Genotypic characterization of *Giardia duodenalis* in children in Menoufiya and Sharkiya governorates, Egypt

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Abstract: *Giardia duodenalis* is among the most common intestinal protozoa and is the most frequent parasitic agent of gastroenteritis worldwide. Chronic or recurrent giardiasis in children have been associated with malnutrition, wasting and stunting, most likely due to malabsorption caused by the parasites. Also, this parasite could cause reduced cognitive functions at later age. Genotypic characterization of *Giardia duodenalis* has been shown to be a useful tool in epidemiological studies and outbreak investigations. Molecular techniques based on polymerase chain reaction (PCR) in combination with techniques such as restriction fragment length polymorphism (RFLP) have been successfully used for differentiation of *Giardia duodenalis* genotypes. Considering the molecular differences and diversity of the prevalence of *Giardia duodenalis* assemblages in different regions of the world, and in view of probable correlation between *Giardia duodenalis* assemblage and clinical symptoms, this study was aimed to assess the genotypes of *Giardia duodenalis* isolates from patients with giardiasis in two governorates of Egypt namely Menoufiya and Sharkiya and its relation to clinical manifestations of the disease. Eighty samples were collected from Menoufiya Governorate and eighty one samples from Sharkiya Governorate. Samples were collected from children aged 5-12 years of both sexes. Samples were examined as wet smear after staining with Lugol's iodine. Negative samples were further examined by Trichrome stain. All positive samples were subjected to examination by PCR-RFLP to detect *Giardia duodenalis* genotypes. Prevalence of giardiasis in Menoufiya Governorate was 30% and it was 28.4% in Sharkiya Governorate. Assemblage AII represents 83.33% of Menoufiya samples and 70.59% of Sharkiya samples while assemblage BIII represents 16.67% of Menoufiya samples and 29.41% of Sharkiya samples. There was a high statistical significant association between assemblage AII and clinical manifestations of the disease, also between assemblage AII and age group 5-8 years. It was concluded that determination of the genetic grouping of *Giardia duodenalis* is a useful way to understand the infection route, to prevent infection effectively, to reveal the critical issues in the molecular epidemiology of this parasite, and finally to address important questions related to human health in Egypt. PCR-RFLP is a sensitive and powerful analytical tool that allows effective genotype discrimination within and between assemblages.

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1. Introduction

Giardia duodenalis (Synonyms: *Giardia intestinalis, Giardia lamblia)* is among the most common intestinal protozoa and is the most frequent parasitic agent of gastroenteritis worldwide. The regional prevalence of infection differs enormously and may be >30% in children in the African and Eastern Mediterranean region (Thompson & Smith, 2011 and Ignatius *et al.,* 2012). Prevalence of *Giardia duodenalis* is found in all age groups, but children are at the greatest risk for contracting clinical giardiasis, especially those attending day care centers (Boontanom *et al.,* 2011). Infection occurs by ingestion of viable cysts, which are transmitted through feco-oral contamination by direct person- to- person transmission, water-borne transmission, food-borne transmission, and animal-to-person transmission or by autoinfection (Helmy *et al.,* 2009 and Boontanom *et al.,* 2011). General characteristics of *Giardia duodenalis* influence the epidemiology of infection, for example, the infective dose in human is about 10 to 100 cysts, also cysts are immediately infectious when excreted in feces. Moreover, cysts are remarkably stable and can survive for weeks to months in the environment (Smith *et al.,* 2006 and Almeida *et al.,* 2010).

