

GIARDIA INTESTINALIS ASSEMBLAGES AMONG EGYPTIAN SYMPTOMATIC CHILDREN: PREVALENCE AND SEASONAL DISTRIBUTION IN CAIRO, EGYPT

By

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Abstract

Giardia intestinalis is one the commonest enteric intestinal protozoan pathogens worldwide, notorious for its seasonal prevalence and wide age span of population infected. *G. intestinalis* causes diarrhea in humans, especially in children. The present study aimed to evaluate the prevalence and genetic diversity of *G. intestinalis* isolated from Egyptian symptomatic children. A total of 176 faecal samples of children, from Aboul-Reesh Pediatrics Hospital, Cairo. Direct wet mount and formalin-ether sedimentation techniques were applied for microscopic determination of *Giardia* infection in faecal samples. DNA was extracted from positive stool samples and amplified by nested polymerase chain reaction (nPCR) using specific primers of *G. intestinalis* Triose Phosphate Isomerase (*TPI*) gene. Assemblages were determined using sequence analysis of the *TPI* gene. Out of the 176 samples, *G. intestinalis* was detected in 33 (18.75%) and 24 (13.63%) cases using Coproscopy and nPCR, respectively. There was a significant predominance of assemblage B (20 cases [83.33%]) versus assemblage A (4 cases [16.66%]) In this study, molecular analysis revealed that assemblages A and B are the most common types with a predominance of assemblage B. These findings suggest that anthroponotic transmission could be a dominant transmission route for giardiasis in Egypt.

Keywords: Children, *TPI* gene, *Giardia intestinalis*, PCR- sequence.

Introduction

Giardia intestinalis (syn. *G. duodenalis*, *G. lamblia*) is one of the commonest worldwide intestinal parasites reached up 30% among Eastern Mediterranean and African children (WHO, 2015). Giardiasis is notorious for its seasonal prevalence and wide age span of population infected (Savioli *et al*, 2006). *G. intestinalis* is the only zoonotic species infects man and animals (Fahmy *et al*, 2015). Its prevalence in Egypt was up to 27.3% among symptomatic children with chronic diarrhea (Bayoumy *et al*, 2010) and considered the most important non-viral infectious agent of human diarrhea in humans. In developing countries it caused about 2.5 million cases of childhood diarrhea annually (Younas *et al*, 2008).

Transmission occurs by faecal-oral route with food, and water or recreational water contaminated with cysts in feces, with some waterborne outbreaks from water contamin-

ated with faeces (Adam *et al*, 2016).

The severity of infectious enteric diseases, including giardiasis, is seasonal in distribution and prevalence (Lai *et al*, 2012). The main drivers of enteric disease seasonality are socio-demographic and behavioral factors as well as environmental factors; these factors affect giardiasis transmission (Ismail *et al*, 2016).

Clinical symptoms of giardiasis varied from asymptomatic infection to acute or chronic diarrhea with abdominal cramps, nausea, vomiting, bloating and malabsorption, which can lead to failure to thrive and retarded growth, as well as, allergic response to *Giardia* parasite or its products (Robertson *et al*, 2010; Hussein *et al*, 2017).

An epidemiological molecular determined genetic diversity of *G. intestinalis*, considered its seasonality, children ages, associated symptoms, and risk factors among Egyptian diarrheic children (Ismail *et al*, 2016).

Most targeted genes for molecular studies of *G. intestinalis* depended upon the analysis of small sub-unit ribosomal RNA (ssu-rRNA), glutamate dehydrogenase (gdh), triose phosphate isomerase (*TPI*), elongation factor 1-alpha (ef-1 α), GLORF-C4 genes, and β -giardin (bg) (Heyworth 2016).

Nucleotide sequencing of *TPI* determined the different genotypes of *G. intestinalis*, confirming the high genetic heterogeneity displayed by *Giardia* spp. at this locus. *TPI* sequences proved that some isolates *G. intestinalis* from animals are of zoonotic potential. Also, the *TPI* was useful in the taxonomy, differentiation, and detection of *Giardia* spp. (Sulaiman *et al*, 2003).

