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Association between *Helicobacter pylori* Infection and Iron Deficiency Anemia among School-Age Children in Sohag University Hospital, Upper Egypt

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Abstract

Background: *Helicobacter pylori* (*H. pylori*) infection has been suggested as a cause of iron deficiency anemia (IDA) refractory to iron therapy. **Objective:** The aim of this work was to evaluate the association between *H. pylori* infection and IDA among school-age children. **Subjects and Methods:** This was a prospective case-control study conducted for one year starting from March 2015 and included 200 school-age children with IDA and 50 age and sex matched non-anemic controls, attending pediatric outpatient clinic at Sohag university Hospital, Sohag, Upper Egypt. All of participants were subjected to clinical evaluation and the following investigations: CBC, serum iron, total iron binding capacity, serum ferritin and a quantitative detection of *H. pylori* IgG antibodies. **Results:** Totally, 72 (36%) children with IDA and 6 (12%) non-anemic controls had positive level for *H. pylori* specific IgG ($P = 0.036$). *H. pylori* IgG antibody titer showed significant positive correlation with age and significant negative correlation with each of Hb level, MCV, HCT and serum ferritin. Age was higher ($p < 0.001$) and hematological parameters, like Hb ($p = 0.001$), MCV ($p = 0.002$), HCT ($p < 0.001$) and serum ferritin ($p = 0.042$), were significantly lower in *H. pylori* positive IDA cases in comparison to *H. pylori* negative IDA cases. **Conclusion:** The results of this study demonstrate significant association between positive serology for *H. pylori* infection and IDA in school-age children. Moreover, infection may increase the severity of anemia.

Keywords

H. pylori, Iron Deficiency Anemia, School-Age Children

1. Introduction

Iron deficiency anemia (IDA) has been recognized as a common nutritional problem in infants and young children in developing countries [1]. It has been reported that more than half of children in developing countries suffer from IDA mainly due to poor nutrition in the majority of cases [2]. Many studies have demonstrated that IDA is associated with poor learning ability, poor memory, lack of concentration, educational failure and affects child cognitive and motor development [3] [4].

Knowledge about the causes of IDA in order to develop treatment and prevention strategies should be in the top of health research agenda, especially in the developing countries. Numerous risk factors for IDA have been recognized such as inadequate iron intake and absorption, increased requirements of iron during growth, and excessive losses of iron. Recently, *Helicobacter pylori* (*H. pylori*) infection has been suggested as a possible etiology for IDA [5].

H. pylori infection is a highly prevalent microbial chronic infection across the world affecting nearly half of the global population with a high variation in prevalence in different countries, age groups, and socioeconomic standards. The prevalence of infection ranges from over 80% in developing low socioeconomic countries to less than 40% among developed countries [6]. It has been reported that 65% of children in developing countries are infected with *H. pylori* [7]. In children, it was proposed that *H. pylori* infection is associated with gastrointestinal disorders as recurrent abdominal pain, dyspepsia, chronic gastritis and peptic ulcers [8]. Moreover, it was reported that *H. pylori* may be also associated with several extra-gastrointestinal diseases such as idiopathic thrombocytopenic purpura, anemia and some allergic diseases [9].

In 1991, Blecker *et al*. [10] suggested the association between *H. pylori* infection and IDA and after that several studies had been conducted to assess the role of infection in IDA [11] [12]. In 2008, a systematic review demonstrated a positive relation between *H. pylori* infection and depletion of iron stores both in adults and children [13]. However, the controversy still exists about association between *H. pylori* infection and IDA [9].

The aim of this study was to evaluate the association between *H. pylori* infection and IDA among school age children in Sohag University Hospital.

2. Subjects and Methods

2.1. Study Design and Place

This was a prospective case-control study conducted in Pediatric Department, Sohag University Hospital, Sohag, Upper Egypt in the period from March 2015 to February 2016.

2.2. Patients

The present study included 200 school-age children (127 males and 73 females) with established diagnosis of IDA and mean age of 7.7 ± 1.35 year (range 5 - 10 years, median = 8 years). All patients were recruited from children attending Pe-

diatric outpatient clinic in Sohag University Hospital, Sohag, Upper Egypt. Also the study included 50 apparently healthy age and sex matched children who attend pediatric outpatient clinic for different complaints (cough, diarrhea, etc.), as a control group.

2.3. Inclusion Criteria

School age children (6 - 12 years) diagnosed with iron deficiency anemia.

2.4. Exclusion Criteria

Children with BMI for age less than 5th percentile.

Children with positive stool analysis for parasitic infestation.

Anemic children due to causes other than IDA.

2.5. Ethical Consideration

The protocol of the study was approved by Sohag University Hospital Ethical Committee and written informed consent was obtained from parents of all participants in accordance to Sohag University Hospital Ethical Committee guidelines.

