

Correlation of Helicobacter Pylori Infection With IL-8 and Recurrence of Abdominal Pain: Our Experience from an Endemic Area

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ABSTRACT

AIM: The prevalence of *H. pylori* infection is widely distributed among young individuals. Interlukin-8 (IL-8) has a role in many gastritis disorders. Although the link between *H. pylori* infection and IL-8 secretion in children was established, its association to abdominal pain recurrence needs further investigation. We aim to study the prevalence of *H. pylori* in children presenting with recurrent abdominal pain and whether it is associated with high serum IL-8 in Egyptian children.

METHODS: This cross-sectional study includes 104 children; 80 children presenting with recurrent abdominal pain and 24 asymptomatic children used as a control. All studied cases were subjected to have family history, and complete general and abdominal examinations. Stool samples were requested from each participating child. The detection of *H. pylori* antigen in stool samples and IL-8 in blood sera were measure by ELISA method.

RESULTS: Our results demonstrate that the prevalence of *H. pylori* was significantly higher (67.50 %; $p = 0.001$) in children presenting with abdominal pain compared to healthy subjects. The level of serum IL-8 was significantly higher and was correlated with the presence of pathogen-positive cases compared to negative volunteers.

CONCLUSION: These findings imply that the infection of *H. pylori*, higher IL-8 in serum and recurrent abdominal pain among infected children were correlated with the age in children.

Key words: Children; *H. pylori*; Prevalence; Serum IL-8; Abdominal pain

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INTRODUCTION

Helicobacter Pylori (*H. pylori*) is widely prevalent Gram-negative bacterium, one of major components of gastric microbiology^[1] and important pathogen of pediatric gastroenterology^[2]. The risk factors for *H. pylori* infection comprise socioeconomic status, household crowding, ethnicity, migration from high prevalence endemic areas, infection status of family members^[3,4] and no sex preponderance was documented^[5]. The prevalence of *H. pylori* is dramatically high in developed countries and it can be detected in most children reach adulthood. In developing countries infants have high infection rate (up to 50%) as early as of 2 year old^[6] and most of them harbor the organism^[7]. *H. pylori* constitutes a real challenge in Egypt as its prevalence in children is noted to be high ranging from 33 - 60% and up to 90 % in adults^[8].

H. pylori colonize the gastric mucosa in 35-70% of people worldwide and it is the main etiologic factor for development of chronic active gastritis and peptic ulcers^[9]. *H. pylori* is suspected in children with history of hyperacidity and among those who have a first degree family member diagnosed with gastric cancer^[10]. It has been known that there is a direct association between gastric cancer and *H. pylori* infection. *H. pylori* increase the release of interleukin-8 (IL-8) in gastric epithelial cells and the higher level of IL-8 is indicative for poor prognosis. This secretion occurs through a dual mechanism which includes a Toll-like receptor (TLR2) 2/5-dependent and independent pathways^[11]. It is worthy to report that over expression of IL-8 induced by *H. pylori* are suggested to play a crucial role in the development and progression of gastric cancer^[12]. Thus, IL-8 is considered as prognostic and predictive gastric cancer biomarker^[13].

The current gold standard for diagnosis of *H. pylori* infection is endoscopic biopsy of the gastric tissue for rapid urease test, histology and culture. However, such invasive procedure has major disadvantages of anesthesia, discomfort and possibly becomes a source of ethical problems. Although the urea breath test (UBT) is the most reliable non-invasive test in the general population, it may be less efficient in pediatric patients and its diagnostic accuracy requires special instrumentation and well-trained staff^[14]. *H. pylori* stool antigen (HpSA) test was approved as a non-invasive, reliable diagnostic tool and it is used to confirm success of eradication therapy^[15]. *H. pylori* stool antigen (HpSA) test has shown high sensitivity and specificity in children^[16] and has been used to screen children with abdominal pain, applicable in mass survey and recommended when endoscopic examination is infeasible^[17].

Thus, our aim was to examine the prevalence of *H. pylori* in a group of children presenting with recurrent abdominal pain and investigate whether *H. pylori*-infected children is correlated with elevated IL-8 in serum.