The parasite can cause gastrointestinal infections ranging from mild to severe as well as chronic disease. Both host factors (e.g. nutrition, immunity, co-infection with other agents) and pathogen factors (e.g. strain, infectious dose) are thought to contribute to the clinical severity of giardiasis (Johnston *et al.,* 2010). However, the majority of cases are asymptomatic or minimally symptomatic in immunocompetent individuals (Helmy *et al.,* 2009). In symptomatic cases, symptoms usually occur in six to fifteen days after infection. In the acute stage, symptoms can last from two to four days, and after that a chronic phase can appear and can last from few weeks to several years (Jaros *et al.,* 2011). Clinical

manifestations of symptomatic giardiasis include diarrhea, greasy stools, flatulence, abdominal cramps, epigastric tenderness as well as steatorrhea accompanied by full-blown malabsorption syndrome (Bertrand *et al.,* 2005 and Helmy *et al.,* 2009). Chronic or recurrent infections in children have been associated with malnutrition, wasting and stunting, most likely due to malabsorption caused by the parasites. Also, reduced cognitive functions may occur at later age (Berkman *et al.,* 2002 and Ignatius *et al.,* 2012).

Diagnosis of *Giardia duodenalis* is traditionally based on the detection of the parasites by light microscopy in direct stool smears or following concentration techniques, e.g. by formalin-ethyl acetate centrifugation. Multiple rather than single sample testing is recommended to improve sensitivity but this is often difficult to implement. Antigen detection immunoassays are of superior sensitivity but also more expensive (Regnath *et al.,* 2006). Highly sensitive molecular methods based on polymerase chain reaction (PCR) have been developed to detect *Giardia duodenalis* cysts in stool. The sensitivity of these methods was proved to be superior to microscopic examination in detecting low number of cysts in stool. Unfortunately, these methods are rarely applied in developing countries so far (Amar *et al.,* 2002; McGlade *et al.,* 2003 and Verweij *et al.,* 2004).

Although *Giardia duodenalis* isolates are morphologically identical, they vary significantly in their biology, virulence and genetics (Thompson *et al.,* 2000 and Sarkari *et al.,* 2012). Genotypic characterization of *Giardia duodenalis* has been shown to be a useful tool in epidemiological studies and outbreak investigations (Robertson *et al.,* 2006). Molecular techniques based on polymerase chain reaction (PCR) in combination with techniques such as restriction fragment length polymorphism (RFLP) have been successfully used for differentiation of *Giardia duodenalis* genotypes (Bialek *et al.,* 2002; Amar *et al.,* 2003; McGlade *et al.,* 2003 and Aydin *et al.,* 2004). Isolates of *Giardia duodenalis* are classified into seven assemblages based on the characterization of the glutamate dehydrogenase (gdh), small-subunit (ssu) rRNA and triosephosphate isomerase (tpi) genes (Van Keulen *et al.,* 2002; Read *et al.,* 2004; Caccio *et al.,* 2005; Papini *et al.,* 2007; Caccio & Ryan, 2008 and Sarkari *et al.,* 2012). These assemblages can infect different hosts. The analysis of human isolates from different geographical locations, examined by PCR amplification of DNA extracted from stool samples demonstrated that in almost all cases only *Giardia duodenalis* assemblages A and B are associated with human infections (Lasek-Nesselquist *et al.,* 2010 and Siripattanapipong *et al.,* 2011). Genetic assemblages C, D, E, F and G seem to be restricted to domestic animals, livestock and wild animals (Adam, 2001). The assemblage A isolates have been further grouped into subgroups I and II. The assemblage B isolates have been divided into subgroups III and IV (Siripattanapipong *et al.,* 2011 and Sarkari *et al.,* 2012). These assemblages also infect a wide range of other hosts, including livestock, cats, dogs and wild mammals (Babaei *et al.,* 2008 and Sarkari *et al.,* 2012). Some researchers believe that *Giardia duodenalis* presents as a risk of zoonosis from these animals (O'Handley *et al.,* 2000; Traub *et al.,* 2004 and Eligio-Garcia *et al.,* 2005). The prevalence of each assemblage varies considerably from country to country; assemblage B seems more common overall, but no strong conclusions can be drawn from current data (Almeida *et al.,* 2010). Assemblages have inconsistently been linked with symptoms: assemblage A parasites have been associated with more severe clinical symptoms as compared to assemblage B parasites in many areas (Read *et al.,* 2002 and Breathnach *et al.,* 2010), but the opposite has been reported from other areas (Homan & Mank, 2001 and Gelnew *et al.,* 2007) and no association in other areas (Kohli *et al.,* 2008).

