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IMPACT OF HELICOBACTER PYLORI-GIARDIASIS COINFECTION ON CHILDREN WITH RECURRENT ABDOMINAL PAIN

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Abstract

Recurrent abdominal pain **(**RAP) affects 10-20% of school-aged children. *Helicobacter pylori* and *Giardia intestinalis* were reported among organic causes of RAP, with different prevalence particularly in developing countries as common association diseases causing agents. This study evaluated the incidence of *H. pylori* and *G. intestinalis* co-infection in RAP Egyptian among 90 children and 90 cross-matched 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 statistically significant difference p=0.001. Giardiasis was found in 47 (52.2%) patients and 30 (33.3%) controls with a statistically significant difference p= 0.02. The incidence of HP infection among cases was higher among age group above 5 years (p=0.001), as a significant predictor for RAP. The association of *H. pylori* and *G. intestinalis* was among 36 (40.0%) patients and 11 (12.2%) controls with a significant difference (p<0.001).

Key words: Egypt, Children, *H. pylori*, *G. intestinalis*, Recurrent abdominal pain.

Introduction

Recurrent abdominal pain (RAP) is one of the commonest complaints of child-hood. RAP is defined as at least three episodic attacks of abdominal pain over at least three months that are severe enough to affect the usual activity of the child (Plunkett and Beattie, 2005). About 10-20% of children suffer from RAP, and about 34% of the worldwide suffer from RAP at some time. (Ramchandani *et al,* 2005). The incidence of organic and non-organic causes of RAP is variable in different studies (Zeyrek *et al*, 2008). *Helicobacter pylori* (*H. pylori*), cholelithiasis and parasitosis were reported as organic causes (Balani *et al*, 2000; Ukarapol *et al,* 2004).

The protozoan *Giardia intestinalis* and the pathogenic bacterium gram-negative *Helicobacter pylori* showed high prevalence in human worldwide (Ankarklev *et al*, 2012). *H. pylori* infects from 20% to >80% of the world population (Graham and Gisbert, 2012), as one of the major causes of the chronic gastritis and duodenal ul-

cer in childhood with a highly risk sector with age increases (Mazigh *et al*, 2012). The infection is mainly contracted during childhood particularly in developing countries among the poor socioeconomic conditions, family overcrowding, and an ethnic or genetic predisposition (Monajemzadeh *et al*, 2010).

Also, *G. intestinalis* (= *lamblia*, or *duodenalis*) infects a wide range of vertebrates, including humans with an annual estimation of 280 million human infection (Ankarklev *et al*, 2012). Infection is characterized by bouts of diarrhea, bloating, flatulence and malnutrition, especially troublesome in the children living in low-income countries where stunted growth and poor cognitive function have been correlated with the disease (Berkman *et al,* 2002). Asymptomatic infections are common and the hosts act as a reservoir for giardiasis (Farthing, 1996).

Cross-sectional studies have reported a potential association between *G. intestinalis* and *H. pylori* (Moreira *et al*, 2005; Zeyrek *et al,* 2008; Isaeva and Efimova, 2010). Both organisms colonize the gastrointestinal tract in the human hosts within a close proximity mainly children at a high rate in low-income countries (Prado *et al*, 2005; Hestvik *et al,* 2010).

H. pylori produces urease which in turn results in reduced gastric acid production, this condition provides a proper environment for *G. intestinalis* (McQuaid, 2006). Both disease agents have a similar mode of transmission (David *et al*, 2006) with co-incidence of concomitant infections (Shafie *et al*, 2009). Both are transmitted by oral-fecal route as well as by non-blood sucking insects (Morsy, 2012), and infect all ages of both sexes (Ukarapol *et al,* 2004). In Egypt, *H. pylori* (Mohammad *et al*, 2008; Allam *et al*, 2010) and *G. intestinalis* (el-Beshbishi *et al*, 2005; Elshazly *et al*, 2007; Soliman *et al*, 2011; Amer, 2013) were reported. But, only one or two studies dealt with their concomitance on the RAP Egyptians patients (Abou-Holw *et al*, 2009).