SUBJECTS AND METHODS

Patients

An ethical approval was taken before starting our study and Informed consent was obtained from all participating subjects before the study. This study was conducted in the Pediatric clinics at the National Hepatology and Tropical Medicine Research Institute, Abou El-Rish Hospital at Cairo University as well as Benha University Hospital after Institutional Review Board (IRB) approval during the period from July, 2012 to October, 2013. In this cross-sectional study, 80 children was included and presenting with recurrent abdominal pain, which is defined as at least 3 discrete episodes of abdominal pain of sufficient severity to interrupt normal daily activities or performance over

a period of not less than 3 months. The presented cases were 62.5 % males with age ranged from 5 - 16 year old. Another 24 gender-matched asymptomatic children whose parents agreed to participate were enrolled as a healthy control group. They were 58.3% males with age ranged from 5 - 17 year old. All demographic information was collected from all participant children in this study. All children were subjected to a record of full history taking with special reference to family history, complete general and abdominal examination. Anthropometric measurements were also assessed such as body weight in Kg, height in cm and body mass index (BMI) in kg/m². Common causes of abdominal pain were excluded by urine analysis, stool analysis and abdominal ultrasound.

Blood sampling and biochemical assays

Fasting venous blood samples (2 mL) were collected for biochemical analyses. Laboratory investigations were performed including complete blood count (CBC), hemoglobin (HB) and white blood cells. Liver function tests were measured including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total and direct bilirubin and albumin. Random blood glucose level, Creatinine, serum TG and cholesterol levels were also assayed using Beckman CX4 chemistry analyzer (Beckman, NY, USA). The level of serum IL-8 was measured by Quantikine Human CXCL8/IL-8 Immunoassay according to the manufacturer instructions (R&D Systems, MN, USA).

The *Helicobacter Pylori* stool Antigen test

Stool sample were requested from each participating child and samples were collected in air tight containers at the time of encounter. The detection of antigen of *H. pylori* in the collected samples was performed using ELISA kit according to the manufacture's protocol (Immunodiagnostic, AG, Bensheim, Germany).

Statistical analysis

Mean and standard deviation (SD) were calculated of quantitative data while frequency and percentage were estimated of qualitative data. Differences in clinical and biochemical characteristics were tested by Student's *t* test or Mann Whitney U test for quantitative data and chi-square test for non-parametric data. A two-sided *p*-value < 0.05 was considered statistically significant. Odds ratio were used to measure the strength of associations of variables. Statistical Package for Social Science (SPSS) program, version 17.0, was used for data analysis (SPSS Inc., IL, USA).

RESULTS AND DISCUSSION

The prevalence rate of *H. pylori* is widely varied based on the living area ranging from 6 % in Finland^[18] to 95% in Perth, Australia^[19]. This variability mainly comes from inadequate living conditions, poor hygiene and overcrowding. Another founding factor contributes to *H. pylori* infection is its transmission through contaminated water^[4,20]. Here, we report that the prevalence of *H. pylori* as measured by the presence of its antigen was significantly higher (67.50 %, *p* = 0.000) in children presenting with abdominal pain compared to control subjects (Table 1). The mean age of children with *H. pylori* infection was 11.5 ± 2.7 compared to 7.4 ± 2.0 in negative cases (Table 2). The prevalence of *H. pylori* had shown a marked increase relative to the age of symptomatic children. The prevalence was 1.9% for children ≤ 5 years compared to 98.1 for children > 5 years (Table 2). Our findings are in accordance with other previously published data^[19,21]. In another study, the authors reported that the global prevalence of

H. pylori infection was 31.6% and such rate can increase by age^[22]. However, other studies have shown that the prevalence of *H. pylori* infection in children is independent of age^[23,24]. The gender as another contributing factor is still controversy. In some studies, the gender has not been identified as a relevant influencing characteristic for *H. pylori* infection^[21,24]. However, it has been reported that females have higher risk of *H. pylori* infection than males^[4,25]. In a recent study conducted in Uganda demonstrated a significant gender difference in the prevalence of *H. pylori* colonization and boys were likely infected compared to girls^[26].

