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GIARDIA INTESTINALIS AND *HELICOBACTER PYLORI* CO-INFECTION: ESTIMATED RISKS AND PREDICTIVE FACTORS IN EGYPT

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Abstract

Giardia intestinalis (*G. intestinalis*) and *Helicobacter pylori* (*H.pylori*) are two intestinal pathogens sharing the same mode of infection. This study determines the prevalence of *G. intestinalis* and *H. pylori* co-infection estimated risks and predictive factors for susceptibility to co-infection. Stool samples were collected from 801 patients suffering gastrointestinal symptoms and living in Greater Cairo. They were subjected to coproscopic examination for detection of intestinal parasites and copro PCR-restriction fragment length polymorphism (PCR-RFLP) and sequencing targeting the glutamate dehydrogenase (gdh) gene for *Giardia*. Positive samples for giardiasis were further subjected to copro-immunoassay to detect *H. pylori* coprontigen. Among 63 cases of giardiasis by both microscopy and PCR (84.1 % as-semblage B and 15.9% AII), 52.5% were co-infected with *H. pylori*. Co-infection was more frequent with assemblage B (50.9%) than assemblage A (40%). Among studied variables of assemblage type, gender, or harboring more than one parasite (polyparasitism), only school age children, was signifi-cantly associated (*P* value: 0.02) with *Giardia* and *H. pylori* co-infection. Physicians in Egypt must consider *G. intestinalis* and *H. pylori* as prevailing intestinal pathogens with predominance of *Giardia* assemblage B. *Giardia* and *H. pylori* co-infection is common in school aged children and modulates gastrointestinal manifestations. Intestinal parasitism and *H. pylori* association is complex and necessitates further genomic studies for a better understanding of the epidemiological and clinical impact of co-infection, as well as possible strategies for their treatment and control.

Key words: Giardiasis, *Helicobacter*, co-infection, PCR-RFLP, gdh, immunochromatograpghy

Introduction

The protozoan *G. intestinalis* is the most common and vital intestinal protozoan (Thompson, 2004). The disease is characterized by number of gastrointestinal symptoms, diarrhea, abdominal pain, flatulence and Malnutrition (Veenemans *et al*, 2011). The source of infection is through oral rout (Thompson and Monis, 2012). Mostly human infections are related to assemblage A and B, the latter is often the most prevalent assemblage in human (Fahmy *et al*., 2015).

H. pylori is a gram negative, microaerophilic bacillus, which colonizes the gastric mucosa giving variations of clinical manifestation ranging from dyspepsia, ulcer and pain till gastric cancer (Gatta *et al*, 2013). The disease often is acquired during early childhood and could persist throughout life as it is widespread intra-familial and from mother to child (Osaki *et al*., 2015). For detection of *H. pylori* copro-antigen, the use of monoclonal antibodies via immunochromatographic technique is a non-invasive diagnostic test with high sensitivity and specificity (Sato *et al*., 2012; Okuda *et al*., 2014).

H.pylori produces a urease causing a state of diminished gastric acidity, a condition which is favored by the parasite *Giardia* (David and William, 2006). In addition, the two organisms share the same route of infection mainly the feco-oral one (Moreira *et al*., 2005). Many studies had discussed the association between giardiasis and *H. pylori* and its impact on each organism (Moreira *et al*, 2005; Grazioli *et al*, 2006; Zeyrek *et al*, 2008; Isaeva and Efimova 2010; Júlio *et al*, 2012). The present study aimed to detect the prevalence of co-existence of giardiasis and *H. pylori* in Egyptian patients and to estimate risks and predictive clinical factors for this co-infection.

Materials and Methods

Study design and populations: A cross sectional study was conducted including 801 patients from greater Cairo suffering from gastrointestinal symptoms. Collected faecal samples were done after informing the adult patients and the parents of young children about the purpose of the study and informed consent was obtained.

