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Correlation between physical signs and clinical symptoms with *Giardia lamblia* genotyping.

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Abstract

Background: *Giardia lamblia* is an intestinal parasite found in a wide range of mammals, including humans. It is one of the most frequent gastrointestinal pathogens that may cause many clinical and other complications.

Objective: To determine the relationship between every *Giardia* sub-assemblage with the clinical picture and physical measurements of the cases. Measurement of some physical features, like weight and height using growth charts for weight, height, weight for height and body mass index.

Methodology: Case history, information about age, gender, and locality were collected and a questionnaire survey about diarrhea, vomiting, abdominal colic, abdominal distension and failure to thrive were done for 93 patients infected with *G. lamblia* molecularly after DNA extraction from stool samples by using (QiAmp® DNA stool Mini kit (Qiagen, UK). Amplification of glutamate dehydrogenase gene using specific primers by semi-nested PCR thermal cycler technique and Restriction of the DNA fragments using two restriction fragment enzymes (NIaIV and RsaI restriction fragment enzymes) was performed. This was followed by measurement of some physical features, like weight and height using growth charts for weight, height, weight for height and body mass index. The cases were taken randomly.

Result: Both *Giardia* assemblage B and *Giardia* assemblage A were poly-symptomatic, especially *Giardia* assemblage B. Diarrhea, abdominal distension, abdominal colic, failure to thrive and the decrease in weight for age, weight for height and body mass index were associated with both the types but more with assemblage B as compared to assemblage A. The decrease in height for age was also associated with both types but more with assemblage A.

Keywords: Clinical picture*, Giardia lamblia,* Physical features, Primers, Assemblage.

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Introduction

Giardia lamblia is one of the most common diarrhea-related parasites in almost all vertebrates, including humans [1]. It affects all age groups; with prevalence rate varying from (2%-5%) in the industrialized world and (20%-30%) in the developing countries. It is more prevalent among children [2].

Giardia is transmitted by the fecal-oral route following direct or indirect exposure to the cyst, through the ingestion of contaminated food, drinking contaminated water [3]. Person to person transmissions may happen directly by fecal-oral contact among family members, children in day-care center and schools [4] and by sexual practices of adults [5]. The parasite has a global distribution with 250-300 million symptomatic human infections reported annually, and its impact is more pronounced in the developing countries, where it is usually associated with poor socioeconomic conditions [6].

Giardiasis can present with a broad range of clinical manifestations from asymptomatic, to acute or chronic diarrheal disease associated with abdominal pain and nausea [7]. Children and immunocompromised individuals are the most affected by *Giardia* spp. The disease has been associated with growth, nutrition, and cognitive retardation in children in poor, disadvantaged settings [8]. The chronic infection of *Giardia* during childhood is mainly due to protein-energy malnutrition, vitamin A deficiency, anemia, mineral deficiency [9].

In 2004, the World Health Organization (WHO) recognized giardiasis as a neglected disease associated with poverty and impaired development [10].

Based on recent molecular and phylogenetic evidence, *G. lamblia* is currently regarded as a species complex consisting of eight (A to H) distinct genetic groups or assemblages with marked differences in host range and specificity [11].

Materials and Methods

Study type

The experimental study was performed at the laboratories of Parasitology Department, Faculty of Medicine, Sohag University and Molecular Biology Center at Assuit University, from October 2017 to October 2018. DNA samples were isolated from the stools of 100 patients infected with *G. lamblia* (after examination by copromicroscopy) and were amplified by using PCR. The 93 positive samples after PCR, were digested with NlaIV and RsaI fermentase restriction fragment enzymes. The samples were then randomly taken and the residence and age for every case were noted down. According to the residence, the cases were divided into urban and rural cases.

Case history and questionnaire surveys were done for cases included in the study. Measurement of some physical features,

like weight and height using growth charts for weight, height, weight for height and body mass index for every case was included in the study. These growth charts were according to the world health organization site.

The research team followed the ethical standards of confidentiality and freedom to participate. The respondents or their parents were informed that the study was voluntary and they were assured that their privacy would be protected and all of them gave written consent for taking and studying their specimens.

Parasitological examination

100 stool samples of patients infected with *Giardia,* were examined using saline and iodine wet mount and all the samples showed positive results.

