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ORIGINAL ARTICLE

Association of *Helicobacter pylori* and parasitic infections in childhood: impact on clinical manifestations and implications

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Abstract The association of *Helicobacter pylori* (*H. pylori*) infection and parasitic infections including *Giardia lamblia* (*G. lamblia*), especially in childhood, is widely recognized to be high in developing communities. We aimed to study the impact of concomitant intestinal parasitic and *H. pylori* infections on the different clinical presentation of infected children and whether this coinfection could in turn cause any alteration in the clinical manifestations of each other. This cross-sectional study included 150 children of both sexes with their age ranging from 1 to 15 years, having gastrointestinal complaints, throughout 8 months duration. All cases were subjected to full history taking, clinical examination and stool analysis by direct wet smear and formalin-ethyl acetate concentration technique, permanent staining with cold acid fast stain in addition to *H. pylori* coproantigen detection in stool. Parasitic infection was recorded in 58.6% of patients, with *G. lamblia* the most detected parasite (35.2%). Cases infected with *H. pylori* were 63 cases (42%) of which 61.9% of cases showed associated parasitic infection. Diarrhea was the most frequent complaint (63.2%) in cases infected with intestinal parasites, while it was less frequently recorded in co-infected cases (35.8%) and in cases with *H. pylori* infection only (29.1%) (*P* value 0.0008). On the other hand, vomiting was less recorded in coinfected cases than cases with *H. pylori* infection. Coinfection with intestinal parasites (including *G. lamblia*) and *H. pylori* could modulate the clinical manifestation of each other especially diarrhea in parasitic infections and vomiting in *H. pylori* infection.

Keywords Children · Gastrointestinal manifestations · Co-infection · *Giardia lamblia* · *Helicobacter pylori*

Introduction

Gastrointestinal (GI) symptoms including abdominal pain and diarrhea are common in children (Abu-Zekry et al. 2013). Globally, there are approximately 1.7 billion cases of childhood diarrhoeal disease annually. Diarrhea is common in childhood due to several etiological infectious agents as viruses, bacteria and parasites including *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* spp. (WHO 2008; Thompson and Ash 2016; World Health Organization (WHO) 2017).

Intestinal parasites are responsible for morbidity and mortality especially in developing countries due to poor sanitation, contaminated water, hot humid weather, poor housing and overcrowding (Oliveira-Silva et al. 2006).

Infection by *Helicobacter pylori*; a gram-negative bacillus which colonizes the gastric mucosa is often acquired during childhood and may persist throughout life (Dore et al. 2012). It may be asymptomatic, or may develop variable GI symptoms including dyspepsia, recurrent epigastric pain, hematemesis, vomiting, ulcer or even gastric cancer. The association between diarrhea and *H. pylori* infection in children is still conflicting (Dore et al. 2012; Gatta et al. 2013). *H. pylori* copro-antigen could be detected in stool using monoclonal antibodies through immune-chromatographic technique with high sensitivity and specificity (Sato et al. 2012; Okuda et al. 2014).

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Several studies had recognized intestinal parasitic infections (especially giardiasis) (Balani et al. 2000; Buch et al. 2002) and *H. pylori* infection as possible etiological factors for GI manifestations including recurrent abdominal pain and diarrhea (Frank et al. 2000; Das et al. 2003) The association of *H. pylori* infection with *G. lamblia* is highly observed in developing countries (Kazemian et al. 2014). Knowing the association of different infectious agents is crucial to select an adequate treatment to ensure eradication of infections (Ortiz-Princz et al. 2016). In our study we focused on the assessment of the impact of associated intestinal parasites and *H. pylori* infections on the clinical presentation of pediatric patients.

Material and methods

Study design and individuals

This cross-sectional study was conducted in Gastroenterology Outpatient Clinic, throughout 8 months duration. Fecal samples were collected from 150 children of both sexes with their age ranging from 1 to 15 years old, who were suspected to be infected with parasites and/or *H. pylori*, with gastrointestinal manifestation. Patients infected with parasites and/or *H. pylori* receiving antiparasitic and/or antibiotic treatment during last month before attending the gastroenterology clinics were excluded. In addition, patients diagnosed to have parasitic and/or *H. pylori* infection were ruled out for other causes of GI manifestations as; viral, bacterial or other dietary or feeding problems.

All patients were subjected to full history taking, source of water supply and history of GI disturbances as diarrhea (number of motions per day), abdominal pain, abdominal distension, vomiting and hematemesis or bleeding per rectum.