Collection and processing of sample: Single stool sample was collected from each patient and divided into three parts, one for coproscopic examination using saline and Lugol's iodine stained direct wet mount before and after formalin-ethyl acetate concentration to detect *G.intestinalis* and other parasites. The other two parts were freshly frozen at -20 °C for copro immuno-molecular assays at Lab of Molecular Medical Parasitology (LMMP), Faculty of Medicine, Cairo University.

Copro immunoassay: Positive stool samples for *Giardia* by microscopy were exposed to *H. pylori* coproantigen detection using chromatographic immunoassay, the On Site *H. pylori* Ag Rapid Test (CTK Biotech, USA).

Copro-PCR assay: All samples 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 instruction. A semi-nested PCR (nPCR) was done targeting the gdh gene, using GDHeF: 5' TCAACGTYAAYCGYGGYTTCCGT 3' and GDHiR: 5' GTTRTCCTTG CACATCT CC 3' primers for first PCR, GDHiF: 5' CA GTACAACTCYGCTCTCGG 3', and GDH iR for semi-nested PCR. The reaction mixture and conditions were performed (Read *et al*, 2004). The amplified products were stained by ethidium bromide and visualized with 1.5% agarose gel electrophoresis.

PCR-RFLP of gdh: The PCR products were digested by two enzymes NIa IV (New England Biolabs. 0141210) which discriminates between AI, AII, B, C, D and E assemblages, and RSaI enzyme (New England Biolabs, FD1124) to differentiate assemblage BIII and BIV according to the manufacturer's instructions. Restriction profiles were visualized on 2 % agarose gel electrophoresis stained with ethidium bromide.

Sequencing: PCR-RFLP results were confirmed by sequencing using Qiagen PCR purification kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, the amplified products were purifand visualized on 1.5% agarose gel electrophoresis. Sequencing was performed with Big-Dye® Terminator v3.1, Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) for semi-nested PCR products. Post sequencing reaction products were cleaned using Big Dye X purification kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. DNA template sequencing was performed on an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA).

Statistical analysis: Statistical package for social sciences (SPSS) version 20 was used for statistical analysis. Descriptive data was analyzed by mean ± standard deviation, while qualitative data was analyzed by frequencies. Co-infection of giardiasis and *H.pylori* was the dependent variable and chi-square test was used to assess their association with each other and with the independent variables: age, sex, polyparasitism, and *Giardia* assemblages. These variables were estimated risks, while clinical symptoms were predictive factors. The odds ratio (OR) and 95% confidence interval (CI) were calculated for each of the variables by logistic regression model. The student T test and mean of age was used to estimate age as a risk factor by comparing patients co-infected with giardiasis and *H.pylori* with patient with *Giardia* infection.

Results

The study included 801 patients with age ranged from 2 to 60 years old with mean of 14.5±7.93. The microscopic prevalence of *Giardia* was about 10% (n=80), among them 63 samples were successfully amplified by

semi-nested PCR and genotyped by PCR-RFLP and sequencing using gdh gene. *Giardia* assemblage B and A (84.1 %, 15.9%, respectively) were detected. Subgenotyping of assemblage B was, BIII, BIV (49.1%, 22.6%, respectively) and 28.3% of assemblage B was not subgenotyped (Tab. 1), and all assemblage A samples were AII.

H. pylori and *G. intestinalis* frequency and risk factors: The co-infection prevalence of *H.pylori* and giardiasis was 52.5% (n=42), *H.pylori* positive coproantigen was more prevalent with assemblage B (50.9%) than assemblage A (40%), without statistical significance. The mean age of co-infected patients was 9.8±8.98, with significant association (*P* value=0.02) (tTabs. 2, 3). None variables other than age was significant associated with co-infection.

Table 1: Demographic and clinical data of individuals.