Molecular examination

Genomic DNA was extracted from stool samples by using DNA extraction (QiAmp® DNA stool Mini kit (Qiagen, UK), amplification of glutamate dehydrogenase gene using specific primers by semi-nested PCR thermal cycler technique and Restriction of the DNA fragments by using two restriction fragments enzymes (NIaIV and RsaI restriction fragment enzymes) were performed. The results are shown in Tables 1-11.

Table 1. *The relation between weight and sub-assemblages AI, AII, BIII, BIV and mixed A and B.*

Sub-assemblage	Sub-assemblage AI	Sub-assemblage AII	Sub-assemblage BIII	Sub-assemblage BIV	Mixed A and B
Weight >50 percentile	2 (2.2%)	13 (14.0%)	10 (10.8%)	5 (5.4%)	7 (7.5%)
Weight <50 percentile	1 (1.1%)	7 (7.5%)	5 (5.4%)	8 (8.6%)	10 (10.8%)
Weight equal to 50 percentile	2 (2.2%)	5 (5.4%)	6 (6.5%)	3 (3.2%)	9 (9.7%)
p-value	0.03 (significant)				
Chi-square (χ^2)	2.51				

Table 2. *The relation between height and sub-assemblages AI, AII, BIII, BIV and mixed A and B.*

Sub-assemblage	Sub-assemblage AI	Sub-assemblage AII	Sub-assemblage BIII	Sub-assemblage BIV	Mixed A and B
Height > 50 percentile	2 (2.2%)	6 (6.5%)	11 (11.8%)	5 (5.4%)	7 (7.5%)
Height < 50 percentile	3 (3.2%)	18 (19.3%)	8 (8.6%)	9 (9.7%)	12 (12.8)
Height equal to 50 percentile	0 (0.0%)	1 (1.1%)	2 (2.2%)	2 (2.2%)	7 (7.5%)
p-value	0.001 (highly significant)				
Chi-square (χ^2)	23.29				

Table 3. *The relation between weight for height and sub-assemblages AI, AII, BIII, BIV and mixed A and B.*

Sub-assemblage	Sub-assemblage AI	Sub-assemblage AII	Sub-assemblage BIII	Sub-assemblage BIV	Mixed A and B
Weight for height >50 percentile	1 (1.1%)	11 (11.8%)	7 (7.5%)	7 (7.5%)	10 (10.8%)
Weight for height <50 percentile	4 (4.3%)	13 (14.0%)	9 (9.7%)	9 (9.7%)	14 (15.1%)
Weight for height equal to 50 percentile	0 (0.0%)	1 (1.1%)	5 (5.4%)	0 (0.0%)	2 (2.2%)
p-value	0.001 (Highly significant)				
Chi-square (χ^2)	28.32				

Table 4. *The relation between body mass index and assemblages A and B assemblages, sub-assemblages and mixed.*

Sub-assemblage	Sub-assemblage AI	Sub-assemblage AII	Sub-assemblage BIII	Sub-assemblage BIV	Mixed A and B
Body mass index > 50 percentile	2 (2.2%)	12 (12.9%)	8 (8.6%)	6 (6.5%)	9 (9.7%)
Body mass index < 50 percentile	1 (1.1%)	11 (11.8%)	11 (11.8%)	9 (9.7%)	13 (14.0%)
Body mass index equal to 50 percentile	2 (2.2%)	2 (2.2%)	2 (2.2%)	1 (1.1%)	4 (4.3%)
p-value	0.001 (Highly significant)				
Chi-square (χ^2)	20.38				

Table 5. *The relation between grades of diarrhea and sub-assemblages AI, AII, BIII, BIV and mixed A and B.*

Sub-assemblage	Sub-assemblage AI	Sub-assemblage AII	Sub-assemblage BIII	Sub-assemblage BIV	Mixed A and B
Mild diarrhea	4 (4.3%)	13 (13.8%)	11 (11.8%)	9 (9.7%)	15 (16.1%)
Moderate diarrhea	0 (0.0%)	7 (7.5%)	9 (9.7%)	6 (6.5%)	9 (9.7%)
Severe diarrhea	1 (1.1%)	3 (3.2%)	2 (2.2%)	2 (2.2%)	2 (2.2%)
p-value	0.03 (significant)				
Chi-square (χ^2)	34.12				

Table 6. *The relation between type of diarrhea and sub-assemblages AI, AII, BIII, BIV and mixed A, B.*