The study aimed to evaluate the frequency of *H. pylori* and *G. intestinalis* concomitant infection among RAP children in Al-Fayoum University Hospital.

Patients, Materials and Methods

This based prospective study was conducted from June 2012 to December 2012 among 90 children attending the outpatient clinic suffered from clinical manifestations suggesting RAP. Besides, cross-matched apparently healthy children were selected as controls. The protocol was approved by the Faculty of Medicine Research Ethical Committee. A waiver and verbal consent was obtained after proper orientation of the care givers regarding the study objectives.

The medical sheets were filled out on each subject including, name, age, sex, complain, same complain in other family members, treatment received outside, school absenteeism and nocturnal pain. They were clinically examined. Also, samples were collected for the laboratory examination included the complete blood picture, routine stool analysis and abdominal ultra-

sound. The stool samples were collected in clean labeled covered plastic containers, and examined for *H. pylori* by using the *H. pylori*-stool antigen (HpSA) ELISA and antibodies against *H. pylori* in sera by using anti *H. pylori* IgG ELISA (Arne *et al*, 2007).

For the giardiasis, the stained stool smears were microscopically examined (El-Taweel and Abou-Holw, 2008).

The unethical *H. pylori* estimated prevalence in patients with RAP as compared to asymptomatic pediatric population by using endoscopy guided biopsies was not done. Thus, the serologic and copro-antigenic diagnosis was only done. Patients with known malignant, genetic or metabolic disease, a history of antibiotic therapy for at least three consecutive days within the previous months, or on proton pump inhibitors or bismuth were excluded.

Results

The patients mean ages was 5.8±2.4 with a range 3-12 years, sexes were equally represented 48 (53.3) males and 42 (46.7%) females, without significant difference between them regarding age, sex or residence. Sixty (66.7%) RAP patients and 37 (41.1%) of controls were *H. pylori* positive with significant difference between them. *G. intestinalis* was present in 47 (52.2%) patients and 30 (33.3%) controls with significant difference between both.

The multivariate (logistic regression model) analysis was conducted to explore the explanatory power of both *H. pylori* and *G. intestinalis* in the recurrent abdominal pain, showed significant X^2=16.9 (df=2), P <0.001, R^2 ranged from 0.09±0.12. H. pylori was a significant predictor for RAP (P=0.002) with OR= 2.7 (95% CI 1.5-5.0), giardiasis was also a significant predictor for RAP with OR= 2.0 (95% CI 1.1-3.8).

H. pylori infection and giardiasis among cases showed no significant difference as regard age, sex and residence. Association of *H. pylori* and giardiasis was in 36 patients (40.0%) and 11 controls (12.2%) with significant difference (p<0.001). Details are shown in tables (1, 2, 3, 4 & 5).

Table 1: Comparison between cases and controls

	Case (n=90)		Control (n=90)		OR	95% CI	P value
	No.	%	No.	%			
Male	48	53.3	53	58.9	0.8	0.4-1.4	0.6
Female	42	46.7	37	41.1			
Age *Mean±SD, range*	5.8± 2.4 (3-12)		6.4± 2.8 (3-12)				0.2
Residence							
Rural	36	40.0	48	53.3	0.6	0.3-1.1	0.1
Urban	54	60.0	42	46.7			
H. pylori	60	66.7	37	41.1	2.9	1.6-5.3	0.001
G. intestinalis	47	52.2	30	33.3	2.2	1.2-4.0	0.02
Concomitant infections	36	40.0	11	12.2	4.8	2.2-10.2	<0.001

Table 2: Relation of *H. pylori* and *G. intestinalis* with RAP

Item	B	S.E.	Wald	df	P value	OR	95% C.I.for OR	
							Lower	Upper
H. pylori	0.995	.314	10.033	1	0.002	2.706	1.462	5.010
G. intestinalis	0.701	.317	4.889	1	0.027	2.015	1.083	3.749
Constant	-0.837	.264	10.037	1	0.002	0.433		