Variables		n (%)
Age group (years old)		
Up to 2		38 (4.7)
>2-6		240 (29.9)
>6-12		216 (26.6)
>12-20		136 (17)
>20-30		114 (14.2)
>30-40		46 (5.7)
>40-50		0 (0)
>50-60		12 (1.5)
Male		432 (53.9)
Female		370 (46.1)
Clinical examination	Pain	238 (29.6)
	Flatulence	124 (15.5)
	Diarrhea	414 (51.6)
	Fatigue	178 (22.2)
	Vomiting	22 (2.7)
Polyparasitism		60 (15)
Microscopic examination		
Giardia cyst/trophozoite		80 (10.0)
Entamoeba complex cyst/trophozoite		21 (2.6)
Entamoeba coli cyst		23 (2.87)
Blastocystis spp.		11 (1.37)
Iodamoeba butschlii		4 (0.5)
Hymenolepis nana		16 (1.99)
Taenia eggs		6 (0.7)
Enterobius vermicularis		4 (0.5)
Ascaris lumbricoides		2 (0.25)

Table 2: Distribution of *H. pylori* coproantigen cases among *Giardia* assemblages

		H.pylori copro antigen			*P* value	OR	95%CI
		positive (n=42)	negative (n=38)	Total			
Giardia assemblage	B (n=53)	27 (50.9%)	26 (49.1%)	53 (66.2%)	0.44	1.20	0.48-3.05
	A (n=10)	4 (40%)	6 (60%)	10 (12.5%)	0.31	1.78	0.46-6.86
	Not-typed (n=17)	11 (64.7%)	6 (35.3%)	17 (21.3%)	0.19	1.89	0.62-5.74

OR= Odds ratio, 95% CI= 95% confidence interval

Table 3: Mean age among *Giardia* and *H. pylori* patients.

	Mean of age	*P* value	95% CI
Co-infection (*Giardia +H.pylori*) (n=42)	9.833±8.9815	0.02*	0.728-6.058
Non co-infection (*Giardia* only) (n=38)	7.168±5.7219		0.662-5.991

*Significant *p* value (<0.05)

Table 4: *Giardia* and *H.pylori* co-infection, estimated risks and predictive clinical symptoms.

Variable		Co-infection (n=42)			
		No.	(%)	*P value*	OR (95% CI)
Age group (years old) Up to 2 >2-6 >6-12 >12-20 >20-30 >30-40		 4 16 11 3 7 1	 9.5 38.1 26.2 7.1 16.7 2.4	0.695	-
Sex	Male	20	47.6	0.176	1.69 (0.69-4.10)
	Female	22	52.4		
Clinical examination	Abdominal Pain	20	47.6	0.176	0.59 (0.24-1.44)
	Flatulence	16	38.1	0.354	1.34 (0.53-3.36)
	Diarrhea	31	73.8	0.595	1.01 (0.37-2.73)
	Fatigue	11	26.2	0.489	0.87 (0.33-2.33)
	Vomiting	4	9.5	0.557	1.23 (0.26-5.88)
Polyparasitism		23	54.8	0.083	2.08 (0.85-5.09)

Discussion

In the present study, there was assemblage B (84.9%) predominance both for the entire study group and for the subgroup of co-infected patients, similar to previous reports of many Egyptians studies (Helmy *et al*, 2014; Fahmy *et al*, 2015; Ghieth *et al*, 2016). The source of *Giardia* infection in a region can be determined by the assemblage profile as it is an extremely diverse organism with a variety of assemblage and sub-assemblage styles (Feng and Xiao, 2011). The predominance of anthroponotic *Giardia* assemblages (B & AII) in the studied population, suggested that man was the source of infection rather than zoonotic. The co-infection prevalence of *H. pylori* and giardiasis was 52.5%, *H. pylori* positive coproantigen was more in assemblage B (50.9%) than assemblage A (40%), while *Giardia* type assemblage was not a risk factor for the association. Infection with *H. pylori* and *Giardia* were a reflection of socio-environmental levels (Patterson *et al*, 2012). Developing countries showed a higher colonization level than developed ones (Vale and Vitor, 2010; Hasosah *et al*, 2015).

