

## **Relationship between Helicobacter pylori infection and Serum Ferritin level in primary school children of Tehran-Iran**

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### **Abstract**

**Objective:** To determine the relationship between serum Ferritin levels and Helicobacter pylori antibody titer (IgG) in primary school children with H.pylori infection.

**Methods:** This cross-sectional study was performed among 6-12 year old healthy primary school children in Tehran during the academic year 2005-2006. Specimen collection was done by cluster and randomization methods (multistage sampling). Personal information and laboratory results were compiled in questionnaires and data was analyzed by descriptive and analytical statistics via SPSSv.15 software.

**Results:** One thousand six hundred sixty five primary school children (43% boys, 57% girls) with mean age  $9.2 \pm 1.5$  years were enrolled into the study. H.pylori IgG antibody titer was positive in 26 % (432 cases) with mean values of  $0.79 \pm 0.42$  ISR units in boys and  $0.75 \pm 0.39$  ISR units in girls, which showed a significant statistical difference ( $P=0.004$ ). H.pylori infection was more common among children of large families or those with low economic status ( $P=0.002$ ). Twenty nine percent of children (477 cases) had low serum Ferritin levels, which was significantly higher among boys ( $P>0.05$ );  $32.82 \pm 33.12$  mg/dL in boys and  $43.23 \pm 2.5$  mg/dL in girls. Out of the children with low serum Ferritin levels, 71% (334 cases) had negative and 28% (132 patients) had positive H.pylori antibody titers (IgG levels). Also, 296 children (25%) with normal Ferritin levels had H.pylori infection.

**Conclusions:** We did not find a significant relationship between H.pylori infection and low serum Ferritin or iron deficiency anaemia.

**Keywords:** Helicobacter pylori infection, Serum Ferritin, Iron deficiency anaemia (JPMA 61:658; 2011).

### **Introduction**

Iron deficiency anaemia is the most common cause of nutritional anaemia throughout the world. The prevalence of iron deficiency anaemia in developing countries has been reported to be more than 50% in children, which is mainly due to poor nutrition.<sup>1</sup> Numerous studies have shown that iron deficiency anaemia is associated with cognitive disorders, poor learning ability, and lack of concentration, poor memory, educational failure, and behavioral or physical problems.<sup>2</sup> Prevention or treatment of iron deficiency anaemia requires knowledge about factors affecting this disease. Unknown factors are also involved in the etiology of anaemia in a considerable percentage of patients.<sup>3</sup> One of the factors affecting iron deficiency anaemia is H.pylori infection, which has a high prevalence in developing countries and some believe that resistant iron deficiency

anaemia, for which a cause has not been found, can be corrected with H.pylori eradication regimen.<sup>4,5</sup> Some studies show that H.pylori seropositive patients are 40% at higher risk of reduced iron reserves.<sup>4</sup> A study on Korean adults showed that H.pylori infection increases the rate of hypoferritinaemia and iron deficiency anaemia.<sup>5</sup> Resistant iron deficiency anaemia can be associated with H.pylori infection without any bleeding cause.<sup>6</sup> Treatment of H.pylori produces a more rapid response to oral iron therapy.<sup>7</sup> Although, this relationship is not significant in some studies,<sup>8</sup> but considering the rising rate of H.pylori infection in developing countries as well as the results of some studies which showed a significant relationship between H.pylori infection and iron deficiency anaemia and low serum ferritin levels,<sup>9-11</sup> we aimed to find the relationship between these two diseases among primary school children in Tehran.

