Association between Iron Deficiency Anemia and *Helicobacter Pylori* Infection among Children Under Six Years in Iran

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Abstract- This study was conducted to evaluate the association between *H. pylori* infection and Iron deficiency anemia (IDA) among preschool children (age range: 40-75 months) at a tertiary referral hospital in Tehran. In a case-control study, the prevalence of *H. pylori* seropositivity was compared between 64 children with IDA diagnosis and 70 healthy non-anemic sex- and age-matched controls. Totally, 52 (81.3%) children with IDA and 10 (14.3%) non-anemic controls had a positive antibody level for *H. pylori* specific IgG and the difference between two groups was statistically significant (*P*<0.0001). *H. pylori* infection had a significant high prevalence among preschool patients with IDA. Eradication of *H. pylori* infection is recommended for patients with refractory IDA.

Introduction

Helicobacter pylori (H. pylori) infection is the most common chronic infection that has involved about half of the people worldwide. Since the discovery of *H. pylori* in 1983 by Marshall and Warren (1), a considerable amount of researches have focused on the role of *H. pylori* infection on a wide spectrum of gastrointestinal disorders that vary from asymptomatic gastritis to peptic ulcer, and also gastric carcinoma and MALT (mucosa-associated lymphoid tissue) lymphoma (2). The prevalence of *H. pylori* infection varies in different populations and within the geographic regions. It has been found that the high rate of infection is associated with low socioeconomic status during childhood (3). However, the rates of infection range from more than 80 percent in developing the world to less than 40 percent among industrial countries (4). One the other hand, widespread use of treatment against *H. pylori* infection has led to decrease dramatically in the prevalence of infection in developed countries. High rates of *H. pylori* was reported from north and south area (>85%) in Iran which was associated with gastrointestinal ulcer and cancer (4-6). During the past

three decades, it is reported that *H. pylori* may influence some extra-gastrointestinal diseases such as idiopathic thrombocytic purpura, anemia and allergic diseases (2,7).

Iron deficiency anemia (IDA) is the most common nutritional problem in infants and young children in developing countries (8). In fact, the most vulnerable subjects for iron deficiency are children aged six months to five years (9). Reports indicate that such deficiencies affect child cognitive and motor development (10,11). Based on the WHO estimation, iron deficiency is responsible for 50 percent of all anemias. The prevalence of anemia during infancy and early childhood is higher than at any other time in the life cycle such as pregnancy. It is estimated that the rate of anemia in young children (before five years old) ranges from 30-90 percent in different countries (8). Researchers from Iran have reported that the prevalence of IDA is about 20-30 percent (~19.1%-29.7%) among children between six months and five years old (12,13). In the absence of gastrointestinal blood loss or hemolytic anemia, an unexplained IDA refractory to iron therapy is a focused subject in researches and *H. pylori* has been suggested as a possible reason. Over the

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past two decades, an association between pediatric IDA and *H. pylori* has been established (14). Dufour *et al.*, (15) in 1993 introduced this possible association between IDA and *H. pylori* infection and after that several research are conducted to assess the role of infection on IDA. However, it is still a controversial issue, despite many studies showing the negative impact of *H. pylori* on iron status (7). The aim of this study was to evaluate the association between *H. pylori* infection and IDA among preschool children at a tertiary referral hospital in Tehran, the capital of Iran.

Materials and Methods

A case-control study was conducted between March 2009 and February 2010 and all children attending the pediatrics clinic in Be'sat Hospital in Tehran; Iran was investigated. The study was approved by the ethics committee of the AJA University of Medical Sciences and was performed in accordance with the Declaration of Helsinki and subsequent updates. An informed consent was obtained from the parents or guardians of participating children. Sixty-four consecutive children with a diagnosis of IDA were enrolled as case group. We included 70 healthy non-anemic sex- and age-matched children as a control group.

All patients had hemoglobin level less than adjusted values for age and sex with a blood film that showed microcytic hypochromic anemia. Serum ferritin was below 20 ng/dl; iron was below 50 µg / dl and total iron binding capacity (TIBC) was more than 350 µg /dl. Serum specimens were collected from participants and tested for IgG against *H. pylori*. All participated individuals underwent a qualitative detection of *H. pylori* IgG antibodies using the Hp-G Screen (Genesis Diagnostics, Cambridgeshire, UK).

Descriptive statistics includes frequency distribution tables and mean ± standard deviations (SD) were generated with the SPSS (SPSS, Chicago, Illinois, USA, version 16) statistical software. A chi-square or Fisher's exact test was used to compare categorical variables. An independent T-test or Mann-Whitney/Kruskal-Wallis tests for non-normally distributed variables was used to compare means. The statistical significant level was considered as $P < 0.05$.

Results

A total of 134 participants (age range: 40-75 months) including 64 cases and 70 controls were enrolled in the study. The mean age in cases and controls was 62.8±7.8 and 61.3±5.4 months, respectively. There were 30 (46.9%) and 34 (48.6%) male in case and control group, respectively. No significant differences were found between the two groups in the proportion of breastfed children and iron supplements consumption (Table 1).

