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IMPACT OF RISK FACTORS ON THE EPIDEMIOLOGICAL PREVALENCE OF *GIARDIA LAMBLIA* ASSEMBLAGES IN KAFRELSHEIKH GOVERNORATE, CAIRO, EGYPT

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Abstract

Although the incidence of *Giardia lamblia* increased, data regarding its possible associated risk factors are scares. The study investigated the genetic diversity of humans *G. lamblia* positive isolates in kafrelsheikh Governorate, and the impact of multiple risk factors on prevalence. A total of 300 fecal samples were collected from patients attended Kafrelsheikh University over a period of one year. Amplification and RFLP analysis of β-giardin gene were used to identify the *G. lamblia* multiple assemblages.

The results showed that, PCR amplification showed 63/300 (21%) positive samples, of them, 12 assemblages A (19.1%) and 52 assemblages B (80.9%). On univariate analysis, there were statistical significant correlation among giardiasis and age group, sex, hand-washing, milk type, flatulence, vomiting, and animal contact (p = 0.028, 0.006, 0.005, 0.02, 0.000, 0.002 & 0.000, respectively). On Multivariate logistic regression analysis showed that children from 2-6 years, adults (< 18 years), females, hand-washing, boiled milk, vomiting, flatulence and animal contact were independent risk factors for giardiasis (p = 0.01, 0.02, 0.007, 0.05, 0.03, 0.000, 0.002 & 0.000, respectively).

Keywords: *Giardia lamblia*, Assemblage, Kaferelsheikh, Cairo, Risk factors.

Introduction

Giardia lamblia constitutes the most prevalence parasite infecting man, domestic and wild animals especially in developing countries with an annual rate of 10-50% or 200 million cases (Noradilah *et al,* 2019). Many water *Giardia* cyst borne outbreaks were reported (Wang *et al,* 2019).

According to genetic characterization of different genetic markers (mall subunit ribosomal RNA (ssurRNA), the glutamate dehydrogenase (gdh), β-giardin (bg), triose phosphate isomerise (tpi), elongation factor 1-alpha (ef-1α), and GLORF-C4 genes), *G. lamblia* is a multispecies complex comprised of at least eight different genetic assemblages (A-H) (Rehbein *et al,* 2019). The majority of human infection was due to zoonotic assemblages A that could infect other mammals and B was mainly reported in man & a small number of animal species, but other assemblages were host specific (Feng *et al,* 2011). Data were lacking about the risk factors affecting the genotypic epidemiology as seasonality, age, sex, consumed water, animal contact and clinical presentations (Asher *et al,* 2016).

This study aimed to investigate the genetic diversity of *G. lamblia* positive isolates in humans in Kafrelsheikh Governorate, and to evaluate demographic, environmental and clinical data on the genotypic prevalence of different *Giardia* assemblages.

Materials and Methods

Study population: after Kafrelsheikh Faculty of Medicine Institutional Board Review and calculation of sample size, a cross sectional study was done over 300 patients of both sexes aged from 1 to 60 years old with either diarrhea and/or associated with abdominal pain, vomiting, fever or flatulence and not received any antidiarrheal drug. Patients attended outpatient clinics in Kafrelsheikh University Hospitals January 2018 to January 2019 (150 in dry season and others in wet season). They were classified by age according to into 5 categories: infant < 2 years, 2-6 years early childhood, 6-12 years

late childhood, 12-18 years adolescent and adult > 18 years. A single fecal sample was collected from each one with recordation to demographic, clinical and environmental data using a designed questionnaire.

Coproscopy of stool samples: All samples were examined microscopically fresh and fixed with sodium acetate acetic acid formalin (SAF) and the others were kept at -20°C for copro- PCR assays Garcia 2007).

Copro DNA extraction and amplification: Extraction of genomic DNA was done using Favor Prep stool DNA isolation Mini Kit (Favorgen Biotech corporation ping-Tung 908, Taiwan, Cat. No. FASTI001) according to manufacturer's instruction with modification in the form of thermal cell disruption (shock): samples were thrown in liquid nitrogen for 5min. and then immediately transferred into water bath 95°C for 5 min. (repeated for 10 cycles). Amplification β-giardin gene was done (Naguib *et al,* 2018) with modification of the annealing temperature to 75°C in primary reaction and 55°C for nested one. Positive and negative controls are incorporated in each reaction. Assemblage specific pattern were obtained by digestion of the amplified nested PR products by Hae III restriction enzyme.