Table 3: *H. pylori* among patients

Test	Positive		Negative		Total	
H. pylori in sera	No.	%	No.	%	No.	%
Positive	43	71.7	0	0.0	43	47.8
Negative	17	28.3	30	100.0	47	52.2
Total	60	100.0	30	100.0	90	100.0
H. pylori in stools						
Positive	59	98.3	0	0.0	59	65.6
Negative	1	1.7	30	100.0	31	34.4
Total	60	100.0	30	100.0	90	100.0

Table 4: *H. pylori* among controls

Test	Positive		Negative		Total	
H. pylori in sera	N	%	N	%	N	%
Positive	27	73.0	0	0.0	27	30.0
Negative	10	27.0	53	100.0	63	70.0
Total	37	100.0	53	100.0	90	100.0
H. pylori in stools						
Positive	36	97.3	0	0.0	36	40.0
Negative	1	2.7	53	100.0	54	60.0
Total	37	100.0	53	100.0	90	100.0

Table 5: Analysis of *H. pylori* and *G. intestinalis* among cases

Sex	Male (n=48)		Female (n=42)		OR	95% CI	P value
	No.	%	No.	%			
H. pylori	34	70.8	26	61.9	1.5	(0.6-3.6)	0.4
G. intestinalis	26	54.2	21	50.0	1.2	(0.5-2.7)	0.8
Residence	Rural (n=36)		Urban (n=54)		OR	95% CI	P value
H. pylori	25	69.4	35	64.8	1.2	(0.5-3.0)	0.8
G. intestinalis	21	58.3	26	48.1	1.5	(0.6-3.5)	0.4
Age group	≤5 years (n=42)		>5–8 years (n=36)		> 8 years (n=12)		P value
H. pylori	20	47.6	32	88.9	8	66.7	0.001
G. intestinalis	22	52.4	18	50.0	7	58.3	0.9

Discussion

RAP is a common problem of difficult clinical concern (Franck *et al,* 2001). Giardiasis and *H. pylori* were reported the commonest organic causes of RAP (Ukarapol *et al,* 2004; Buch *et al*, 2002).

In the present study, the incidence of *H. pylori* infection and giardiasis in children with RAP was 66.7% and 52% respectively, indicating the high contribution of both organisms in the RAP aetiology.

The incidence of *H. pylori* infection among children with RAP varied. The higher incidence among cases than controls was reported in Czech (Sedlackova *et al,* 2003) where infection was 33% of RAP cases and 7 % in controls and in Iran (Paolzi *et al,* 2010) where infection was 40% of cases versus 25% of controls. Others reported

low rate of infection in both cases and controls as in Canada (Macarthur, 2001) and Netherlands (Van Der Meer *et al,* 2002). In the present study the incidence of *H. pylori* infection was 66.7% in cases versus 41% in controls with a significant difference (p=0.001) indicating high rate of *H. pylori* infection in children with RAP.

Giardiasis was defined as a leading cause for RAP in developing countries (Balani *et al*, 2000). But, the role of *H. pylori* infection as cause of RAP is controversial (Mansour *et al,* 2012). The relation of *H. pylori* infection to RAP was reported (Özen *et al*, 2001; Örmeci *et al*, 2003), while others reported no role at all (De Giacomo *et al*, 2002; Bode *et al*, 2003).

In this study the high incidence of *H. pylori* infection among children with RAP together with the use of multivariate (logistic regression model) analysis of the subjected data revealed that *H. pylori* infection is an important etiologic cause of RAP in children.

The present incidence of giardiasis was 52.2% among cases and 33.3% in controls with a significant difference (p=0.02). This high incidence correlated with the study from Uganda (Johnston *et al*, 2010) another developing country that reported high incidence of giardiasis among children with RAP.

The incidence of giardiasis among cases was not affected by age, sex and residence while *H. pylori* infection was significantly higher at older ages. The increasing of age, low socio-economic standard of living and consumption of contaminated water were described as the major risk factors for *H. pylori* infection (Frenck *et al,* 2006; Pardo *et al,* 2011).