Considering the molecular differences and diversity of the prevalence of *Giardia duodenalis* assemblages in different regions of the world, and in view of probable correlation between *Giardia duodenalis* assemblage and clinical symptoms, this study was aimed to assess the genotypes of *Giardia duodenalis* isolates from patients with giardiasis in two governorates of Egypt namely Menoufiya and Sharkiya and its relation to clinical manifestations of the disease.

2.Materials and Methods

Study design and study population:

From January 2012 to October 2012, fresh stool samples were collected in two governorates in Egypt namely Menoufiya Governorate and Sharkiya Governorate. Eighty samples were collected from Menoufiya Governorate and eighty one samples from Sharkiya Governorate. Samples were collected from children aged 5-12 years. They were classified according to age into two groups: group 1 including children between 5-8 years and group 2 including children between 9-12 years. Children were both males and females. Each child was asked about the presence of some clinical manifestations namely diarrhea, flatulence and abdominal cramps, also mothers were asked about presence of greasy stool.

Microscopic examination of stool samples:

Each stool sample was divided into two parts. The first part was concentrated by using zinc sulfate floatation technique according to the method of Sloss *et al.* (1999) and washed with phosphate buffer saline, then examined as wet smear after staining with Lugol's iodine to detect trophozoites and cysts of *Giardia duodenalis* by using x40

objective and then x100 objective. Negative samples were further examined by Trichrome staining after fixation in Shaudinn's fixative according to method of Wheatley (1951).The second part was kept at -20°C until use.

DNA extraction:

Stool samples which were proved to be positive by microscopic examination were subjected to DNA extraction using QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Extraction was done on the frozen parts of the samples.

PCR amplification of the gdh gene:

A DNA fragment (about 432 bp) of the gdh gene was amplified using a single PCR protocol by using the forward primer (GDHiF) 5'-CAGTACAACTCTGCTCTCGG-' 3 and the reverse primer (GDHiR) 5'GTTGTCCTTGCACATCTCC-'3 (Leonhard *et al.,* 2007 and Qader & Bakir, 2011). Amplification was done on the PCR mix which consisted of 2 ul of each primer, 5 ul of template DNA, and 20 ul of Dream Taq Green PCR Master Mix (Product No. K1081, Thermo Scientific, USA). Amplification consisted of one cycle of 94°C for 10 minutes (Initial heat inactivation step), 50 cycles of 35 seconds at 94°C, 35 seconds at 61°C and 50 seconds at 72°C, with a final extension of 7 minutes at 72°C. PCR products were separated by electrophoresis on 1.5% agarose gel and visualized under a transilluminator after staining with ethidium bromide (Qader and Bakir, 2011). PCR was conducted in Clinical Pathology Department at National Research Center.

RFLP analysis:

The restriction fragment length polymorphism (RFLP) analysis was performed by digesting 10 ul of PCR product (432 bp) with 2 ul of RsaI (product No. ER1121, Thermo Scientific, USA) or 2 ul of N1a IV (product No. ER1151, Thermo Scientific, USA) in 1X enzyme buffer in a final volume of 25 ul for 3 hours at 37°C (Qader and Bakir, 2011).

Statistical analysis:

Data were collected, tabulated, statistically analyzed by computer using SPSS version 16, two types of statistics were done:

1- Descriptive statistics:

*Qualitative data expressed in number and percentage.

*Z test was used to compare between two proportions.

2- Analytic statistics:

Fischer exact test and Chi-square (x^2) tests were used to compare categorical outcomes.

• ***P* value** > 0.05 was considered statistically non significant.

• ***P* value** < 0.05 was considered statistically significant.