Sub-assemblage	Sub-assemblage AI	Sub-assemblage AII	Sub-assemblage BIII	Sub-assemblage BIV	Mixed A and B
Acute diarrhea	2 (2.2%)	22 (23.7%)	19 (20.4%)	10 (10.8%)	19 (20.4%)
Chronic diarrhea	3 (3.2%)	3 (3.2%)	2 (2.2%)	6 (6.5%)	7 (7.5%)
p-value	0.03 (significant)				
Chi-square (χ^2)	15.76				

Table 7. *The relation between abdominal distension and sub-assemblages AI, AII, BIII, BIV and mixed (A, B).*

Sub-assemblage	Sub-assemblage AI	Sub-assemblage AII	Sub-assemblage BIII	Sub-assemblage BIV	Mixed A and B
Abdominal distension	0 (0.0%)	7 (7.5%)	8 (8.6%)	6 (6.5%)	5 (5.4%)
No abdominal distension	5 (5.4%)	18 (19.4%)	13 (14.0%)	10 (10.8%)	21 (22.6%)
p-value	0.001 (Highly significant)				
Chi-square (χ^2)	18.75				

Table 8. *The relation between abdominal colic and sub-assemblages AI, AII, BIII, BIV and mixed A, B.*

Sub-assemblage	Sub-assemblage AI	Sub-assemblage AII	Sub-assemblage BIII	Sub-assemblage BIV	Mixed A and B
Abdominal colic	5 (5.4%)	24 (25.8%)	19 (20.4%)	13 (14.0%)	23 (24.7%)
No abdominal colic	0 (0.0%)	1 (1.1%)	2 (2.2%)	3 (3.2%)	3 (3.2%)
p-value	0.001 (Highly significant)				
Chi-square (χ^2)	60.48				

Table 9. *The relation between failure to thrive and sub-assemblages AI, AII, BIII, BIV and mixed A, B.*

Sub-assemblage	Sub-assemblage AI	Sub-assemblage AII	Sub-assemblage BIII	Sub-assemblage BIV	Mixed A and B
Failure to thrive	2 (2.2%)	6 (6.5%)	5 (5.4%)	8 (8.6%)	11 (11.8%)
No failure to thrive	3 (3.2%)	19 (20.4%)	16 (17.2%)	8 (8.6%)	15 (16.1%)
p-value	0.03 (significant)				
Chi-square (χ^2)	7.83				

Table 10. *The relationship between anthropometric parameters with genotypes.*

		Gender		Locality		Grades of diarrhea			Types of diarrhea		Abdominal Distension		Abdominal Colic		Failure to thrive	
		Female	Male	Rural	Urban	Severe Diarrhea	Moderate diarrhea	Mild diarrhea	Chronic diarrhea	Acute diarrhea	Yes	No	Yes	No	Yes	No
Assemblage A	Sub-assemblage AI	2 (2.1%)	3 (3.2%)	4 (4.3%)	1 (1.1%)	1 (1.1%)	0	4 (4.3%)	3 (3.2%)	2 (2.2%)	0	5 (5.4%)	5 (5.4%)	0	2 (2.2%)	3 (3.2%)
	Sub-assemblage AII	8 (8.6%)	17 (18.2%)	18 (19.4%)	7 (7.5%)	3 (3.2%)	7 (7.5%)	13 (13.8%)	3 (3.2%)	22 (23.7%)	7 (7.5%)	18 (19.4%)	24 (25.8%)	1 (1.1%)	6 (6.5%)	19 (20.4%)
Assemblage B	Sub-assemblage BIII	7 (7.5%)	14 (15%)	19 (20.4%)	2 (2.2%)	2 (2.2%)	9 (9.7%)	11 (11.8%)	2 (2.2%)	19 (20.4%)	8 (8.6%)	13 (14%0	19 (19.4%)	2 (2.2%)	5 (5.4%)	16 (17.2%)
	Sub-assemblage BIV	4 (4.4%)	12 (12.9%)	13 (14%)	3 (3.2%)	2 (2.2%)	6 (6.5%)	9 (9.7%)	6 (6.5%)	10 (10.8%)	6 (6.5%)	10 (10.8%)	13 (13.4%)	3 (3.2%)	8 (8.6%)	8 (8.6%)
Mixed A and B		12 (12.9%)	14 (15%)	20 (21.5%)	6 (6.4%)	2 (2.2%)	9 (9.7%)	15 (16.1%)	7 (7.5%)	19 (20.4%)	5 (5.4%)	21 (22.6%)	23 (24.7%)	3 (3.2%)	11 (11.8%)	15 (16.1%)
p-value		0.05 (signifi cant)		0.03 (signifi cant)		0.03 (signifi cant)			0.03 (signifi cant)		0.001 (highly significant)		0.001 (highly significant)		0.03 (significant)	
Chi-square (χ^2)		7.83		32.52		34.12			15.76		18.75		60.48		7.83	