Ethical considerations

The study protocol was approved by the institutional ethical committee and carried out in compliance with the Helsinki Declaration (2008). All patients were enrolled in the study after an informed consent was obtained from their parent/guardian.

Sample collection and processing

All stool samples were examined in the Diagnostic Research laboratory of Parasitology Department, Faculty of Medicine, Cairo University, to detect helminthic and/or protozoal infections besides *H. pylori* stool antigen testing. The following methods were applied:

Three consecutive fecal samples were obtained from all participants and each stool sample was collected in a clean, dry, labeled, wide-mouth plastic cup (Fleck and Moody 1988). The stool cups were immediately transported to the laboratory for examination and were subjected to the following:

1. Direct wet smear technique: It is examined microscopically at low and high power magnifications. A drop of iodine was then placed on the edge of the coverslip and the specimen was then checked for the presence of protozoa (Garcia 2007).
2. Concentration by formalin-ethyl acetate (FEA) sedimentation technique: and the sediment was transferred onto a clean glass slide for examination. Every slide was examined for at least 10 min before being considered negative (Garcia 2007).
3. Permanent Stained Smear: 10% Formalin preserved stool was used for staining using Kinyoun's Acid-Fast stain (cold method) (Garcia 2007). The stain was prepared manually in the laboratory of Parasitology department, Faculty of medicine, Cairo University.
4. *H. pylori* antigen detection using immunochromatographic immunoassay test (ICT): Every stool sample was subjected to coproantigen detection using on site *H. pylori* Ag rapid test from CTK BIOTECH.

Statistical methods

Microsoft excel 2010 was used for data entry and the statistical package for social science (SPSS version 21) was used for data analysis. Frequencies were used for qualitative data. Bivariate relationship was displayed in cross tabulations and comparison of proportions was performed using the chi-square and Fisher's exact tests where appropriate.

Results

The study included 150 children with their age ranging between 1 and 15 years old presenting with gastrointestinal manifestations. Sixty-three patients were positive for fecal antigen *H. pylori* test (42%) and 88 (58.6%) presented with intestinal parasitic infection. Intestinal protozoa were much more frequent (92%) than intestinal helminths.

Seventy- five patients showed monoparasitic infection, with the most frequently detected parasites including *G. lamblia* (35.2%), *Entamoeba histolytica/E. dispar* complex (22.7%) followed by *Blastocystis hominis* and *Hymenolepis nana* parasites. Mixed parasitic infection was evidenced in 13 patients with the combinations detected; *Blastocystis hominis* and *Entamoeba histolytica/E. dispar complex* (5

patients), *B. hominis* and *G. lamblia* (3 patients), *G. lamblia* and *Entamoeba histolytica/E. dispar complex* (4 patients)and *Entamoeba histolytica/E. dispar complex* and *Cryptosporidium* (one patient) with no statistical significance (Table 1).

Patients were classified according to the results of coproparasitological examination and *H. pylori* stool antigen detection test into four groups; group A: included 49 cases positive for parasitic infection, group B: included 24 cases positive for *H. pylori* infection, group C: included 39 cases positive for both parasitic and *H. pylori* co-infection and group D: included 38 children negative for both parasitic and *H. pylori* infection.

The 39 coinfected cases represented 61.9% of the *H. pylori* positive group and 44.3% of the group with parasitic infection. *G. lamblia* was the most frequently detected parasite associated with *H. pylori* infection (35.9%). (Table 1).

Regarding clinical presentation, abdominal pain (85.3%), abdominal distension (52%) and diarrhea (40.6%) were the most frequently recorded complaints among the different groups (Fig. 1). These symptoms were found to be more frequent in cases of parasitic infection alone than those with *H. pylori* infection only, while vomiting and hematemesis were more frequent in this latter group.

Diarrhea of more than 3 motions per day was recorded in 35.8% and 29.1% of the coinfected and *H. pylori* positive cases, respectively, which was much less than that recorded in patients with parasitic infection alone (63.2%) with statistically significant difference among groups. Additionally, both the co-infected cases and those that presented parasitic infection only showed a lower frequency of vomiting than those infected by *H. pylori* (18%, 17.9% and 37.5% respectively).