•***P* value** < 0.001 was considered statistically highly significant.

3. Results

By examination of stool samples of children in Menoufiya and Sharkiya governorates, it was found that there were 24 positive cases out of 80 (30%) in Menoufiya Governorate and 23 positive cases out of 81 (28.4%) in Sharkiya Governorate. However, the difference between the prevalence in the two governorates was not significant (p>0.05) {Table 1, Figures 1& 2}.

Out of the 24 microscopically positive samples in Menoufiya Governorate, gdh gene was successfully amplified in 18 samples by PCR (75%) and out of the 23 microscopically positive samples in Sharkiya Governorate, gdh gene was successfully amplified in 17 samples by PCR (73.9%). However, the difference between them was not significant (p>0.05) {Table 2 & Figure 3}.

In Menoufiya Governorate, RFLP analysis showed that 15 samples out of the total 18 samples with successful amplification of the gdh gene by PCR were proved to be classified as assemblage AII (83.33%) while the remaining 3 samples were proved to be classified as assemblage BIII (16.67%). No samples examined by RFLP analysis were found to be related to either assemblage AI or BIV. In Sharkiya Governorate, RFLP analysis showed that 12 samples out of the total 17 samples with successful amplification of the gdh gene by PCR were proved to be classified as assemblage AII (70.59%) while the remaining 5 samples were proved to be classified as assemblage BIII (29.41%). No samples examined by RFLP analysis were found to be related to either group A1 or BIV. Statistical analysis showed that there was no significant statistical difference between the two governorates as regards distribution of *Giardia duodenalis* assemblages in positive stool samples (p>0.05) {Table 3 and Figure 4}. Expected fragment sizes were taken according to Qader & Bakir (2011) {Table 4}.

From the above mentioned data, total cases which were proved to be related to assemblage AII in both two governorates were 27 cases; also total cases which were proved to be related to assemblage BIII in both two governorates were 8 cases. It was found that out of 27 assemblage AII cases, 25 were positive for one or more clinical manifestations (92.6%) while the remaining 2 cases were negative for any clinical manifestations (7.4%). Also, it was found that out of the 8 assemblage BIII cases, 2 were positive for one or more clinical manifestations (25%) while the remaining 6 cases were negative for any clinical manifestations (75%). Difference between assemblage AII and BIII regarding correlation to clinical manifestations was highly statistically significant (p<0.001) {Table 5}.

By analysis of the correlation between age of infected children and *Giardia duodenalis* assemblage, it was found that all 19 cases related to group 1 (5-8 years) were proved to be related to assemblage AII (100%) while no cases were related to assemblage BIII (0%). In group 2, 8 cases were found to be related to assemblage AII (50%) and the other 8 cases were found to be related to assemblage BIII (50%). There was a high significant statistical difference between groups 1&2 regarding distribution of *Giardia duodenalis* assemblage AII & BIII (p<0.001) {Table 6}.

Table 1: Prevalence of giardiasis in children in Menoufiya and Sharkiya governorates as detected by microscopic examination.

Prevalence of giardiasis	Menoufiya Governorate (n=80)	Sharkiya Governorate (n=81)	*Z* test	*P* value
Total number infected	24	23	0.05	>0.05*
%	30%	28.4%		

*p>0.05= not significant.

Table 2: Results of PCR amplification of the gdh gene in microscopically positive samples in Menoufiya and Sharkiya governorates.

Results of PCR amplification of gdh gene	Menoufiya Governorate	Sharkiya Governorate	*Z* test	*P* value
No. of microscopically positive samples	24	23	0.25	>0.05*
No. of samples with successful amplification of gdh gene by PCR	18	17		
%	75%	73.9%		

*p>0.05= not significant.

Table 3: *Giardia duodenalis* assemblages in positive stool samples with successful amplification of gdh gene in both Menoufiya and Sharkiya governorates as detected by PCR- RFLP.