In the present study association of both was found in 36(40%) of children with RAP and 11 (12.2%) of controls with a significant difference p<0.001. A significant relationship of *H. pylori*-giardiasis co infection at the events of RAP and treatment of one agent would not improve the complaint of patients and treatment of both organisms was recommended (Zeyrek *et al*, 2008). This association was reported in different studies (Ankarklev *et al*, 2012; Moreira *et al*, 2005; Pardo *et al*, 2011).

This co-infection was presumed to be due to the common route of infection; fecal-oral route (Vale and Vitor, 2010) or synergistic polymicrobial infection in which one microbe creates a favorable environment for another (Moreira *et al*, 2005; Shafie *et al*, 2009; Ankarklev *et al*, 2012). Zeyrek *et al* (2008) suggested that the achlorhydria and atrophic gastritis developing after *H. pylori* infection facilitates for *Giardia* infection. Both pathogens can be cultured in-vitro and in-vivo assays paving the way to understand the correlation (Sainsus *et al,* 2008; Benere *et al*, 2010).

Conclusion

The outcome results showed high incidence of *H. pylori* and *G. intestinalis* concomitant infection in RAP children, with *H. pylori* as a leading cause for RAP in children. The upper gastrointestinal symptoms (epigastric pain and anorexia) are common clinical

picture in patients with giardiasis and *H. pylori*. The relationship of *H. pylori* infection and giardiasis represent an important etiologic factor in children with recurrent abdominal pain.

Concomitant infection with both was the commonest feature and magnified the pathogenesis of recurrent abdominal pain.

References

Abou Holw, SA, Anwar, MM, Heshmat, MG, Enany, AY, Rashad, MM, 2009: Effect of concommitant *Helicobacter pylori* infection in patients with giardiasis *lamblia* in Egypt. J. Egypt. Soc. Parasitol. 39, 2:439-46.

Allam, MA, El-Shafie, AM, Elwan, A M, Soliman, GM, Abu-Alfotuh, A, *et al*, 2010: Haematological side effect of *Helicobacter pylori* eradication. J. Egypt. Soc. Parasitol. 40, 3: 583-90.

Amer, SE, 2013: Genotypic and phylogenetic characterization of *Giardia intestinalis* from human and dairy cattle in Kafr El Sheikh Governorate, Egypt. J. Egypt. Soc. Parasitol. 43, 1:133-46.

Ankarklev, J, Hestvik, E, Lebbad, M, Lindh, J, Deogratias, H, *et al*, 2012: Common coinfection of *Giardia intestinalis* and *Helicobacter pylori* in non-symptomatic Ugandan childr-en. Plos. Negl. Trop. Dis. 6, 8: e1780.

Arne, S, Gaustad, P, Stray-Pedersen, B, 2007: Detection rate of *H. pylori* stool antigen in newborn infants and children. J. Perinat. Med. 35, 2:155-8

Balani, B, Patwari, AK, Baja, P, Diwan, N, Anand, VK, 2000: Recurrent abdominal pain: A reappraisal. Indian Pediatr. 37: 876-881.

Benere, E, Geurden, T, Robertson, L, Van Assch, T, Cos, P, *et al*, 2010: Infectivity of *Giardia duodenalis* assemblages A & E for the gerbil and axenisation of duodenal trophozoites. Parasitol. Int. 59: 634-7.

Berkman, DS, Lescano, AG, Gilman, RH, Lopez, SL, Black, M, 2002: Effects of stunting, diarrheal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. Lancet 359:564-71.

Bode, G, Brenner, H, Adler, G, Rothenbacher, D, 2003: Recurrent abdominal pain in children: evidence from a population-based study that social and familial factors play a major role but not *Helicobacter pylori* infection. J. Psychosom. Res. 54, 5: 417-21.

Buch, NA, Ahmad, SM, Ahmad, S Z, Ali, SW, Charoo, BA, *et al,* 2002: Recurrent abdominal pain in children. Indian Pediatr. 39:830-4.