## Subjects and Methods

This cross-sectional study was performed on primary school children in the 19 educational sectors of Tehran during the academic year 2005-2006. Exclusion criteria were history of epigastric pain, vague abdominal pain, and prolonged diarrhoea, girls who had passed menarche, chronic or haemorrhagic diseases, and children without written paternal consent or incomplete questionnaires. Using a multistage sampling specimen collection method, two girl's and two boy's primary schools were randomly chosen from each of the educational sectors of Tehran, without the intervention of the executive researcher and the study was conducted on 1779 healthy school children. Subjects excluded were 114 due to inadequate haemolyzed blood samples or absence of consent forms, and finally 1665 subjects were enrolled into the study. After making the necessary arrangements which related to higher authorities of the Ministry of Health, the Health officials of each educational sector, and with the school health officials, blood samples were taken from the cases, by an expert laboratory technician. At the end of blood sample collection, a questionnaire comprising of personal health information and place of education of school children was also completed for all subjects. After transferring the samples to the laboratory and after serum separation, the samples were stored at -20°C and H.pylori IgG level was gradually estimated using ELISA (Trinity Biotic, Ireland, Capita. TMH Pylori 2326400-96 test). The results of this test were reported according to ISR value (Immune status Ratio), such that IgG levels  $\leq 0.9$ , 0.91-1, and  $\geq 1.1$  were considered as negative, borderline, and positive, respectively. ELISA has a relative specificity of 96% for the diagnosis of HP IgG.<sup>12</sup> Also, serum Ferritin level was estimated by CLIA method (chemiluminescent Immunoassay Technology, Liaison, Italy, REF 313, 551). Serum Ferritin  $\geq 12$  mg/dL was considered as normal and values below this were considered as abnormal. After compiling the test results of all patients, data was entered in a SPSS version II computer software data bank and later analyzed by descriptive and analytical statistics using chi-square and t-test.

## Results

Samples were taken from 1779 pupils, of whom 114 subjects were excluded from the study due to various reasons. Thus 1665 subjects were studied, of whom 945 (56.77%) were boys and 720 (43.23%) were girls. The mean age was  $9.2 \pm 1.5$  years. H.pylori antibody titer was positive in 429 (26%) subjects, of whom 220 (23.4%) were girls with mean  $0.75 \pm 0.39$  ISR and 209 (29.1%) were boys with mean  $0.79 \pm 0.42$  ISR. H.pylori infection was therefore significantly more common among boys than girls ( $P=0.029$ ). A significant difference was seen between age groups ( $P=0.0001$ ) and HP antibody titer increased with

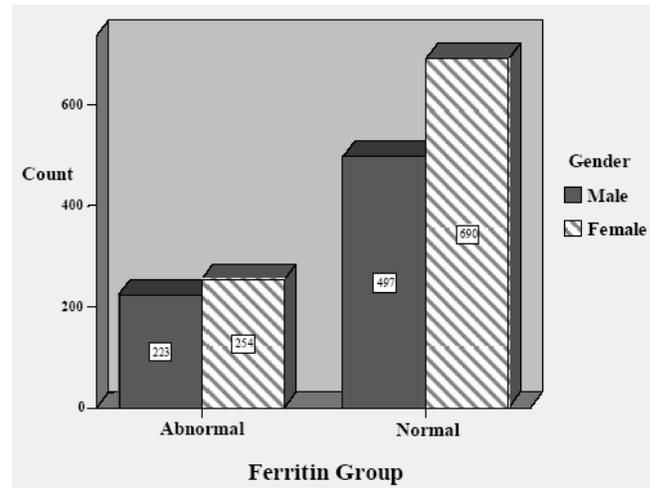


Figure: Frequency of low Ferritin level in both sexes.

Table: Frequency of H.pylori Antibody positive according to Ferritin level.

HP-Ab Ferritin	Negative N (%)	Borderline N (%)	Positive N (%)	Total N (%)
Abnormal	334(132)	11(2,3)	132 (27,7)	477(100)
Normal	857(72,7)	34(2,9)	296(24,9)	1187(100)
Total	1191(71,6)	45(2,7)	428(25,7)	1664(100)

advancing age, such that maximum infection rate occurred in 12 year olds (43%). Infection rate was highest in South Tehran (33%) with a statistically significant difference between boys (39.4%) and girls (27.9%) ( $P=0.002$ ). HP infection rate was higher in highly populated areas and in low socio-economic status areas, and was related to poor personal hygiene such as failure to wash hands with soap after using the toilet ( $P=0.004$ ). H.pylori infection was also related to type of nutrition; use of tinned food ( $P=0.015$ ), sandwiches ( $P=0.002$ ) and sausages ( $P=0.07$ ), but there was no such relationship with water or other foodstuff. Mean serum Ferritin level was  $33.63 \pm 22.22$  mg/dL, among whom 1187 (71.3%) had normal ( $\geq 12$  mg/dL) and 477(28.7%) had subnormal Ferritin levels ( $=12$  mg/dL), which was more common among boys (Figure). A significant statistical difference was not found between serum Ferritin level and gender ( $P=0.078$ ). Low serum Ferritin levels were most common among  $< 9$  year old boys (36.6%) and significantly least common among  $> 12$  year olds. However, mean serum Ferritin level was not statistically different between different age groups ( $P=0.203$ ). A significant difference was not found between different geographical regions ( $P=0.141$ ) and low serum Ferritin levels. There was no relationship between low serum Ferritin level, nutrition and health status. Among the 477 children with low Ferritin levels, 334 (70.7%), 11

(2.3%) and 132 (27.7%) had negative, borderline, and positive HP antibody (IgG) levels, respectively. H.pylori infection was found in 296 (24.9%) children with normal serum Ferritin levels (Table).