The high occurrence rate of *H. pylori* (52.5%) in patients with positive giardiasis in the results supported the theory that conditions for *Giardia* survival are heightened by the bacterium *H. pylori*. A much higher rate of co-infection was observed in Iran by Shafie *et al*. (2009) who found that all *Giardia* positive patients were infected with *H. pylori*. Studies differ in explaining which organism was agonist in the presence of the other, Júlio *et al*. (2012) reported that the presence of *H. pylori* infection was a risk factor for giardiasis, and Moreira *et al.* (2005) mentioned that, *H pylori* infection was significantly associated with *G. lamblia.*

The present study showed that late childhood was the most vulnerable age group (9.8±8.98) for co-infection, with statistical significance (*P* value=0.02), while *Giardia* infection alone was more prevalent in preschool age children. Bin Mohanna *et al.* (2014) also reported higher prevalence of co-infection in late childhood, and for *H. pylori* infection the age above 10 was a risk factor (Hasosah *et al*, 2015). In contrast Ankarklev *et al*. (2012) reported a higher prevalence of co-infection for a younger age group (3-5 years old). Giardiasis usually affects 2-6 year old children, while co-infection with *H. pylori* causes giardiasis to reach a peak at an older age. This can be explained by the fact that *H. pylori* create favorable conditions for the sustenance of *Giardia* colonization.

In the present study, co-infection was associated with polyparasitism (other than *Giardia*) in more than half of patients and was more prevalent in females (52.4%) than males, without statistical significance. The results showed that, diarrhea and abdominal pain were most frequent (73.8%, 47.6%, respectively) while vomiting was least present (9.5%), however no clinical symptoms were

predictive for co-infection. There is controversy concerning association between *G. intestinalis* and *H. pylori* co-infection and clinical symptoms. Zeyrek *et al.* (2008) in Turkey reported that such association has an impact on patients with recurrent abdominal pain. Others reported that gastric colonization by *Giardia* was preceded by *H. pylori* (Doglioni *et al,* 1992; Moreira *et al,* 2005). A significant association was found between the two infections in patients with irritable bowel diseases by Grazioli *et al.* (2006).

In Egypt Kader *et al*. (1998) in Ain-Shams University's Hospitals examined thirty patients treated with proton pump inhibitor but had symptoms related to gastritis or peptic ulcers to upper gastrointestinal endoscopy and gastric biopsy for giardiasis. They reported gastric giardiasis in 3 (10%) cases of intestinal metaplasia and *H. pylori* in all cases, and concluded that there might be a relation between the presence of gastric giardiasis and the intake of proton pump inhibitor and that endoscopists have to search for gastric giardiasis especially in *H. pylori* and/or intestinal metaplasia. Abou Holw *et al.* (2009) in Alexandria reported that giardiasis was one of the most common enteroprotozoal diseases associated with *H. pylori*. Among fifty patients parasitologically proven giardiasis cases and ten normal healthy controls, they found significant upper gastrointestinal symptoms (epigastric pain and anorexia) in giardiasis patients with H. pylori. Also, endoscopic and histopathologic examination showed significant gastric lesions in this group of patients as compared to those suffering only *G. lamblia.* Eldash *et al*. (2013) in Al-Fayoum evaluated the incidence of H. pylori and G. intestinalis co-infection in RAP Egyptian among 90 children and 90 crossmatched healthy controls. H. pylori (HP) infection was diagnosed by detection of HP stool antigen (HPSA), ELISA and/or HP antibody (IgG), ELISA in serum, while *G. intestinalis* by stained stool smears. The HP infection was detected in 60 (66.7%) patients and 37 (41%) controls with a significant difference p=0.001. Giardiasis was found in 47 (52.2%) patients and 30 (33.3%) controls with a significant difference p= 0.02. Incidence was higher among children above 5 years, as a significant predictor for RAP. They concluded that recurrent abdominal pain affected 10-20% of school-aged children due to coinfection with *H. pylori* and *G, intestinalis* as organic causes.

Conclusion

Assemblage B and AII were the profiles of giardiasis in symptomized patients at Great Cairo. *H. pylori* coexisted in more than half of patients infected with giardiasis, it was more frequent with assemblage B. it is recommended in giardiasis patients with the age of late childhood and early young to do an easy, none invasive *H. pylori* coproantigen test. None of assemblage type, gender or polyparasitism was risk factors for such coinfection.

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