Statistical analysis: Data were tabulated and analyzed using statistical package SPSS version 21 (Chicago, IL, USA). Data were described using frequency and percentage with $P < 0.05$ was considered significant.

Results

Out of 300 samples, wet mount examination detected *Giardia* cyst in 21 samples (7%), while, molecular detection of *Giardia* copro-DNA showed 63 positive (21%). Out of 63 positive samples 43 (68.2 %) were females and 20 (31.8 %) were males.

Two assemblages A & B were detected in amplified *Giardia* DNA products with highest prevalence of assemblage B than A; 51 (80.9%) vs. 12 (19.1%) and no mixed infection (Tab.1). Univariate analysis showed significant correlation between giardiasis and age group (2-6 year), vomiting, females, proper washing hands before meals, flatulence, animal contact and kind of consumed milk (P < 0.05). Multivariate logistic regression analysis showed that children from 2-6 years, adults, females, hand-washing, boiled milk, vomiting, flatulence and contact with animals were the high risk factors for giardiasis (Tab. 2). There were marked discrepancy between associated risk factors and 2 assemblages, but without significance correlation between assemblages' types and risk factors (Tab. 3).

Table 1: Prevalence & univariate analysis of possible risk factors associated with *Giardia lamblia* among group (n=300).

		Giardia positive (N=63)	Giardia negative(N=237)	P value
Age group	Infant (<2 Y)	6 (2%)	46 (15.3%)	0.028
	Early childhood (2-6 Y)	32 (10.7%)	88 (29.3%)	
	Late childhood (6-12Y)	3 (1%)	8(2.7%)	
	Adolescence(12-18 Y)	11 (3.7%)	24(8%)	
	Adult (> 18Y)	11 (3.7%)	71(23.6%)	
Gender	Female	43 (14.3%)	116(38.7%)	0.006
	Male	20 (6.7%)	121 (40.3%)	
Associated complaint	Weight loss	18 (28.5%)	282 (17.5%)	0.161
	Loss of appetite	32 (50.7%)	268 (49.7%)	0.35
	Fever	17 (5.7%)	283 (83%)	0.14
	Abdominal pain	58 (19.3%)	242 (80.7%)	0.099
	Flatulence	59 (19.6%)	241(80.4%)	0.000
	Vomiting	40 (13.3%)	260 (86.7%)	0.002
Animal contact	Yes	28 (9.3%)	272 (90.7%)	0.000
Hand washing	Yes	53 (17.7%)	199 (66.3%)	0.005
	NO	10 (3.3%)	38 (12.7%)	
Milk type	Boiled	56 (18.7%)	182 (60.7%)	0.028
	Pasteurized	7 (2.3%)	55 (18.3%)	
Season	Dry	29(9.7%)	121(40.3%)	0.47
	Wet	34(11.3%)	116(38.7%)	

*Data presented as No., percentages and P value <0.05.

Table 2: Multivariate analysis of possible associated risk factors.

	Items	P value	OR	CI
Age group	Infant	0.75	0.84	2.91-2.43
	Early childhood	0.01	2.6	1.22-5.75
	Late childhood	0.68	1.29	0.37-4.49
	Adolescence	0.23	2.42	0.55-10.53
	Adult	0.02	2.95	1.13-7.69
Sex	Female/ male	0.007	2.24	1.24-4.03
Associated complaint	Flatulence	0.000	0.08	0.02-0.228
	Vomiting	0.002	0.4	0.22-0.72
Animal contact	Yes	0.000	0.23	0.12-0.42
Hand washing	Yes	0.005	0.24	0.09-0.65
Milk type	Boiled /Pasteurized	0.03	2.4	1.06-5.73

Table 3: Distribution of Giardia assemblages in comparison to potential risk factor

	Items	Assemblage A (N= 12)	Assemblage B (N=51)	P value
Age group	Infant	0	6	0.73
	Early childhood	7	25	
	Late childhood	1	2	
	Adolescence	2	9	
	Adult	2	9	
Gender	Female	8	35	0.89
	Male	4	16	
Associated complaint	Weight loss	5	13	0.26
	Loss of appetite	6	26	0.95
	Fever	2	15	0.35
	Abdominal pain	12	46	0.25
	Flatulence	10	49	0.10
	Vomiting	9	31	0.37
Animal contact	Yes	3	25	0.12
Hand washing	Yes	10	43	0.99
	NO	2	8	
Milk type	Boiled	9	47	0.11
	Pasteurized	3	7	
Season	Dry	11	18	0.87
	Wet	1	33	

Discussion

In the present study, genotyping results revealed the occurrence of both assemblages B and A with predominance of B suggesting that the infection is primarily anthroponotic which may be through non hygienic personal practices including feeding habits and inefficient environmental sanitation.