Table 1. Demographic characteristics in case and control groups

	Case (N=64)	Control (N=70)	*P*-value
Age (months)	62.8±7.8	61.3±5.4	NS
Sex			
Male	30 (46.9%)	34 (48.6%)	NS
Female	34 (53.1%)	36 (51.4%)	
Infant nutritional status			
Breast fed	52 (81.2%)	59 (84.3%)	NS
Formula fed	12 (18.8%)	11 (15.8%)	
Iron supplements			
Yes	53 (82.8%)	56 (80%)	NS
No	11 (17.2%)	14 (20%)	

NS: non-significant

Table 2 shows the comparison between case and control groups with regard to the results of laboratory tests including hematological, biochemical and serological parameters. As expected, the mean hemoglobin concentration, as well as serum ferritin and iron stores, was significantly lower, and TIBC was significantly higher in IDA patients than the control group (*P*<0.0001). The serological investigation for *H. pylori* infection revealed that 52 (81.3%) patients with IDA and 10 (14.3%) non-anemic controls had a positive antibody level for *H. pylori specific* IgG and the difference between two groups was statistically significant (*P*<0.0001).

The occurrence of serology positive for *H. pylori* infection was compared between male and female children separately in cases and controls. Table 3 shows that 80% of boys versus 82.4% of girls in IDA patients (*P*=0.53), and 17.6% of boys compared to 11.1% of girls

in control group (*P*=0.33) had a positive serology for *H.* *pylori* infection (Table 3).

Table 2. Comparison of laboratory results between case and controls

	Case (N=64)	Control (N=70)	*P*-value
Hemoglobin	10.0±0.68	10.8±1.3	<0.0001
Ferritin	17.2±4.6	89.6±2.1	<0.0001
Iron	50.1±9.9	134.1±1.9	<0.0001
TIBC	371.2±18.1	350.7±3.2	<0.0001
Serology-positive *H. pylori* infection	52 (81.3%)	10 (14.3%)	<0.0001

TIBC: total iron binding capacity

Table 3. Comparison of serology-positive *H. pylori* infection between male and female children in case and control group

	Positive for *H. pylori* infection		*P*- value
	Male	**Female**	
Case	24 (80%)	28 (82.4%)	0.53
Control	6 (17.6%)	4 (11.1%)	0.33

Discussion

The results of this study indicated that the prevalence of *H. pylori* infection was significantly higher among IDA children than non-anemic healthy controls. An Iranian cross-sectional study was carried out by Zamani *et al*., during 2005 and 2006 on 1665 students aged between 6 and 12 years old in Tehran to evaluate the relationship between serum ferritin levels and *H. pylori* IgG antibody (16). The rate of *H. pylori* seropositivity was 26 percent and 29 percent of children had low serum ferritin levels. They found no association between *H. pylori* infection and low serum ferritin levels or IDA. This finding is comparable to another study in Tehran on children aged 2-14 (median: 7.1) years with a case-control design including 100 consecutive cases with *H. pylori* infection and 109 age-matched consecutive non-infected controls (17). They reported that 19% of cases and 21.1% of controls had IDA (*P*=0.7), and there was no association between *H. pylori* infection and IDA. However, an analytical study in 2008 on 100 IDA children aged 7- 12 years in Ilam city (located in west of Iran) found a significant negative correlation of *H. pylori* antibody level with serum iron and ferritin, and its positive correlation with TIBC levels (*P* < 0.001) (18). Furthermore, recent meta-analyses have indicated that *H. pylori* infection is associated with IDA. A meta-analysis in 2010 performed on 15,183 patients from 20 studies (15 observational studies and 5 RCTs) indicated an association between *H. pylori* and IDA and the pooled odds ratio (OR) with 95% confidence interval

(CI) was calculated 2.22 (95%CI, 1.52-3.24) for positive *H. pylori* patients (19). Another study reported the pooled OR 2.8 (95%CI, 1.9- 4.2) for iron deficiency and also pooled OR 1.38 (95%CI, 1.16-1.65) among *H. pylori* infection (20).

Although, studies still have controversy about the association between *H. pylori* infection and iron stores, some authors believe that variation in *H. pylori* species is one of the possible reasons for disagreement with findings in the literature (21,22). Furthermore, studies have compared different parameters to evaluate iron and also included different age groups that may influence their results. For example, since serum ferritin level is an acute phase protein that is elevated during infections and inflammations, its comparison between *H. pylori* infected and non-infected individuals for iron status may have some effects on the results. Thus, in this study we compared the rate of *H. pylori* infection in IDA established children with their matched (for age and sex) controls. On the other hand, some meta-analyses of randomized trials have showed that eradication of the infection may improve the IDA treatment. Zhang *et al*., conducted a meta-analysis in 2010 and included 800 participants in eight trials (23). They found that *H. pylori* infection may influence the absorption of oral ferrous and elimination of the infection may improve the treatment of iron-deficiency. In another meta-analysis, Yuan *et al.,* had similar results and suggested that the effect of *H. pylori* eradication is more valuable for patients with moderate or severe anemia (24).

Although the mechanisms responsible for effect of *H. pylori* on iron status remains unclear, some theories argue that several pathways may be involved which include 1) consumption of iron by the bacterium; 2) gastrointestinal blood loss due to gastritis or duodenitis; and 3) decrease in iron absorption due to low levels of gastric acid (20,25).

In present study the age of participants was lower than other similar studies and it, as strength of the study, indicated that in developing countries infection with *H. pylori* in early childhood may be a reason for refractory IDA. Moreover, because of the low age of current patients some covariates such as menstruation, which might contribute additional residual confounding factor for iron deficiency or the difference between two sexes, were automatically ruled out.

In conclusion, this study determined an association between *H. pylori* and IDA among pediatric patients. It is suggested that in children with refractory IDA the possible infection of *H. pylori* be considered and eradication of infection should be started and organized prior to IDA treatment in case of positive serologic status for *H. pylori* infection. According to the high rate of *H. pylori* infection in our community, the screening strategies for vulnerable pediatric population to detect *H. pylori* infected children and to perform an appropriate treatment may prevent the possible consequences of such infection.

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