Table 11*. The relationship between symptoms with genotype.*

		Weight			Height			Weight for height			Body mass index		
		>50 percentile	<50 percentile	=50 percentile	>50 percentile	<50 percentile	=50 percentile	>50 percentile	<50 percentile	=50 percentile	>50 percentile	<50 percentile	=50 percentile
Assem-blage A	Sub-assemblage AI	2 (2.2%)	1 (1.1%)	2 (2.2%)	2 (2.2%)	3 (3.2%)	0	1 (1.1)	4 (4.3%)	0	2 (2.2%)	1 (1.1)	2 (2.2%)
	Sub-assemblage AII	13 (14%)	7 (7.5%)	5 (5.4%)	6 (6.5%)	18 (19.3)	1 (1.1%)	11 (11.8)	13 (14%)	1 (1.1%)	12 (12.9)	11 (11.8)	2 (2.2%)
Assem-blage B	Sub-assemblage BIII	10 (10.8)	5 (5.4%)	6 (6.5%)	11 (11.8)	8 (8.6%)	2 (2.2%)	7 (7.5%)	9 (9.7%)	5 (5.4%)	8 (8.6%)	11 (11.8)	2 (2.2%)
	Sub-assemblage BIV	5 (5.4%)	8 (8.6%)	3 (3.2%)	5 (5.4%)	9 (9.7%)	2 (2.2%)	7 (7.5%)	9 (9.7%)	0	6 (6.5%)	9 (9.7%)	1 (1.1%)
Mixed A and B		7 (7.5%)	10 (10.8)	9 (9.7%)	7 (7.5%)	12 (12.8)	7 (7.5%)	10 (10.8)	14 (15.1)	2 (2.2%)	9 (9.7%)	13 (14%)	4 (4.3%)
p-value		0.03 (significant)			0.001 (highly significant)			0.001 (highly significant)			0.001 (highly significant)		
Chi-square (χ^2)		2.51			23.29			28.32			20.38		

Discussion

The intestinal protozoan *Giardia lamblia* is frequently found in diarrheal disease throughout the world affecting humans and other mammalian species [12].

93 samples were found to be positive molecularly after amplification of the glutamate dehydrogenase (gdh) gene using two restriction fragments enzymes (NIaIV and RsaI restriction fragments enzymes).

In the present study, it was found that patients with *Giardia* assemblage B (BIII, BIV) were more poly-symptomatic than assemblage A (AI, AII). These results agreed with [13] in Italy, [14] in Argentina, [2] in Egypt, and [15] in Cuba, and disagreed

with [16] in Equador and [17] in Portugal who found no correlation between gastrointestinal manifestations and *Giardia* assemblages.

In regards to the severity of diarrhea, the current results showed that mild diarrhea was higher in prevalence than moderate and severe. The predominant assemblage here in all types of diarrhea was assemblage B (42.1%). The results also showed that acute and chronic diarrhea, (31.2%) and (8.7%) respectively were associated mainly with assemblage B and the prevalence of acute diarrhea is more than chronic diarrhea. These results agreed with [18] in Ethiopia, [19] in Saudia Arabia, [2] and [15] in Cuba who assumed that assemblage B is associated with all types of diarrhea especially from moderate to severe types.

On the other hand, the results of this study disagreed with [16] in Equador who found no correlation between digestive manifestations and *Giardia* assemblages, [17] in Portugal and [20] in Bangladesh who found that diarrhea was more associated with assemblage A than assemblage B and [21] in Thailand, found that all subjects with AI assemblage were symptomatic while only 50% of the subjects with BIII assemblages were symptomatic.