G. intestinalis complex is found in the intestine of a wide variety of vertebrate hosts. This complex is morphologically similar, but with genetically different assemblages (Cacciò and Ryan 2008). Some assemblages of *G. intestinalis* infect a wide range of mammal hosts, whereas others appear to be restricted to groups such as cats or dogs, and some are known to infect only one host species. The assemblages include A to H. Assemblages A & B isolates infect the broadest panel of hosts, including humans, and various other mammals. C and D infect dogs and cats, while E infects cattle, sheep, and goats. Furthermore, F infects only cats, G infects rats, and H infects seals (Lasek-Nesselquist *et al*, 2010; Feng and Xiao 2011).

Giardiasis is one of the most important public health problems due to its high prevalence and major impact on the patients' quality of life. *G. intestinalis* causes diarrhea in humans, especially in children.

The present copro-molecular study aimed to evaluate the prevalence and genetic diversity of *G. intestinalis* isolated from Egyptian children

Materials and Methods

Fecal samples were collected from 176 children of both sexes with ages ranged from 1 to 12 years, Aboul-Reesh Hospital, Cairo from the period of August 2016 to July 2017 throughout the different seasons of the year.

This study has been approved by the ethical committee of Aboul-Reesh Pediatric Hospital and performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Families of all children were informed verbally about the purpose of the study, and the collection of samples was performed after obtaining consents. Participation in the study was optional.

Microscopic examination: All fecal samples were collected from every child; a specimen part was preserved in formalin saline and examined directly as wet mount before and after concentration. Samples were then stored at -20°C, without preservative, for PCR analyses (Lab. of Molecular Medical Parasitology (LMMP), Faculty of Medicine, Cairo University, Egypt).

PCR: DNA extraction was performed with the favor stool DNA spin columns isolation mini Kit (Favorgen Biotech Corporation ping-Tung 908, Taiwan, Cat. No. FAS-TI001) with a modification of prolongation of incubation to 95°C for 1hr after thermal shock (cycling of deep freezing in liquid nitrogen for 5min and immediately transferred into a 95°C water bath for 5min, repeated for 5 cycles). Purified DNA was measured for concentration and purity.

nPCR of *TPI* gene: Nested PCR was done by analysis of the *TPI* gene included two consecutive PCR reactions. The first reaction amplified the 605 bp fragment by using an external pair of primer, and the second reaction amplified the 530 bp fragment, using two sets of oligonucleotide primers (Tab. 1). The reaction mixture consisted of 12.5 μ l PCR Master Mix, 1 μ l of each primer, 2.5 μ l of template DNA for the primary reaction and 1 μ l for the secondary reaction, 0.1 μ l Taq polymerase (as an activator), and 7.9 μ l of sterile distilled water to complete a total volume of 25 μ l. Cycling conditions were done (Sulaiman *et al*, 2003). Reactions were performed in a gradient thermal cycler after adjusting the thermal profile to initial dena-

turation at 95°C for 4min, followed by 35 cycles of amplification consisting of denaturation at 94°C for 60second, annealing at 65°C for 60 second, and extension at 72°C for 60 second. Final elongation was performed at 72°C for 10 min. The second-round PCR was identical to the first-round PCR except for denaturation at 94°C for 50second, annealing at 54°C for 35second, and extension at 72°C for 50 second. Amplified PCR products were separated by electrophoresis on 2% agarose gel in TBE buffer and visualized under a trans-illuminator after staining with ethidium bromide.

Sequence analysis of *TPI* gene: Genotyping was performed using sequence analysis of the nPCR products of *G. intestinalis*. The secondary PCR products were sequenced using AL3544 primer. Nucleotide sequences were edited manually and the sequence representatives for each of the assemblages were identified. The resulted sequences were analyzed and compared with similar *G. intestinalis* sequences deposited in the GenBank at NCBI database.

Statistical analysis: Aassessed potential association between the infection with *Giardia* and various studied risk factors (age, sex and seasonality), by a chi-square test of association for infected samples and the estimated risk factors. Significance of association was: $P > 0.05$: non-significant, $P < 0.05$: significant. Analyses were conducted using Graph Pad Prism V.5 software (Graph Pad Software, Inc., San Diego, California).