Assemblage	Menoufiya Governorate (Total samples=18)		Sharkiya Governorate (Total samples= 17)		Chi square test (x^2)	*P* value
	No.	**%**	**No.**	**%**	0.81	>0.05*
AI	0	0.00%	0	0.00%		
AII	15	(83.33%)	12	(70.59%)		
BIII	3	(16.67%)	5	(29.41%)		
BIV	0	0.00%	0	0.00%		

*p>0.05= not significant.

Table 4: The RFLP profiles of *Giardia duodenalis* assemblages after digesting with N1aIV and RsaI.

Assemblage	Restriction enzymes	Expected fragment sizes (bp)	Diagnostic fragment sizes (bp)
AII	N1aIV	20, 40, 50, 70, 80, 90, 120	80, 90, 120
BIII	N1aIV	50, 120, 290	120, 290
BIII	RsaI	30, 130 300	130, 300

Table 5: Correlation between *Giardia duodenalis* assemblage and clinical manifestations in patients with successfully amplified gdh gene in stool samples (Total= 35 samples).

Assemblage	No. of positive cases for one or more clinical manifestations		No. of negative cases for any clinical manifestations		Fischer exact test	*P* value
	No	**%**	**No**	**%**	15.99	<0.001*
AII (27 samples)	25	92.6%	2	7.4%		
BIII (8 samples)	2	25%	6	75%		

*p<0.001=highly significant.

Table 6: Correlation between *Giardia duodenalis* assemblage and age of patients with successfully amplified gdh gene in stool samples (group1=19 cases, group 2=16 cases).

	Age				Fischer exact test	*P* value
	Positive cases in group 1 (5-8 years)		**Positive cases in group 2 (9-12 years)**		12.31	<0.001*
Assemblage AII	No.	%	No.	%		
	19	100%	8	50%		
Assemblage BIII	0	0%	8	50%		

* p<0.001=highly significant.

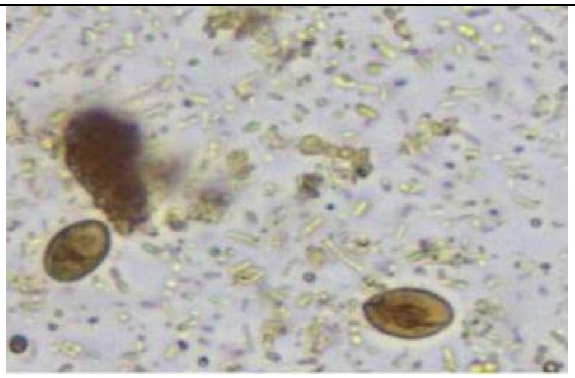

Figure 2: *Giardia duodenalis* cysts stained by Lugol's Iodine (x1000).

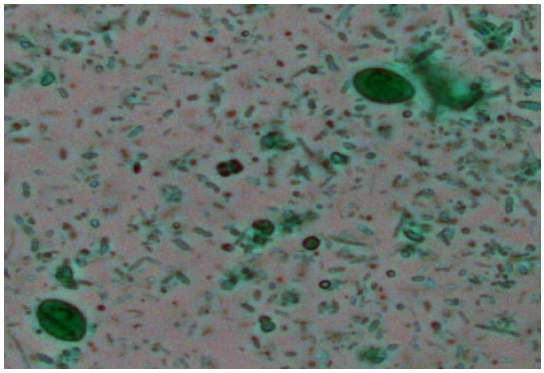

Figure 2: *Giardia duodenalis* cysts stained by Trichrome stain (x1000).