2.6. Methods

Blood was withdrawn into standard EDTA vacutainers for the assay of CBC, plain tubes for the assay of ferritin, iron, TIBC, and *H. pylori* IgG antibodies. Separated serum was divided in aliquots. One aliquot was used for estimation of ferritin, iron and TIBC and the rest of aliquots were stored at −70˚C to be used for estimation of *H. pylori* IgG antibodies.

Laboratory Investigations

Complete blood count (CBC) was done by the use of cell dyne-3700 (Abbott Diagnostics, Dallas, USA). Serum ferritin was done by the use of Architect 2000 system (Abbott Diagnostics, Dallas, USA). *H. pylori* IgG was measured using ELIZA kit (R-Biopharm AG, Anderneuren Bergstrabe 17, 64,297 Darmstadt, Germany). Serum iron and total iron binding capacity (TIBC) were done by the use of Cobas-C311 (Roch Diagnostics, Mannheim, Germany). All of the included individuals underwent a quantitative detection of *H. pylori* IgG antibodies

2.7. Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS version 22). All data was expressed as mean ± SD. Student t test was used to compare means between different groups. Correlation Coefficient (r) was used for showing positive and negative correlation between variables. *P* value is considered significant if less than 0.05.

3. Results

The study was carried out on 200 children with IDA (127 males and 73 females)

and 50 apparently healthy age and sex matched controls. Age, hematological data and *H. pylori* specific IgG titer of patients are represented in Table 1.

The serological investigations for *H. pylori* infection revealed that 72 cases (36%) of IDA patients and 6 cases (12%) of non-anemic controls had positive antibody level for *H. pylori* specific IgG and the difference between the two groups was statistically significant ($P = 0.036$) Table 2.

H. pylori specific IgG antibody titer showed significant positive correlation with age and significant negative correlation with each of Hb level, MCV, HCT and serum ferritin, Table 3 and Figure 1.

Comparison between *H. pylori* positive and *H. pylori* negative IDA patients revealed that the mean age of *H. pylori* positive IDA cases is significantly higher than that of *H. pylori* negative IDA cases, with male predominance in *H. pylori* positive patients. Also it showed that hematological parameters (Hb, MCV & HCT) and serum ferritin were significantly lower in *H. pylori* positive IDA patients than *H. pylori* negative IDA cases, Table 4.

Table 1. Hematological data of the study group.

	Mean	Median	SD	Min	Max
Age	7.70	8.00	1.349	5	10
Hb	7.210	6.90	0.908	5.8	9.6
MCV	58.344	58.75	5.647	50.0	69.6
MHC	15.704	15.35	1.668	13.2	19.4
RBCs	4.589	4.55	0.246	4.20	5.30
HCT	26.70	26.00	2.714	21	33
Ferritin	4.556	4.50	0.945	2.8	8.0
Iron	14.92	14.50	2.202	11	20
TIBC	421.14	422.50	18.99	380	450
H. pylori antibody titer	7.84	5.50	5.973	3	24

Table 2. Case-control comparison.

	Case (n = 200)	Control (n = 50)	P value
Age	7.70 ± 1.35	7.50 ± 1.30	0.337
Male sex	127 (63.5%)	31 (62%)	0.844
Hb	7.21 ± 0.91	10.75 ± 0.87	**<0.001**
MCV	58.34 ± 5.64	82.69 ± 5.79	**<0.001**
MHC	15.70 ± 1.67	24.32 ± 3.45	**<0.001**
RBCs	4.59 ± 0.25	4.66 ± 0.29	0.090
HCT	26.70 ± 2.71	35.14 ± 3.79	**<0.001**
Ferritin	4.56 ± 0.95	21.46 ± 4.90	**<0.001**
Iron	14.92 ± 2.20	89.11 ± 8.34	**<0.001**
TIBC	421.14 ± 18.99	323.54 ± 24.87	**<0.001**
H. pylori antibody titer	7.84 ± 5.97	4.90 ± 4.88	**<0.001**
H. pylori positive cases	72 (36%)	6 (12%)	**0.036**

Table 3. Pearson correlation statistics between *H. pylori* antibody titer and both of age and laboratory investigations.

	r (correlation coefficient)	p value
Age	0.298	**<0.001**
Hb	−0.157	**0.026**
MCV	−0.158	**0.025**
MHC	0.005	0.941
RBCs	−0.089	0.211
HCT	−0.193	**0.006**
Ferritin	−0.145	**0.041**
Iron	−0.055	0.440
TIBC	−0.023	0.744

4. Discussion

H. pylori infection has a higher prevalence rate in developing than developed countries and has been reported in 50% of 10 year old children [14]. In Egypt, the prevalence of *H. pylori* infection among school age was 72.38% as reported by Mohamed *et al*., in 2008 [15]. *H. pylori* was discovered in 1983 by Marshall and Warren [16] in the stomach of peptic ulcer patients. However, it is now agreed that *H. pylori* is not only associated with peptic ulcer, but also with wide spectrum of gastrointestinal disorders varying from asymptomatic gastritis to gastric carcinoma [17]. Moreover, it was reported that *H. pylori* may be also associated with several extra-gastrointestinal diseases such as IDA, idiopathic thrombocytopenic purpura, and some allergic diseases [9]. On the other hand, *H. pylori* infection is mostly asymptomatic and nearly 20% of infected people develop a clinical disease, usually in adulthood [18].