Results

This cross-sectional study was conducted with 176 samples of children to detect of *G. intestinalis* by using microscopy and molecular differentiation of the parasite genotypes.

By microscopy 33/143 samples (18.75%) were positive and 81.25% negative (Tab. 2). The prevalence of *G. intestinalis* was detected in both sex, but the infection in males exceeded that of females. *G. intestinalis* was detected in samples during summer, spring and autumn, but none in winter. All examined age groups, with infants (less than 2

years) showed the lowest prevalence (Tab. 3), But, without association between the assemblages of giardiasis and any risk factors as age, sex or infection seasonality (Tab. 4).

The PCR products produced targeting *TPI* were sequenced. Blast analyses of the obtained nucleotide sequences showed high similarity with *G. intestinalis* the *TPI* gene sequences were available in the NCBI database. The retrieved reference sequences together with the study sequences were subjected to multiple sequence alignment. Patterns of nucleotide variations were among the study sequences (Fig. 2), 20 (83.33%) sequences showed high similarity with *G. intestinalis* assemblage B (from G1-20) & 4 (16.66%) sequences showed high similarity with assemblage A (from G21-24).

Discussion

In the present study, 33 out of 176 (18.75%) examined fecal samples from symptomatic children were microscopically positive, but only 24 samples were positive using nPCR targeting *TPI* gene. Fahmy *et al.* (2015) reported that of 96 *Giardia* positive fecal specimens by microscopy, 77 samples were positive by nPCR. The negative results may be due to the presence of inhibitors in faecal samples, cyst quantity and quality, samples storage conditions, DNA extraction method, the gene targeted and the number of copies, and choice of primers and cycling conditions (Wilke and Robertson 2009).

The prevalence of giardiasis varied among children from different geographic locations between 1% and 36% and as high as 72% depending on the age group and country (Asher *et al.*, 2014). In Egypt, there was a discrepancy in the prevalence rate of *G. intestinalis*, which varied between 7.9% (el-Beshbishi *et al.*, 2005) and 35% (Fahmy *et al.*, 2015). El Shazly *et al.* (2006) reported 19.6% among inpatients in Mansoura University Pediatric Hospital and Ismail *et al.* (2016) in Cairo reported prevalence of giardiasis in cohort of Egyptian children.

In the present study, *G. intestinalis* prevalence among the children based on micro-

scopic examination was higher in summer (25%) than spring (20%) and autumn (15.15%), and zero in winter. In contrast, molecular prevalence using nPCR was higher in the spring (28%) than in autumn (15.15%) and lastly summer (6.9%). Using nPCR, there was significant association between the season in which the sample was positive and the status of *Giardia* infection ($P= 0.006$). Eraky *et al.* (2014) in Benha reported that the high risk of acquiring parasitic infection was in summer due to the consumption of raw vegetables. Ismail *et al.* (2016) reported that *G. intestinalis* was detected throughout the year with a seasonal peak in mid-summer and late winter. Pasley *et al.* (1989) in Arkansas, USA, reported that higher *Giardia* prevalence was in summer related to the corresponding increase in outdoor activity, water source usage and drinking of untreated surface water. González-Moreno *et al.* (2011) in Catalonia, Spain, reported no seasonality related to *G. intestinalis* prevalence

In the current study, the giardiasis prevalence by microscopy and nPCR was more in males than in females, but without significant difference. These results agreed with others (Abbass *et al.*, 2011; Ismail *et al.*, 2016; Hussein *et al.*, 2017), probably due to the higher activity of male children especially outdoor in contaminated environment. But, Al-Mekhlafi *et al.* (2010) in Ismailia reported that the predominance of *Giardia* was higher in females than males, while Helmy *et al.* (2014) stated that sexes did not correlate with giardiasis in Ismailia Governorate.

The occurrence and prevalence of giardiasis varies according to the age of patients (Abbass *et al.*, 2011; Ismail *et al.*, 2016). In the present study, children from 3 to 8 years showed highest infection rate (20.68%), followed by those from 9-12 years (17.18%) and the lowest was in infant from one month to 2 years (16%). This might be due to the outdoor activity of children from 3 to 8 years with lack the personal hygiene. This

evidence was reported in young child ren (Abbass *et al.*, 2011; Ismail *et al.*, 2016).