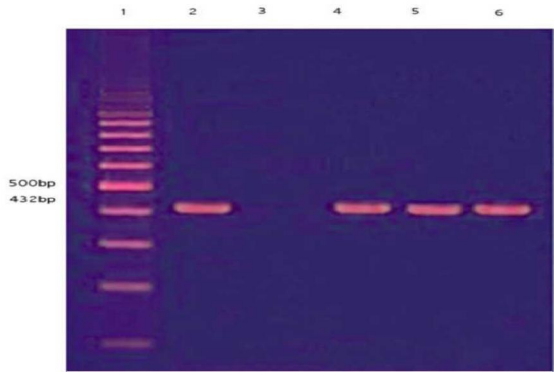

Figure 3: Agarose electrophoresis of PCR amplification for the gdh gene using GDHiF and GDHiR primers. Lanes 2, 4, 5 and 6 represent PCR product. Lane 1 represents 100 bp ladder.

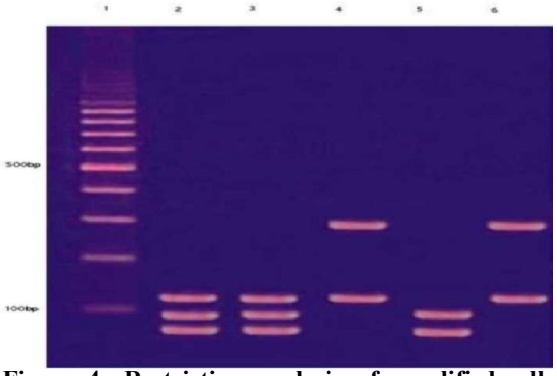

Figure 4: Restriction analysis of amplified gdh gene by PCR using N1aIV on an ethidium bromide-stained 1.5% gel. Lanes 2, 3 & 5 *Giardia lamblia* assemblage A II, lanes 4 & 6 assemblage BIII and lane 1 represents 100 bp ladder.

4.Discussion

Giardia duodenalis is a universal and well-known enteric protozoan that is found in the intestines of mammalian hosts, including both domestic & wild animals and humans. It is one of the most common gastrointestinal pathogens in children, causing severe intestinal disorder and growth retardation (Thompson and Monis, 2004). This protozoan was recently taken into the World Health Organization's (WHO's) " Neglected Diseases Initiative" (Savioli *et al.,* 2006).

Human giardiasis is caused by two genetically very distinct assemblages (A and B) of *Giardia duodenalis,* and a number of molecular assays have been developed for their specific detection in stool and environmental samples (Almeida *et al.,* 2010). All those assays are based on the in vitro amplification of specific gene fragments, but the detection formats differ, ranging from the simple analysis of restriction patterns to sequence analysis or to more versatile platforms, like microarrays and quantitative PCR (qPCR) {Guy *et al.,* 2003 and Guerden *et al.,* 2009}.

PCR-RFLP is a sensitive and powerful analytical tool that is capable of providing the level of genotype discrimination between and within assemblages by targeting some loci such as gdh and tpi, making it possible to identify the presence of mixed genotypes (Cedillo- Rivera *et al.,* 2003; Sulaiman *et al.,* 2003 and Itagaki *et al.,* 2005). It is important to note that all loci enable successful grouping at level assemblage of *Giardia duodenalis* isolates, and could characterize the sub-assemblages AI and AII, whereas only a few loci allow sub-assemblages differentiation within the B assemblage. Besides, markers like the small-subunit rRNA, the elongation factor 1α gene can only be used to discriminate major assemblages, whereas the gdh, tpi and β giardin genes allow us to distinguish between the subgroups of the assemblages A and B (Wielinga and Thompson, 2007).

Although the genotypic separation of *Giardia duodenalis* assemblages is relatively well established, the clinical or epidemiological significance of infection with assemblage A versus B is poorly understood (Helmy *et al.,* 2009). Hence, the present work was aimed to assess the genotypes of *Giardia duodenalis* isolates from patients with giardiasis in two governorates in Egypt, namely Menoufiya Governorate and Sharkiya Governorate and their relation to age of patients and clinical

manifestations of the disease that mentioned by those patients.