Our studies show that there was a significant difference in the incidence of *H. pylori* infection among the symptomatic children. This implies the correlation between the recurrence of abdominal pain and *H. pylori* infection^[3,27]. However, the association of recurrent abdominal pain and *H. pylori* is still controversy since other studies have reported there was no association between both factors^[28,29]. The response in recent abdominal pain children after *H. pylori* eradication was investigated and the results showed that the bacterial eradication and healing of gastric inflammation did not lead to symptomatic relief of recurrent abdominal pain in children^[30]. In our study, children presented with positive *H. pylori* antigen were significantly older and with more prevalent burning sensation, unprotected water source, no or low level education, living in rural areas with positive family history than non-symptomatic control group. *H. pylori*-positive children showed higher WBCs, IL-8 and lower hemoglobin than negative subjects (Table 2 and Figure 1). However, there was no statistical difference in glucose level, liver panel, creatinine, triglycerides and cholesterol among children with positive and negative *H. pylori*. The frequency of *H. pylori* was rising with the increased age, age group of 14 - 17 years which demonstrated the highest distribution of *H. pylori* ($p = 0.000$) as shown in Figure 2. The age of children and IL-8 were positively correlated with level of *H. pylori* antigen in stools (Figure 3). The risk of harboring *H. pylori* Ag was increased with age above 5 years who having burning sensation and unprotected water source. A large body of evidence shows that the prevalence of *H. pylori* was significantly higher among children with reflux oesophagitis than in children with hyperemic gastropathy^[31]. However, we could not find any significant difference between *H. pylori* positive and negative cases in the incidence of nausea, and vomiting^[32], or diarrhea^[33]. However, other studies reported positive correlation between the infection and diarrhea^[3,18], and in some cases it correlates with vomiting^[3].

Our results also demonstrated that there was no association with the increased risk of children's gender and positive family history (Table 3). Other studies showed that the family history with gastroduodenal disease was positive in about 44% of *H. pylori* positive cases^[34]. Although the exact impact of environmental factors on *H. pylori* infection is unclear, bodily fluids and personal contact may be the reason in the spread of disease among family members^[35]. Lower socioeconomic status, crowded families and sharing of common living areas with compromised hygiene can also lead to increase the dissemination of *H. pylori*^[36].

In our study, *H. pylori* positive cases revealed a significant difference in the incidence of anemia (Table 2) and these results are in accordance with another previous study done by Kurekci *et al*^[37]. Development of anemia during *H. pylori* infection could be originated from poor absorption of iron due to low gastric acid secretion and/or poor dietary intake and utilization of iron by the bacterial flora^[29]. A recent meta-analysis study demonstrated an association between *H. pylori* and iron deficiency anemia^[38].

Most of *H. pylori* infections are acquired early in life and

Table 1 Demographic and laboratory data collected from children experienced abdominal pain.

	Control (N=24)	Cases (N=80)	p-value
Gender: N (%)			0.7
Male	14 (58.30 %)	50 (62.50 %)	
Female	10 (41.70 %)	30 (37.50 %)	
Age (year): mean ± SD	11.80 ± 3.03	10.20 ± 3.10	0.020*
Positivity to <i>H. pylori</i> Ag: N (%)	0	54 (67.50 %)	0.0001*

*depicts significant at $p < 0.05$ and $p < 0.001$

Table 2 Comparison of children with positive and negative *H. pylori* antigen (Ag).

	<i>H. pylori</i> Ag group		p-value
	Negative (n=26)	Positive (n=54)	
Age: Mean ± SD	7.40 ± 2.00	11.50 ± 2.70	0.000*
Gender: N (%)			0.500
Male	15 (57.70%)	35 (64.80 %)	
Female	11 (42.30 %)	19 (35.20 %)	
Burning sense: N (%)	3 (11.50 %)	29 (53.70 %)	0.000*
Nausea: N (%)	10 (38.50 %)	18 (33.30 %)	0.700
Vomiting: N (%)	16 (61.50 %)	24 (44.40 %)	0.200
Gastritis: N (%)	0	8 (14.80 %)	0.050
Bowel habits: N (%)			0.300
Diarrhea	4 (15.40 %)	14 (25.90 %)	
Constipation	22 (84.60 %)	40 (74.10 %)	
Bleeding: N (%)	0	7 (13.00 %)	0.090
Education: N (%)			0.000*
No	2 (7.70 %)	22 (40.70 %)	
Low	3 (11.50 %)	18 (33.30 %)	
Moderate	12 (46.20 %)	11 (20.40 %)	
High	9 (34.60 %)	3 (5.60 %)	
Water source: N (%)			0.000*
Protected	24 (92.30 %)	23 (42.60 %)	
Unprotected	2 (7.70 %)	31 (57.40 %)	
Habitat: N (%)			0.000*
Urban	26 (100 %)	21 (38.90 %)	
Rural	0	33 (61.10 %)	
Positive family history: N (%)	0	24 (44.40 %)	0.000*
WBCs count (x 10 ³):mean ± SD	10.10 ± 3.70	12.10 ± 3.60	0.030*
HB: mean ± SD	11.80 ± 0.70	9.20 ± 1.20	0.000*
IL 8: mean ± SD	125.80 ± 40.10	208.80 ± 43.70	0.000*

N: number; SD: standard deviation; *depicts significant at $p < 0.05$.