In the present study, in regards relation to *Giardia* assemblages and sub-assemblages with abdominal distension, abdominal colic and failure to thrive: for abdominal distension only (28%) are affected, assemblage B (15.1%), assemblage A (AII, 7.5%), and mixed (A+B) (5.4%), the most predominant sub-assemblage is BIII (8.6%). For abdominal colic (90.3%) were affected, assemblage B (34.4%), assemblage (A 31.2%), and mixed (A+B) (24.7%). Moreover, this study showed that failure to thrive was associated with all assemblages and sub-assemblages of *Giardia lamblia* by different ratios, mainly assemblage B (14%) was associated more than mixed (A+B) (11.8%) and A (8.7%). From the previous results regarding diarrhea, abdominal distension, abdominal colic, failure to thrive, and abdominal colic is the second most common complaint affecting (90.3%) cases after diarrhea, followed by failure to thrive affecting (34.5%) cases, and lastly, abdominal distension affecting (28%) cases.

These results agreed with [22] in Egypt, [1] in Sewed, [2] in Egypt, [23] in Spain and [15] in Cuba, who assumed that assemblage B was more associated with clinical manifestations than assemblage A.

These results disagreed with [24], in India, who stated that giardiasis was associated with little gastrointestinal symptoms, [25] in London who assumed that both assemblages A and assemblage B caused similar illness, [26] in Rwanda, who assumed that children infected with assemblage A were more associated with abdominal pain, while those infected with Assemblage B had clinically assessed severe malnutrition and disagreed with [27] in Iran who found that both assemblages caused similar illness, but assemblage AII was more frequently associated with abdominal pain, nausea, and vomiting.

In regards of relation to *Giardia* assemblages and sub-assemblages with some physical features like weight for age (indicate underweight), height for age (indicate stunting), weight for height (indicate wasting) and body mass index (indicate wasting also but with more confirmation): the present cases are classified into 3 groups, the first group whose measurements were more than 50 percentile, The second group whose measurements equal to 50 percentile and the third group whose measurements are less than 50 percentile. The first two groups helped us as we could consider them as an indicator for the non-affected individuals with *Giardia lamblia* infection. In the third group, we can consider it as an indicator of association with *Giardia lamblia* infection.

In regards to weight for age, the results indicated that for weight less than 50 percentile {assemblage B (14%), mixed (A+B) (10.8%), and assemblage A (8.6%)}, the predominant sub-assemblage was sub-assemblage BIV (8.6%).

In regards to height for age, (53.8%) of individuals were affected. In height less than 50 percentile {assemblage A (22.6%), assemblage B (18.3%) and mixed (A+B) (12.9%)} the predominant sub-assemblage was sub-assemblage AII (19.4%).

In regards to weight for height, (52.8%) of individuals were affected. In weight for height less than 50 percentile {assemblage B (19.4%) assemblage A (18.3%), and (A+B) (15.1%)}, the predominant sub-assemblage was assemblage AII (14%).

In regards to body mass index, (48.4%) of individuals were affected. In body mass index less than 50 percentile {assemblage B (21.5%), mixed (A+B) (14%) and, assemblage A (12.9%)} the predominant sub-assemblage was sub-assemblage AII (11.8%) and sub-assemblage BIII (11.8%).

The results of this study showed that there was a decrease in weight, height, weight for height, and body mass index of children infected with *Giardia* in different proportions.

This was consistent with previous findings in many different studies as [28] in Brazil, [29] in Peru [30] in Malaysia, [31] in Iran, [32] in Colombia, and [33] in Turkey. All these results found a strong association between *Giardia* infection and undernutrition, wasting and stunting among children but without determining assemblages of *Giardia lamblia*.

Also, there was a previous study among Brazilian children which showed that *Giardia*-infected children had a double risk for stunted growth as compared to other children [34].

The results in this study disagreed with [35] in Gambia and [36], they found that there was no significant association between *Giardia lamblia* infection and malnutrition. The difference with these results could be attributed to the low prevalence of *G. lamblia* reported by these studies as compared to the present study.

Conclusion

The results in this study showed that there was an association with some physical features like underweight, stunting and wasting with *Giardia lamblia* infection. However, it is very probable that *Giardia* infection is one of the several factors associated with low nutritional status, together with sanitary and socioeconomic conditions.

Lastly, according to our knowledge, the present study is the first study that tried to find an association between *Giardia* lamblia assemblages and the previous physical features.

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