In the present work, it was found that the prevalence of *Giardia duodenalis* in Menoufiya Governorate was 30% and in Sharkiya governorate was 28.4%. However, difference between them was not significant. These ratios support the ratios previously reported in many studies in Egypt. For example, Shukry *et al.* (1986) reported a prevalence of 33% in Cairo residents. Curtale *et al*. (1998) detected *Giardia duodenalis* in 24.7% of fecal samples in Behera Governorate. On the other hand, Fawzi *et al.* (2004) found *Giardia duodenalis* in 10.4% in El-Prince (Alexandria) in Egypt. The low prevalence in this area may be attributed to the fact that the examined patients might have better living conditions.

In the current work, gdh gene was successfully amplified in 75% of samples in Menoufiya Governorate and in 73.9% of samples in Sharkiya Governorate, however difference between them was not significant. Our results are in agreement with the finding of El-Shazly *et al.* (2004) who reported an amplification percentage of 73.3% when working with fresh feces. However, our results exceed those of Van der Giessen *et al.* (2006) who reported amplification percentage of 52.9% in human fecal samples when they worked on the same gene with fresh feces. Moreover, our results exceed those reported by Qader and Bakir (2011) who found amplification percentage of 56.6% when working with the same primers and on the same gene. On the contrary, some researchers reported higher percentages, for example, Read *et al.* (2002) and Babaei *et al.* (2008) reported amplification percentages of 100% and 98% respectively when they worked on the gdh gene. The failure in the amplification of some fecal smears could derive from the quantity of samples DNA, either due to their degradation by time or may be due to presence of some of PCR inhibitors such as lipids, hemoglobin, bile salts, polysaccharides from mucus, bacteria & food degradation products (Abbaszadegan *et al.,* 2007). PCR inhibitors may act in 3 levels: interference in cellular lysis, degradation or uptake of nucleic acids or inactivation of thermostable polymerases (Molina *et al.,* 2007). However, it was proved that QIA amp DNA Stool Mini Kit is able to remove these inhibitors by absorbing them in the inhibitex matrix (Molina *et al.,* 2007 and Babaei *et al.,* 2008). Also, fecal samples used for molecular method diagnosis must be collected without preservatives since these reduce the sensitivity of the techniques due to DNA degradation. Therefore, the use of fresh feces would prevent the false negative results (Molina *et al.,* 2007).

By RFLP, it was found that no stool samples were related to *Giardia duodenalis* assemblage AI or BIV in both Menoufiya Governorate and Sharkiya Governorate. AII assemblage was found in 83.33% of Menoufiya samples and in 70.59% of Sharkiya samples. Also, BIII assemblage was found in 16.67% of Menoufiya samples and in 29.41% of Sharkiya samples. However, difference between the two governorates regarding distribution of *Giardia duodenalis* assemblages was not significant. Regarding absence of assemblage AI, our result is consistent with those of Amar *et al.* (2002) in the United Kingdom and Bertrand *et al.* (2005) in France.

Concerning the distribution of assemblage AII & BIII, results of the present work found that the prevalence of assemblage AII is far higher than that of BIII. These results are in agreement with the results of Babaei *et al.* (2008) who reported that the majority (87%) of the samples belonged to *Giardia duodenali*s assemblage AII in Tehran. Also, our results are in accordance with results of an Italian study that reported 80% of samples to be assemblage A by sequencing or PCR-RFLP analysis of β giardin gene (Caccio *et al.,* 2002). Moreover, our results are consistent with those obtained by Sarkari *et al.* (2012) who mentioned that the main assemblage of the isolates was AII followed by BIII. The same authors added that genotype A, especially AII is the frequent genotype in human isolates in various areas of the world. On the other hand, our results are contradictory with those obtained by Amar *et al.* (2002) in the United Kingdom who reported assemblage B in 64% of samples, Solaiman *et al.* (2003) in Peru who found assemblage B in 76% of samples, Guy *et al.* (2004) in the United States who mentioned that prevalence of assemblage B in the examined samples was 80%. Besides, our results contradict those obtained by Bertrand *et al.* (2005) in France who found that assemblage B prevalence was 61.5%. Moreover, results of the present work disagree those stated by Ignatius *et al.* (2012) who found that the majority of samples (85.9%) were assemblage B and only 12.7% were assemblage AII.