The mechanism of association between *H. pylori* infection and IDA was proposed by several pathways which may include consumption of iron by the organism itself, gastrointestinal blood loss and decrease in iron absorption due to low levels of gastric acid [12] [13].

In this study we aimed to evaluate the association between *H. pylori* infection and IDA among school-age children. The results of this study revealed that there was a significantly higher rate of *H. pylori* infection among studied group than healthy controls, demonstrating significant association between *H. pylori* infection and IDA among school age children. This came in agreement with Darvishi *et al*., [19] who reported an association between *H. pylori* infection and IDA among pediatric patients where they found that 80.3% of IDA cases and 14% of non-anemic controls had a positive antibody titer for *H. pylori* infection and the difference between two group was statistically significant ($P < 0.0001$). In addition several other studies have shown this association [20] [21] [22] [23] [24]. Moreover, four meta-analyses including both pediatric and adult patients have shown an association between *H. pylori* infection and IDA [5] [13] [25] [26]. Based on the results of these studies, the new guidelines of the last Maastricht Florence Consensus Report recommended to search and eradicate *H. pylori*

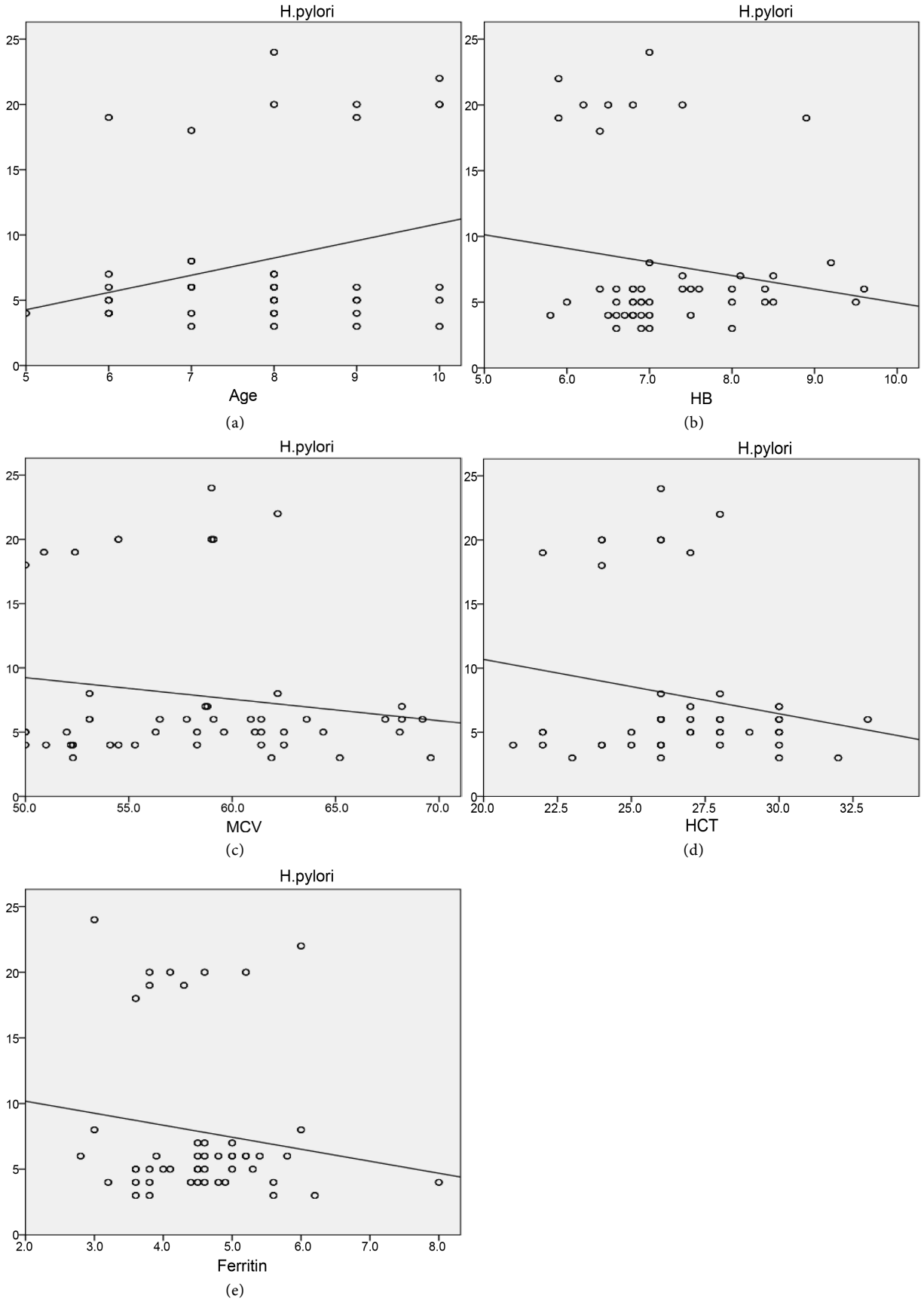

Figure 1. Correlation between *H. pylori* IgG titer and (a) age, (b) hemoglobin, (c) MCV, (d) HCT and (e) ferritin level.

Table 4. Comparison between *H. pylori* positive and *H. pylori* negative IDA patients.

	H. pylori positive (n = 72)	*H. pylori* negative (n = 128)	P value
Age	8.56 ± 1.36	7.51 ± 1.28	**<0.001**
Male:Female	3.5:1	1.4:1	**0.019**
Hb	6.78 ± 0.90	7.31 ± 0.89	**0.001**
MCV	55.73 ± 4.07	58.92 ± 5.79	**0.002**
MHC	15.43 ± 1.44	15.76 ± 1.71	0.283
RBCs	4.53 ± 0.31	4.60 ± 0.23	0.134
HCT	25.22 ± 1.78	27.02 ± 2.78	**<0.001**
Ferritin	4.27 ± 0.86	4.62 ± 0.95	**0.042**
Iron	14.78 ± 2.02	14.95 ± 2.23	0.667
TIBC	422.67 ± 13.67	420.80 ± 19.82	0.501
H. pylori antibody titer	20.22 ± 1.71	5.12 ± 1.28	**<0.001**

infection in IDA after exclusion of bleeding from gastrointestinal tract [27] [28]. On the other hand some studies did not support the association between *H. pylori* infection and IDA [14] [29] [30] [31]. Discrepancies of results among different studies may be attributed to differences in study design, number of patients, inclusion criteria, ethnicity and *H. pylori* species [19] [28] [32].

Results of the current study revealed that *H. pylori* IgG specific antibody titer had a significant negative correlation with each of Hb level, MCV, HCT and serum ferritin. This agree with an analytical Iranian study on school-age children, where it showed a significant negative correlation between *H. pylori* antibody level with serum iron and ferritin ($P < 0.001$) [33]. Also in another study, *H. pylori* seropositivity was associated with a tendency to a lower ferritin levels in Arab children in Palestine [34].