Table 3 Risk factors of *H. pylori* among children presented with abdominal pain.

	No.	<i>H. Pylori</i> positive N (%)	p	Odds Ratio	95% Confidence Interval	
					Lower	Upper
Age			0.040*	9.60 *	1.02	91.20
< 5 yrs	15	3 (20.0 %)				
> 5 yrs	65	53 (81.53 %)				
Gender			0.500	0.70	0.30	1.90
Male	50	35 (70.0 %)				
Female	30	19 (54.29%)				
Burning sense	32	29 (90.63 %)	0.001*	8.90 *	2.40	33.20
Unprotected water source	33	31 (93.93 %)	0.001*	16.20*	3.50	75.40
Positive family history	24	24 (100 %)	0.001*	0.50	0.40	0.70

N: number; SD: standard deviation; *depicts significant at $p < 0.05$.

may persist throughout the life of the individual^[39]. Despite the development of chronic inflammatory response accompanied by adaptive immune response in infected individuals, these responses failed in clearing the infection^[40]. Remarkably, *H. pylori* can persistently colonize the stomach for decades even after the acquired immune response was initiated^[41]. Recent evidence suggests that the persistence of infection is attained through early interactions

between *H. pylori* adhesions and host cellular receptors, after which the bacteria will avoid clearance by immune system. Understanding the mechanisms underlying the process of colonization, persistence factors of the pathogen as well as the developing the immune

response of the host are key factors for development of new strategies to circumvent the development of *H. pylori*-induced gastroduodenal diseases^[42].

Our study revealed that serum level of IL-8 is significantly higher ($p = 0.000, r = 0.6$) in *H. pylori*-positive cases (Figure 3). There was a positive correlation between IL-8 level and the age at diagnosis, family history and the level of circulating antigen of the bacteria. *H. pylori* can induce pro-inflammatory cytokines such as IL-1, IL-8, and TNF- α . These cytokines can enhance mucosal inflammation and gastric acid secretion^[43]. In other previous studies, the levels of IL-6, IL-8 and TNF-alpha had no difference in infected cases^[44]. They anticipated that the pathogen-related cytokine activation becomes concentrated on gastric mucosa and this accompanied local inflammatory cascade does not cause any significant changes in their levels. Some of our study limitations are the relatively low

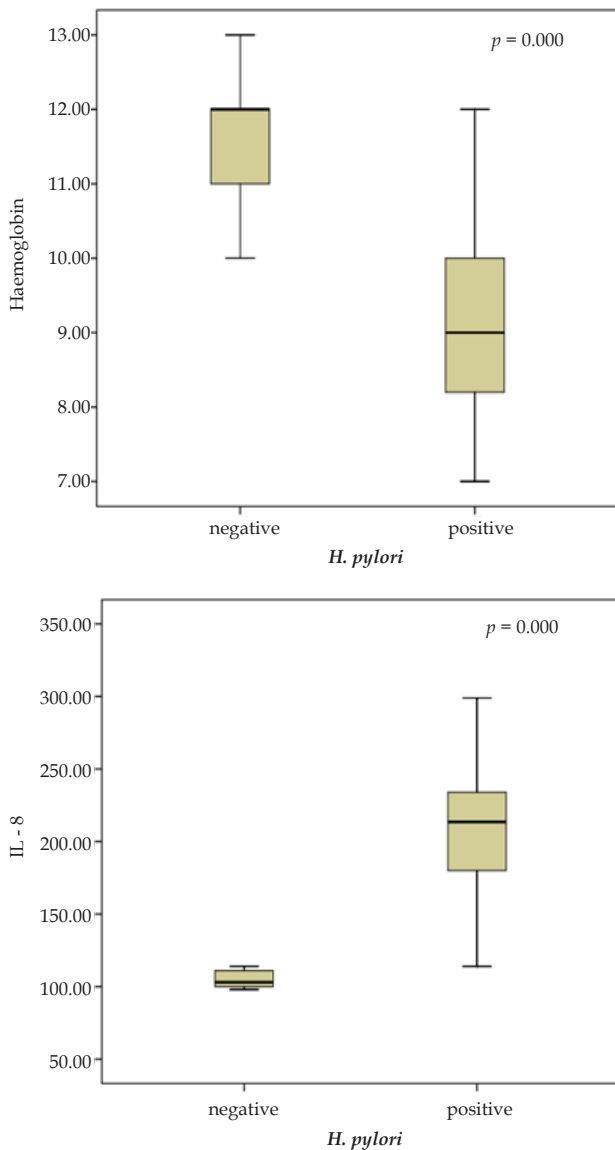


Figure 1 Correlation of Hemoglobin, serum IL-8 level and *H. pylori* antigen in children.