Since *G. lamblia* was the most frequently diagnosed parasitic species (31 cases, 35.2% of the total of cases with parasitic infection), the occurrence of GI symptoms was compared among those monoparasitized by this species (17/31 positive cases for the protozoan), those infected only by *H. pylori* (24/150), and those coinfected with both microorganisms (14/31). Although the comparisons among these groups were not statistically significant, both patients with giardiasis and coinfected patients tended to present a higher frequency of diarrhea than those infected only by *H. pylori* (64.7% and 50%, respectively, vs. 29.1%); as well as a lower frequency of vomiting (23.5% and 21.4%, respectively, vs. 37.5%). On the other hand, in the coinfected group, the frequency of abdominal distension was higher than in the other groups (71.4%, vs. 58.8% and 45.8%, respectively) (Table 2).

Predominance of GI manifestations among cases infected with parasites other than *G. lamblia* (after exclusion of mixed parasitic infections with *G. lamblia*) and those coinfected cases with *H. pylori* compared with cases infected with *H. pylori* only was performed. The most prevailing manifestation in the former group was diarrhea (62.5%) while the most predominant manifestation in the other two groups was epigastric abdominal pain (54.1% and 44% respectively). Conversely, vomiting was least recorded in the first two groups (15.6% and 16% respectively) with increased frequency (37.5%) in group of *H. pylori* infection only.

Among the studied variables, age, gender, residency and water supply showed no statistical association among the different groups (Table 3).

Discussion

Diarrhea, vomiting and other gastrointestinal (GI) manifestations are common complaints among children (Abu-Zekry et al. 2013). Intestinal parasites (including *G. lamblia*) have been found to elicit symptoms of GI morbidity (Hanevik et al. 2009). *H. pylori* infection is believed to be the most prevalent infectious disease occuring in humans,

Table 1 Parasites distribution among the studied groups A and C

	Giardia lamblia	*E. histolytica/E. dispar complex*	*B. hominis*	*Cryptosporidium*	*H. nana*	*E. vermicularis*	Mixed infection	Total
Group A [n(%)]	17 (34.4%)	13 (26.5%)	9 (18.4%)	1 (2%)	3 (6.1%)	1 (2%)	5 (10.2%)	49 (100%)
Group C [n(%)]	14 (35.9%)	7 (17.9%)	7 (17.9%)	0 (0%)	2 (5.4%)	1 (2.6%)	8 (20.5%)	39 (100%)
Total	31 (35.2%)	20 (22.7%)	16 (18.1%)	1 (1.1%)	5 (5.6%)	2 (2.2%)	13 (14.7%)	88 (100%)

P value = 0.79

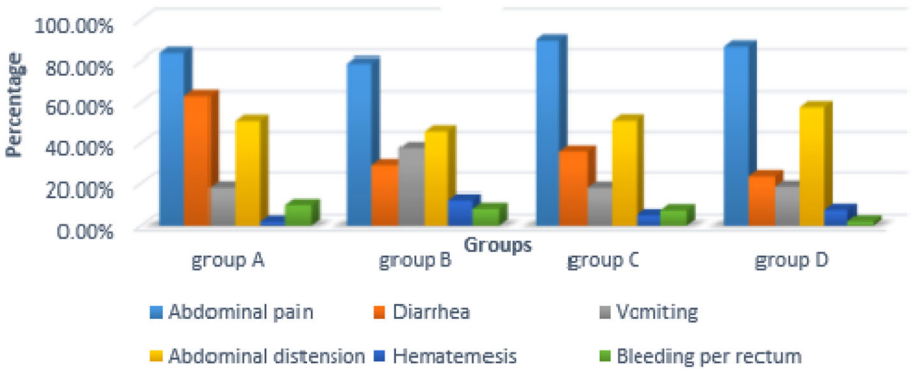

Fig. 1 Gastrointestinal manifestations among different studied groups

Table 2 Distribution of GI manifestation in cases with *G. lamblia* infection compared to cases of *H pylori* infection only and cases with co-infection of both

Manifestations	*Giardia lamblia* infection (n = 17)	*H. pylori* infection (n = 24)	*Giardia lamblia* with *H pylori* coinfection (n = 14)	*P* values
Abdominal pain [n(%)]	10 (58.8)	13 (54.1)	7 (50)	0.885
Diarrhea [n(%)]	11 (64.7)	7 (29.1)	7 (50)	0.073
Vomiting [n(%)]	4 (23.5)	9 (37.5)	3 (21.4)	0.478
Abdominal distension [n(%)]	10 (58.8)	11 (45.8)	10 (71.4)	0.298