In the present study, there was a significant positive correlation between *H. pylori* IgG titer and age and the mean age of *H. pylori* positive IDA cases was significantly higher than that of *H. pylori* negative IDA cases. This was in agreement with Baggett *et al*., [22] who reported that *H. pylori* infection was associated with older age and the association between iron deficiency and *H. pylori* infection was modified by age, with the strongest association in children who were aged ≥9 years. The increased risk in older children seems biologically reasonable as older children are likely to have been infected for longer than younger children, allowing more time for iron deficiency to develop. Similarly, an age-dependent association between *H. pylori* and IDA was reported among South Korean children [35].

The present work showed significant male predominance in *H. pylori* positive IDA patients. This came in agreement with the study done by Zamani *et al*., who reported that *H. pylori* infection was significantly more common in boys than girls ($p = 0.029$) [2].

The current study showed that parameters of iron deficiency anemia (Hb, MCV, HCT and serum ferritin) were significantly lower in *H. pylori* positive IDA patients than those of *H. pylori* negative IDA cases. This finding showed

the negative effect of *H. pylori* infection on iron stores and indicated that *H. pylori* infection may increase the severity of anemia and support the findings of other studies which suggested that eradication of *H. pylori* infection improve IDA treatment and help improvements in iron status [10] [19] [36]. In 2010, Zhang *et al*., in a meta-analysis [37], found that *H. pylori* infection may impair the absorption of oral ferrous and elimination of the infection may improve the treatment of iron-deficiency. Also, in another meta-analysis, Yuan *et al*., [26] had reported similar results.

5. Conclusion

In conclusion, the results of this study demonstrate significant association between positive serology for *H. pylori* infection and IDA in school-age children. Moreover, infection may increase the severity of anemia.

6. Limitations of the Study

In this study, due to limited resources, the existence of *H. pylori* infection was assessed using only one test (*H pylori* specific IgG antibody titer). High prevalence of *H. pylori* infection in Egypt, and local prevalence could affect positive predictive value of the used test.

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Conflict of Interest

The authors declare that they have no competing interests.

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