From the above mentioned results, it seems that the prevalence of assemblage AII versus B especially BIII differs greatly according to the examined locality. Results of the present work concerning higher prevalence of assemblage AII than assemblage BIII in Menoufiya and Sharkiya governorates are consistent with an anthroponotic origin of infection and ensure the fact that for human infection to occur, other humans are the main reservoirs while zoonotic source plays a minor role in the examined areas.

Several authors reported that origin of infection by assemblage AII is anthroponotic like Read *et al*. (2002) and Sprong *et al.* (2009). Also, Babaei *et al.* (2008) added that assemblage AI and genotype B have a more zoonotic potential than subgroup AII and have a more host range.

In our opinion, high prevalence of assemblage AII in the examined areas may be attributed to contamination of the drinking water especially by residential activities (e.g. sewage discharge). Several authors reported that contamination of drinking water is an important source of infection. Hilber (1988) analyzed 4,423 water samples from 301 municipalities in 28 states in the United States and cysts of *Giardia duodenalis* were detected in 26% of source water samples and in 11% of finished water samples. Besides, Wallis *et al.* (1996) detected *Giardia duodenalis* cysts in 18% of treated water samples from 72 Canadian cities. Moreover, Liang *et al.* (2012) stated that assemblage A isolates were obtained from 4 out 22 water samples and added that their results suggest that the risk of *Giardia duodenalis* transmission is greater from water borne than animal transmission in the study area.

In the current work, it was found that 92.6% of cases with assemblage AII were complaining of one or more clinical manifestations while only 25% of cases with assemblage BIII were complaining of one or more clinical manifestations. Difference between them was highly statistically significant ($p<0.001$). These results are in agreement with those of Homan & Mank (2001) who found that children infected with genotype A had a 26 folds greater risk of having diarrhea. Haque *et al.* (2005) stated that diarrhea was more associated with assemblage A than with assemblage B in Bangladesh. On the other hand, other studies have found a connection between symptomatic infection and assemblage B; also a connection between asymptomatic infection and assemblage A (Aydin *et al.,* 2004). The correlation between clinical symptoms of *Giardia duodenalis* and assemblage is controversial and some authors like Eligio-Garcia *et al.*(2002) stated that there was no correlation between clinical manifestations and genotypes. Anyhow, difference between results of the current work and other works may be attributed to difference in the age of patients or the level of their immunity.

The present work has found that 100% of children between 5-8 years (Group 1) were proved to be related to assemblage AII while 50% of children between 9-12 years (Group 2) were proved to be related to assemblage AII and the rest were related to assemblage BIII. Difference between them was highly statistically significant ($p<0.001$). These results are not unexpected as children of lower age (Group 1) have a lower immunity than older children (Group 2) so, it is a normal sequel to have the symptomatizing AII assemblage.

In conclusion, determination of the genetic grouping of *Giardia duodenalis* is a useful way to understand the infection route, to prevent infection effectively, to reveal the critical issues in the molecular epidemiology of this parasite, and finally to address important questions related to human health in Egypt. PCR-RFLP is a sensitive and powerful analytical tool that allows effective genotype discrimination within and between assemblages at targeting gdh gene. Our data suggests that there is an anthroponotic origin of the infection route, so it seems that the main reservoir of *Giardia duodenalis* infection in the two studied areas is human. Finally, effective isolation and lysis method of cysts from stool samples and purification of genetic materials are needed to obtain results with a higher sensitivity.

Author contribution:

Gehan S. Sadek and Magda A. El-Settawy proposed the research idea, proposed the study design, shared in collecting the samples & performing the laboratory work and wrote the manuscript. Soha A. Nasr shared in performing the laboratory work, interpreted the results of PCR and shared in reviewing the manuscript.

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