC-3 for nPCR. The reaction mixture and conditions were performed according to Sulaiman et al.

**Statistical analysis:** Data entry and analysis were performed using the statistical package for social sciences (SPSS) version 20. Mean ± standard deviation were used for analysis of descriptive data, while frequencies were used to analyze qualitative data. *Giardia* infection was the dependent variable and chi-square test was used to assess its significance of association with the independent variables (demographic data and clinical symptoms).

**Ethical considerations:** Fecal samples were collected from children after informing their parents about the purpose of the study and informed consent was obtained from parents of all children included in the study.

### RESULTS

*G. intestinalis* was detected in 38 samples (30.2%). Among them 36 (28.6%) samples were successfully amplified by nPCR and genotyped by sequencing using *tpi* gene (Fig. 1). Their age ranged from 5 to 12 years old with mean of 7.34 ± 2.33 without statistical significance (*P*=0.96). Both *Giardia* assemblage B and A (94.4% [n=34], 5.6% [n=2], respectively) were detected. All assemblage A samples were AII while subgenotyping of assemblage B was BIII and BIV (29.4% [n=10], 70.6% [n=24], respectively) (Table 1). All studied variables showed no significant association with *Giardia* infection or detected *Giardia* assemblage (Table 2).

### Table 1: Diagnostic yield of nPCR and sequencing among microscopically positive samples.

<table>
<thead>
<tr>
<th>Assemblage A</th>
<th>Assemblage B</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AII</td>
<td>BII</td>
<td>24</td>
</tr>
<tr>
<td>BIV</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td><strong>Positive microscopy</strong></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td></td>
<td>38</td>
</tr>
</tbody>
</table>

Ethical considerations: Fecal samples were collected from children after informing their parents about the purpose of the study and informed consent was obtained from parents of all children included in the study.
Table 2: Demographic and clinical data of the studied children positive by nPCR (n=36).

<table>
<thead>
<tr>
<th>Variables</th>
<th>n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group (years old)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;2-6</td>
<td>16 (44.5)</td>
<td>0.96</td>
</tr>
<tr>
<td>&gt;6-12</td>
<td>20 (55.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (66.7)</td>
<td>0.33</td>
</tr>
<tr>
<td>Female</td>
<td>12 (33.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical examination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>13 (36.1)</td>
<td>0.27</td>
</tr>
<tr>
<td>Flatulence</td>
<td>5 (13.9)</td>
<td>0.56</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>5 (13.9)</td>
<td>0.56</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7 (19.4)</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Stool consistency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loose</td>
<td>11 (30.6)</td>
<td></td>
</tr>
<tr>
<td>Soft</td>
<td>15 (41.7)</td>
<td>0.23</td>
</tr>
<tr>
<td>Greasy</td>
<td>10 (27.8)</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

Diarrheal disease is one of the worldwide leading causes of children morbidity and mortality with 2 billion cases yearly[^18]. Giardiasis causes most of diarrhea cases worldwide[^19]. *G. intestinalis* was detected in 30.2% of diarrhoeic children from Zagazig city in our study. Phylogenetically, within genus *Giardia* there are 6 species, amongst which only *G. intestinalis* has been documented as infecting humans[^11,13]. Based on molecular analyses, 8 assemblages A-H of *G. intestinalis* species were recognized; assemblages A and B infect mammals including humans, while, assemblages C-H are zoonotic and more host specific[^10,12]. Lebbad *et al.*[^20] reported that there is limited zoonotic transmission among human *Giardia* genotypes. Different loci on multiple genes have been targeted for *G. intestinalis* molecular sub-typing, including small subunit ribosomal RNA, β-giardin, glutamate dehydrogenase, elongation factor 1-alpha (*gdh*), *tpi*, GLORF-C4 and the inter-genomic rRNA spacer region[^21].

β-giardin gene was considered a target for the molecular detection of *Giardia* spp. as it is unique to *Giardia[^22]*. Nevertheless, *tpi* gene is preferred by many studies due to its high genetic heterogenicity[^14,23]. The ssrRNA gene is the least used because it lacks specificity[^24,25]. Direct sequencing of an amplified DNA sequence remains the “gold standard” for accurate typing and sub-typing of *Giardia[^26]*.

Targeting the *gdh* gene by PCR-RFLP revealed that assemblage AII was much higher than assemblage BIII in cases from Menofeya and Sharkia Governorates[^27]. Also from Egypt, El Tantawy and Taman[^15], targeting the *tpi* gene, reported 62.14% as assemblage B, 31.07% as A, and mixed A and B in 2.91% with 3.88% of undetermined species. Hence with variable reports on prevalence, assemblage B seems to be the most prevailing assemblage for *Giardia* infections in Egypt as documented in many Egyptian studies in Cairo[^2,28], Kafr Elshiekh[^29], and Ismailia[^13,30].

Our study revealed a much higher assemblage B predominance (94.4%), with no association between *G. intestinalis* and the patient’s demographic and clinical data. There continues to be controversial reports
concerning correlation between *Giardia* assemblage (A or B) and clinical symptoms. Although there are reports on the lack of correlation between *Giardia* assemblage (A or B) and clinical symptoms, a few studies report significant correlation. In the report by El Tantawy and Taman, the detection rate of assemblage B was higher in samples from children with persistent diarrhea, whereas assemblage A detection rate was higher in samples from acute diarrhea. Thus, we corroborate the report of Rafiei et al. on the need for more studies to understand these relations.

*Giardia* was the prevailing enteric pathogen (38/126 [30.2%]) among our study group, indicating that physicians and public health professionals should consider *Giardia* in diarrhoeic children. Giardiasis seems to be endemic in Zagazig as none of the study population had travel history.

We amplified *Giardia* copro-DNA by nPCR for 36 of the 38 microscopically positive samples. Negativity of positive microscopic samples by PCR may be attributed to the different *Giardia* strains, which may include single-nucleotide polymorphisms, insertion-deletion, and rearrangements of chromosomes. The negative results could also be explained by the presence of inhibitors in stool that may affect the DNA amplification.

In conclusion, *G. intestinalis* is the prevailing intestinal pathogen found in diarrhoeic Egyptian children, with a predominance of *Giardia* assemblage B. These findings necessitate physicians’ attention when diagnosing diarrhoeic patients. Further genetic studies are needed, in Egypt and other endemic areas, targeting different genetic loci, with the inclusion of larger population samples. This will lead to a better understanding of the ecological and clinical impact of *Giardia* infection and its management and control.

**Author contribution:** All authors contributed equally in the work.

**REFERENCES**

15. El Tantawy NL, Taman AI. The epidemiology of *Giardia intestinalis* assemblages A and B among...