David, TJ, William, AP, Markell, A, Voge, S, 2006: Medical Parasitoogy. 9th ed. Elsevier New York: Saunders.

De Giacomo, C, Valdambrini, V, Lizzoli, F, Gissi, A, Palestra, M, *et al,* 2002: A population-based survey on gastrointestinal tract symptom and *Helicobacter pylori* infection in children and adolescent. Helicobacter 7, 6: 356-63.

el-Beshbishi, SN, Abdel-Magied, A A, el-Nahas, HA, Azab, MS, el-Shazly AM, Morsy AT, *et al*, 2005: Geoparasites in rural Dakahlia Governorate, a preliminary based study for development of community-based intervention programs. J. Egypt. Soc. Parasitol. 35, 3:1051-70.

Elshazly, AM, Elsheikha, HM, Soltan, DM, Mohammad, KA, Morsy, T A, 2007: Protozoal pollution of surface water sources in Dakahlia Governorate, Egypt. J. Egypt. Soc. Parasitol. 37, 1: 51-64.

El-Taweel, HA, Abou Holw, SA, 2008: Use of a non-mercury containing fixative for diagnosis of giardiasis. J. Egypt. Soc. Parasitol. 38, 1:65-72.

Farthing, MJ, 1996: Giardiasis. Gastroenterol. Clin. North Am. 25:493-515.

Franck, F, Sicker, T, Stallmach, T, *et al*, 2001: *Helicobacter pylori* in recurrent abdominal pain. J. Pediatr. Gastroenterol. Nutr. 32, 4:504-9.

Frenck, RW, Jr, Fathy, HM, Sherif, M, *et al,* 2006: Sensitivity and specificity of various tests for the diagnosis of *Helicobacter pylori* in Egyptian children. Pediatrics 118:e1195-202.

Graham, DY, Gisbert, JP, 2012: *Helicobacter pylori*: Tailored therapy with novel sequential quadruple therapies. Nat. Rev. Gastroenterol. Hepatol. 27. doi:10. 1038/nrgastro.232

Hestvik, E, Tylleskar, T, Kaddu-Mulindwa, DH, Ndeezi, G, Grahnquist, L, *et al*, 2010: *Helicobacter pylori* in apparently healthy children aged 0-12 years in urban Kampala, Uganda: a community-based cross se-ctional survey. BMC-Gastroenterol. 10:62-70.

Isaeva, G, Efimova, NG, 2010: Gastrointestinal giardiasis associated with *Helicobacter pylori*. Eksp. Klin. Gastroenterol. 15:30-4.

Johnston, AR, Gillespie, TR, Rwe-go, IB, McLachlan, TL, Kent, AD, *et al,* 2010: Molecular epidemiology of the cross-species *Giardia duodenalis* transmission in western Uganda. PLoS. Negl. Trop. Dis. 4: e683.

Macarthur, C, 2001: *Helicobacter pylori*: Non-ulcer dyspepsia and childhood recurrent abdominal pain. Pediatr. Res. 32:49-140

Mansour, MM, Al Hadidi, KhM, Omar, MA, 2012: *Helicobacter pylori* and recurrent abdominal pain in children: Is there any relation? Trop. Gastroenterol. 33, 1:55-61.

Mazigh, M, Abidi. K, Brini, I, Boukthir, S, Sammoud, A, 2012: Nodular gastritis: An endoscopic indicator of *Helicobacter pylori* infection in children. Tunis Med. J. 11:789-92.

McQuaid, KR, 2006: Gastrointestinal and liver Disease: Ppathophysiology, diagnosis, management. In: Dyspepsia. 8th ed. eds. Sleisenger and Fordtran's: Saunders; Elsevier, Canada.

Mohammad, MA, Hussein, L, Coward, A, Jackson, SJ, 2008: Prevalence of *Helicobacter pylori* infection among Egyptian children: The impact of social background and effect on growth. Pub. Hlth. Nutr.11, 3:230-6

Monajemzadeh, M, Ashtiani, MTH, Ali, AM, Sami, MN, Shams, S, *et al,* 2000: *Helicobacter pylori* infection in children: association with giardiasis. Br. J. Biomed. Sci. 67, 2:86.