G. intestinalis has two major genotypes, assemblage A and B the most common types of human giardiasis. Assemblage A can be further classified into sub-assemblage AI, which is found in humans and also in many animals; sub-assemblage AII were in humans, as well as in a few studies; and sub-assemblage AIII is found exclusively in animals (Cacciò *et al.*, 2008; Heyworth 2016). Assemblage B subgroups are unresolved, and numerous assemblage B variants contributed to human and animal infections (Feng and Xiao 2011; Ryan and Cacciò 2013). Anthroponotic infections were more frequent with assemblage B compared to A (Bahrami *et al.*, 2017).

In the present study, two genetically distinct assemblages A and B of *Giardia* infections were recorded, assemblage B was predominant (83.33%) in comparison with assemblage A (16.66%). Similar assemblage distributions were reported in India (Traub *et al.*, 2004), Egypt (Foronda *et al.*, 2008), and Kuala Lumpur (Mahdy *et al.*, 2009). But, this differed from that in Saudi Arabia, Yemen, Iran and Ethiopia where assemblage A was higher than assemblage B (Gelanew *et al.*, 2007; Al-Mohammed 2011; Sarkari *et al.*, 2012; Alyousefi *et al.*, 2013). Others found that both assemblage A and assemblage B were present approximately with equal distribution (Roointan *et al.*, 2013; Choy *et al.*, 2014; Bahrami *et al.*, 2017).

Assemblage A was more zoonotic transmission, with a wide range of animals acting as reservoir hosts. Although assemblage B was most likely transmitted from man to man, and also detected in animals, representing a zoonotic potential as well (Feng and Xiao 2011; Lebbad *et al.*, 2011).

In the present study, though assemblage B was predominant among symptomatic persons, without association between giardiasis and any risk factors. This agreed with many studies (Molina *et al.*, 2011; Alyousefi *et al.*, 2013; Choy *et al.*, 2014; Puebla *et al.*, 2014;

Fahmy *et al.*, 2015).

Some studies reported a relation between *G. intestinalis* assemblages and clinical pictures, especially in children (Helmy *et al.*, 2014; Hussein *et al.*, 2017). El-Badry *et al.* (2017) and Hussein *et al.* (2017) reported a clear predominance of assemblage B among symptomatic children. Haque *et al.* (2005) in Bangladesh reported association between *G. intestinalis* assemblage B and asymptomatic infection. But, others reported a significant correlation between symptoms and assemblage A (Read *et al.*, 2004; Sahagún *et al.*, 2008; Sadek *et al.*, 2013; Fouad *et al.*, 2014). Nevertheless, many studies found no correlation between assemblages and symptoms (Choy *et al.*, 2014; Bahrami *et al.*, 2017). However, the relation between giardiasis assemblages and clinical manifestations remained unclear (Rafiei *et al.*, 2013).

Conclusion

In this study, molecular analysis revealed that assemblages A and B are the most common types of *Giardia* infection among Egyptian children, with a predominance of assemblage B. These data suggested that anthroponotic transmission could be a dominant transmission route for the giardiasis in Egypt.

Conflict of interest: The authors declared that they neither have conflict of interest nor received fund.

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Explanation of figures

Fig. 1: Agarose gel electrophoresis for products of nPCR targeting *TPI* gene of *G. intestinalis* at 530 bp .Lane 1 to 7: Positive samples. Lane 8: Negative control. Lane 9: Positive control. Lane 10: 100 bp DNA molecular weight marker "ladder".
 Fig. 2: Multiple sequence alignment of sequenced DNA and reference *G. intestinalis* sequences on NCBI-BLAST, with 514 nucleotide positions in this matrix data. Similarity regions between sequences shown as dots, while dispersed letters indicate nucleotide variations. Gaps represented in dashes, showed 2 patterns of nucleotide diversity, assemblage B pattern (G1-G20) and assemblage A pattern (G21-G24).