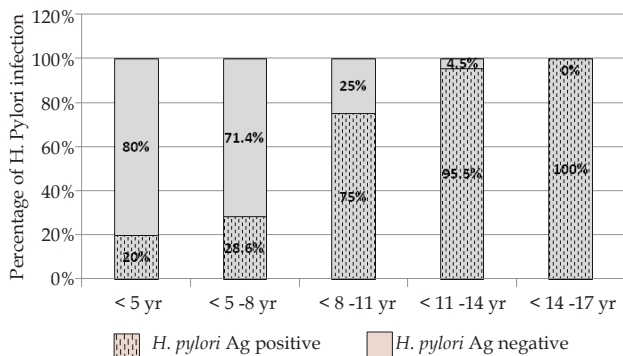


Figure 2 Frequency of *H. pylori* among different age categories in cases.

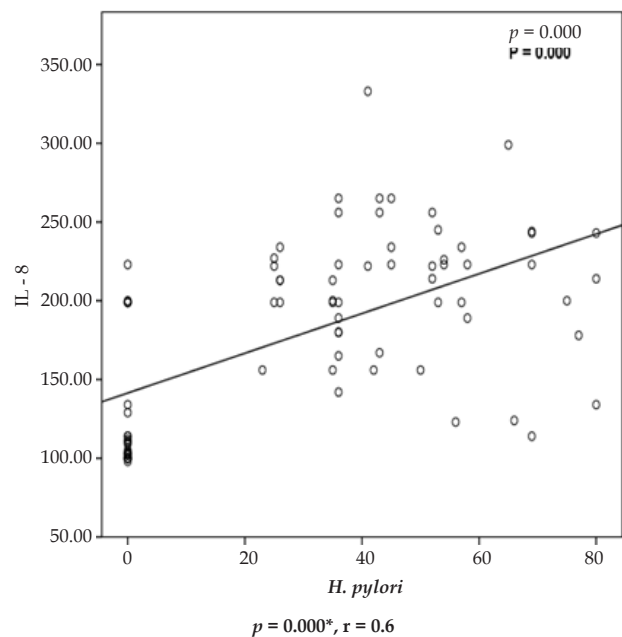
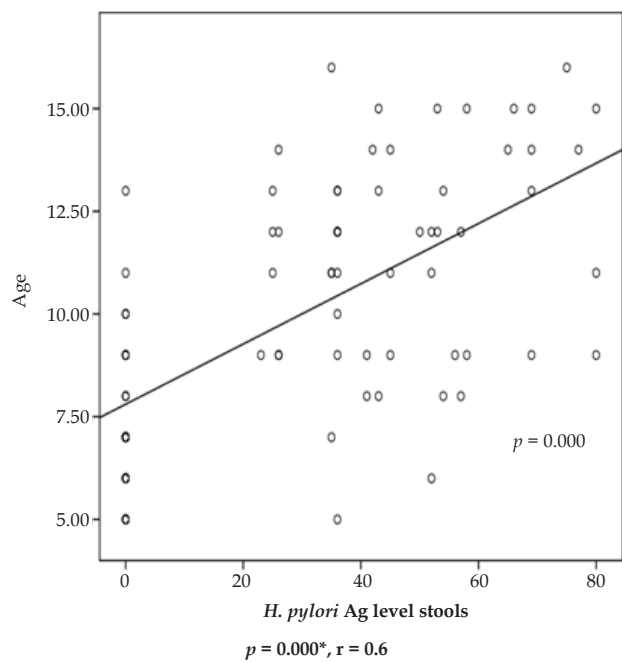


Figure 3 Correlation between age & IL-8 and *H. pylori* level in stools.

sample size used and more reliable, precise method for measuring interleukins should be considered and further studies are needed to address the role of IL-8 in gastroduodenal diseases.

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