P value $\leq$ 0.05 is statistically significant

Table 3 Demographic data of all study groups

	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)
Age group (*P* value = 0.258)				
< 1 year	0 (0%)	1(4.1%)	0 (0%)	0 (0%)
1–5 year	21 (42.8%)	6 (25%)	16 (41%)	11 (29%)
> 5 < 10	17 (34.7%)	11 (45.8%)	10 (25.6%)	18 (47.3%)
10–15	11 (22.4%)	6 (25%)	13 (33.3%)	9 (23.6%)
Gender (*P* value = 0.23)				
Males	27 (55.1%)	14 (58.3%)	17 (43.5%)	14 (36.8%)
Females	22 (44.8%)	10 (41.6%)	22 (56.4%)	24 (63.1%)
Residency (*P* value = 0.25)				
Urban	37 (75.5%)	21 (87.5%)	25 (64.1%)	24 (63.1%)
Rural	7 (14.2%)	3 (12.5%)	9 (23%)	11 (28.9%)
Suburban	5 (10.2%)	0 (0.0%)	5 (12.8%)	3 (7.8%)
Type of water supply (*P* value = 0.58)				
Tap water	44 (89.7%)	22 (91.6%)	37 (94.8%)	36 (94.7%)
Filtered water	5 (10.2%)	2 (8.3%)	2 (5.1%)	1 (2.6%)
Pumped water	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.6%)

especially in low-income countries where infection is usually acquired at an early age (Rosenberg 2010).

In our study, intestinal parasites were recorded in 88 cases (58.6%) with intestinal protozoa (92%) more frequently recorded than intestinal helminths. These findings were in accordance with the results of Al-Shammari et al. (2001) in Saudi Arabia and Mbae et al. (2013) in Kenya. However higher prevalence rates were detected in

other studies by Morales-Espinoza et al. (2003) and Legesse and Erko (2004) recording 67% and 83% of cases respectively, these differences might be attributed to study conduction in areas of high poverty and in rural communities in addition to different sources of water supply.

Our study showed that overall detection of *H. pylori* infection was 42%, which was close to the results obtained by Cheng et al. (2009) in China and Hestvik et al. (2010) in Uganda who identified overall prevalence of 46.6% and 44.3% respectively. Coinfection between *H. pylori* and parasitic infections had been studied in previous literature (Kazemian et al. 2016; Seid et al. 2018). Both *H. pylori* bacterium and intestinal parasites share in colonizing the gastrointestinal tract (GIT) and in being abundant in childhood (Escobar-Pardo et al. 2011; Sabah et al. 2015). In addition, co-existence of both infections may be owed to possible alike modes of transmission (e.g. feco-oral route), low socioeconomic status and poor sanitary conditions (Moreira et al. 2005; Yakoob et al. 2005).

In this study, intestinal parasites were detected in 61.9% among *H. pylori* infected cases, signifying high percentage of parasitic and *H. pylori* co-infection with *G. lamblia*, being the most detected parasite. These results were in agreement with many other studies (Kazemian et al. 2014; Heras-Cañas et al. 2015).

Our study results showed no statistical significance in age, gender, residency or source of drinking water between all studies groups with almost the same composition of different variables in all groups.

Abdominal pain, distension and diarrhea were the most frequently recorded complaints among different cases and were found to be more frequent in cases of parasitic infection alone than those with *H. pylori* infection. Similarly, Okyay et al. (2004) and Escobedo et al. (2008) demonstrated strong association between parasitic infections and abdominal pain or diarrhea. On the other hand, vomiting and hematemesis were more frequently recorded in cases with *H. pylori* infection individually than other groups. Dore et al. (2012) reported that *H. pylori* infection was significantly higher among children having nausea or vomiting than among those who did not show these symptoms. This difference in manifestations may be attributed to the difference in the habitat of infection for *H. pylori* (upper gastrointestinal tract) and intestinal parasites (lower gastrointestinal tract). Different gastrointestinal manifestations detected in group D were attributed to the presence of other diagnosed infectious (bacterial and viral) and noninfectious (dietary and feeding) causes.

Diarrhea wasn't reported to be a prominent symptom in group with *H. pylori* infection (29.1%); which was in lower frequency than other groups. This may be attributed to that the virulence factor of *H. pylori* (as certain VAC A cytotoxin) in our study might not be that one which promotes diarrhea with *H. pylori* (Passaro et al. 2001).