Moreira, ED, Nassir, VB, Santos, R S, Matos, JF, de Carvalho, W, *et al,* 2005: Association of *Helicobacter pylori* infection and giardiasis: results from a study of surrogate markers for fecal exposure among children. World J. Gastroenterol. 11:2759-63.

Örmeci, A, Hekimoğlu, Ü, Kronik, K,

Çocuklarda, A, 2003: *Helicobacter py pylori* İnfeksiyonu: Prevalans, Tanı, Tedavive Risk Faktörl. Çocuk Dergisi. 3, 2:144-50.

Özen, H, Dinler, G, Akyön, Y, Koçak, N, Yüce, A, *et al,* 2001: *Helicobacter pylori* infection and recurrent abdominal pain in Turkish children. Helicobacter 6, 3:234-8.

Paolzi, OA, Visconti, E, Andrei, F, *et al,* 2010: Ten and eight day sequential therapy in comparison to standard triple therapy for eradicating *Helicobacter pylori* infection: A randomized controlled study on efficacy and tolerability. J. Clin. Gastroenterol. 44, 4: 261-8.

Pardo, ML, Godoy, AP, Machado, R S, Rodrigues, D, Neto, U, *et al,* 2011: Prevalence of *Helicobacter pylori* infection and intestinal parasitosis in children of the Xingu Indian reservation. J. Pediatr. Rio. J. 87, 5:393-8.

Plunkett, A, Beattie, RM, 2005: Recurrent abdominal pain in childhood. J. Roy. Soc. Trop. Med. 98, 3:101-6.

Prado, MS, Cairncross, S, Strina, A, Barreto, ML, Oliveira, AM, *et al,* 2005: Asymptomatic giardiasis and growth in young children; a longitudinal study in Salvador, Brazil. Parasitology 131:51-6.

Ramchandani, PG, Hotopf, M, Sandhu, B, *et al,* 2005: The epidemiology of recurrent abdominal pain from 2 to 6 years of age: Results of a large, population-based study. Pediatrics 116:46-52.

Sainsus, N, Cattori, V, Lepadatu, C, Hofmann, LR, 2008: Liquid culture medium for the rapid cultivation of *Helicobacter pylori* from biopsy specimens. Eur. J. Clin. Microbiol. Infect. Dis. 27:1209-17.

Sedlackova, M, Volf, V, Malaty, H, 2003: *Helicobacter pylori* infection in a group of symptomatic and asymptomatic children and adolescents in the Czech Republic. Cas. Lek. Cesk. 142, 2:102-5.

Shafie, R, Jahani, MR, Rezaeian, M, Amini, M, Metvayi, AR, *et al,* 2009: *Giardia lamblia* and *Helicobacter pylori* coinfection. Iranian J. Publ. Hlth. 38, 1: 127-30.

Soliman, RH, Fuentes, I, Rubio, J M, 2011: Identification of a novel assemblage B subgenotype and a zoonotic assemblage C in human isolates of *Giardia intestinalis* in Egypt. Parasitol. Int. 60, 4:507-11.

Ukarapol, N, Lertprasertsuk, N, Wongsawasdi, L, 2004: Recurrent abdominal pain in children: the utility of upper endoscopy and histopathology. Singapore Med. J. 45, 3:121-4.

Vale, FF, Vitor, JM, 2010: Transmission pathway of Helicobacter pylori: does food play a role in rural and urban areas? Int. J. Food Microbiol. 138:1-12.

Van der Meer, SB, Forgte, PP, Loffled, RJLF, *et al,* 2002: The prevalence of *Helicobacter pylori* serum antibodies in children with recurrent abdominal pain. Eur. J. Pediatr. 151: 799-801.

Zeyrek, D, Zeyrek, F, Cakmak, A, Cekin, A, 2008: Association of *Helicobacter pylori* and giardiasis in children with recurrent abdominal pain. Turkiye Parazitol. Derg. 32, 1:4-7