The current study showed that early childhood had higher prevalence of giardiasis with statistical significance between age groups and giardiasis (*P* value= 0.028). Similar results were obtained in one previous study in Egypt, where children had a higher frequency of assemblage B (24/34 or 70.6%) than assemblage A (10/34 or 29.4%), while adults had a similar frequency of both assemblages (12/26 or 46.2% & 14/26 or 53.8%, respectively) (Mohammad et al. 2011). This agreed with Choy *et al.* (2014) and El-Badry *et al.* (2017). This may be attributed to the higher risk of exposure of young children to a wide range of infectious sources that could be due to lower personal hygienic standards when compared to the older children or adults. This was in contrary to Ignatius et al. 2012 who documented that there was no correlation between age and giardiasis.

In this study, 49% (25/51) of *Giardia* children were infected with assemblage B, compared with 17.6% (9/51) in both adolescence and adults. Similarly, in on previous Egyptian study children had a higher frequency of assemblage B than assemblage A, but adults had same frequency of both assemblages (El Basha *et al,* 2016). Also, Ignatius *et al.* (2012) found that children had a higher frequency of assemblage B (24/34 or 70.6%) than assemblage A (10/34 or 29.4%), while

adults had a same frequency of both assemblages (12/26 or 46.2% & 14/26 or 53.8%, respectively)

In contrast, in United Kingdom in which assemblages A & B were equally distributed in children of 0-9 years, assemblage B was more predominant in adults of 30-49 years, and assemblage A was more common in adults >50 years (Minetti *et al,* 2015). This indicated that dominance of *G. lamblia* genotypes could be changed in humans over age reflecting the differences in exposures and /or the development of acquired immunity.

Variation in sex distribution was highly significant (P = 0.006). This agreed with Duldova *et al.* (2012) who found a higher prevalence in females than males, and added that females caring for their children or working in care-day centers or nurseries. But, this disagreed with Julio *et al.* (2012) and De Lucio *et al.* (2015) who found a higher giardiasis incidence in males than in females.

Baldursson and Karanis (2011) considered giardiasis the most common causes of waterborne diseases outbreaks worldwide.

None of the drinking water sources in the study was significantly associated with the development of giardiasis risk (P = 0.464). This may be attributed to the fact that most of studied individuals mainly depended on tap water as the main source of water supply. This agreed with Okojokwu *et al.* (2014). Others reported significant association between type of water and giardiasis (Choy *et al,* 2014; Osman *et al,* 2016).

In this study, association was between animal contact and giardiasis (P= 0.000). Also, Júlio *et al.* (2012) showed a positive association regarding the giardiasis pre-valence and the animal contact. But, others did not find association between animal contact and giardiasis (Hunter *et al,* 2005).

In the present study, flatulence was the most common clinical symptom followed by abdominal pain and then vomiting, but there was significant correlation between the presence of flatulence, vomiting and giardiasis (P = 0.000 & 0.002 respectively). This agreed with Lebbad *et al.* (2011) who found that flatulence was significantly commonly associated with giardiasis. But, Torabi *et al.* (2014) did not find any association between flatulence and giardiasis (P= 0.925)

In the present study, there was no correlation between infections in dry and/or wet seasons. But, Noradilah *et al*. (2019) found that seasonality played a major role in protozoa mainly in the river water during the dry season, and using infected untreated water or water-related activities during this season reduce the burden of *G. lamblia* infection.

The present study showed no significant difference between clinical symptoms and infection with assemblages A & B. This may be related to multiple factors affected variability of *G. lamblia* clinical outcomes genotypes as: hosts, nutrition, immunity, and pathogenicity. The present data agreed with Ismail *et al.* (2016) who found many variations in socioeconomic status in same geographical areas and population densities played a major role in giadiasis prevalence.

Conclusion

A higher giardiasis incidence was found in Kafrelsheikh Governorate. Predominance of assemblage B indicated that infection was mostly anthroponotic. Children aged from 2-6 years, adults <18 years, females, hand-washing, boiled milk, vomiting, flatulence and animal contact were all independent risk factors.

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