On the contrary to our expectations, in our study the frequency of diarrhea in cases with parasitic and *H. pylori* co-infection (35.8%) was less reported than that recorded in cases of parasitic infection alone (63.2%) with statistically significant difference between groups. Different studies had reported the protective outcome of *H. pylori* infection on the course of the diarrheal illness in *H. pylori* infected patients which might be related to activation of the gut immune response (Perry et al. 2004). Convincingly, co-infection with *H. pylori* and intestinal parasites may show lower frequency of diarrhea than that occurring with parasitic infection only.

Although, vomiting is one of common symptoms of *H. pylori* infection, it was less recorded in coinfected cases (17.9%) than cases of *H. pylori* infection alone (37.5%), and similarly was hematemesis. To the best of our knowledge, no previous literature had studied the impact of coinfection of parasitic and *H. pylori* infections on the clinical presentation of each other. This study revealed that *H. pylori* infection could probably decrease the diarrheal illness associated with intestinal parasites. Alternatively, parasitic infection might decrease the frequency of vomiting associated with *H. pylori* infection in coinfected cases.

In our study, the most frequently detected parasite was *G. lamblia* (35.2% of all parasitized individuals) with 45.1% out of these cases in coinfection with *H. pylori*, so co-infection by both organisms is very frequent as previously reported by many studies (Passaro et al. 2001; Ankarklev et al. 2012). A study done in Iran by Shafie et al. (2009) stated that all patients infected with *G. lamblia* showed concomitant infection with *H. pylori* infection. Ankarklev et al. (2012) and McQuaid et al. (2006) attributed this high occurrence rate of co-existence of both infections to production of a urease that reduces acid levels, which in turn provides a suitable environment for *G. lamblia* survival. Additionally, this co-infection might be important for vitality of *G. lamblia* as that described in case of *E. histolytica* and gut flora, however it does not accentuate the clinical manifestation of *G. lamblia* infection (Berrilli et al. 2012).

Diarrhea was well documented in *G. lamblia* infection (64.7%). However, it was not found to be a prominent symptom in cases infected with single *H. pylori* infection. These results were similar to those conducted by Fouad et al. where diarrhea was the most frequent complaint among the group with *G. lamblia* only while it was less recorded in the group coinfected with *G. lamblia* and *H. pylori* and the group of *H. pylori* infection only (Fouad et al. 2014). On the other hand, vomiting was less recorded in coinfected group and *group with G. lamblia* infection

alone than cases with single *H. pylori* infection, however there was no statistically significant difference between groups.

Comprehensively, the presence of *H. pylori* may play a role in modifying the clinical morbidity of *G. lamblia* as co-infection of *G. lamblia* with *H. pylori* did not accentuate the clinical manifestations of *G. lamblia*, on the contrary it might be associated with less frequency of diarrhea which is an important symptom of *G. lamblia* infection.

Regarding, GI manifestations among cases infected with parasites other than *G. lamblia*, the most predominant manifestation in cases with parasitic infection was diarrhea versus abdominal pain in the other two groups. While the least recorded manifestation in group of parasitic infection other than *G. lamblia* and group of coinfections with *H. pylori* and other parasites other than *G. lamblia* was vomiting (15.6% and 16% respectively). This could support our postulation concerning amendments of clinical manifestation of each of parasitic and *H. pylori* infections in co infected cases.

Further studies reporting TEM analysis on co infection effects on both *H. pylori* and other parasites' fine structures might be required to clarify the effects of both organisms on each other and subsequently on the appearance of different clinical presentation of patients.

Conclusion

Infection with intestinal parasites, *H. pylori* and co-infection with both is common in childhood. Abdominal pain is a common clinical manifestation in childhood, not specifically attributed to *H. pylori* infection. Coinfection of intestinal parasites with *H. pylori* could modulate the clinical manifestations of each other. *H. pylori* infection could probably decrease the intensity of diarrhea associated with intestinal parasites. On the other hand, parasitic infection could modulate vomiting associated with *H. pylori* infection. Coinfection with *G. lamblia* and *H. pylori* is common, accordingly, routine detection of parasitic infection mainly *G lamblia* in cases infected with *H. pylori* in symptomatic children and vice versa should be put in mind, especially that both infections can be easily diagnosed in stool samples.

Author's contribution All manuscript authors contributed to every activity of it; idea of paper, study design, collection of materials, methodology, writing the paper and revising it.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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