## Discussion

H pylori is one of the factors affecting iron deficiency anaemia and hypoferritinaemia, which has a higher prevalence in developing than developed countries and has been reported in 50% of 10 year old children.<sup>13</sup> The relationships between H.pylori infection and diseases outside the gastrointestinal system have been considered by researchers. One of these is the association of HP and iron metabolism.<sup>3</sup> Some researchers consider this relationship as significant.<sup>1,9,10</sup> HP is the main factor involved in peptic ulcer and chronic gastritis. Some studies have reported the relationship between this disease, low serum Ferritin levels and iron deficiency anaemia, especially with the resistant type.<sup>5</sup> Other reports show that resistant iron deficiency anaemia, of unknown cause, improves after HP treatment.<sup>4,5</sup> Some ferrokinetic iron mechanism problems have been associated with HP, including the secretion of Hyatoline by hepatocytes which cause decreased iron absorption.<sup>3</sup> Also, some believe that presence of HP in the gastric antrum can cause lactoferrin production, which causes the iron derived from transferrin to attach to the HP bacteria, and to be subsequently lost in the faeces after the bacteria is destroyed. Another form of association is the increased iron absorption by 19K Da iron binding protein surface receptors and as a result of erosive gastritis and low iron absorption secondary to the hypochlorite conditions which results in occult blood loss.<sup>3,13</sup> For this purpose, in order to detect HP, ELISA serology (sensitivity=100%, specificity=96 %) was used to detect HP IgG.<sup>12</sup> One of the most accurate tests used to determine body iron stores, is serum Ferritin level, which is an index for the condition of iron stores and iron deficiency anaemia.<sup>14</sup> This test was performed by the CLIA method. The current study showed that 26% of school students had H.pylori infection (positive IgG), which as compared to Karachi (40%)<sup>11</sup> is lower than neighboring countries. Our study showed that boys are at higher risk of developing H.pylori infection than girls, which was statistically significant ( $P=0.004$ ). This is similar to the findings of some studies<sup>13</sup> while others report equal proportions.<sup>15</sup> Also, a J-shaped relationship was found between the child's age and IgG antibody level ( $P=0.001$ ), such that it was higher among 6 year old than 7 or 8 year old children. Serum IgG levels were higher in 9-12 year old children, and they were even higher than that of 6 year olds. On the whole, the rate of H.pylori infection rises with advancing age. Such findings have also been reported in the studies performed by Baggett in Alaska,<sup>9</sup> Suogla in Turkey,<sup>10</sup> Aziz in Pakistan,<sup>11</sup> Jais<sup>15</sup> and

Hveem.<sup>13</sup> In our study 29% of children had low serum Ferritin levels, which did not show a significant statistical relationship between boys and girls ( $P=0.051$ ) (Figure). Serum ferritin level was lower in boys, even though lowest values were seen in 11 year old girls. It seems that the reduced serum Ferritin levels in this age group is most probably due to the rapid growth of girls and the loss of blood through menstruation as well as inadequate dietary iron intake in this age. We did not find a significant relationship between low serum ferritin levels, highly populated families, low socioeconomic status, and type of diet, level of personal hygiene or geographical area. Another, study also showed no difference to exist between serum ferritin levels of poor and wealthy children.<sup>16</sup> Correlation coefficient shows the relationship between serum Ferritin and HP serum IgG titer to be 86%. In our study, 28% of children with Ferritin levels  $<12\text{mg/dL}$ , had positive HP antibody (IgG). Also, 25% of children with normal Ferritin levels had H.pylori infection (IgG positive). A widespread study showed that HP seropositive individuals are 40% at higher risk of developing low iron reserves.<sup>4</sup> Another study showed that H.pylori infection causes a 17% reduction in serum Ferritin levels,<sup>17</sup> whereas in our study, 28% of the children had normal Ferritin levels. Our study shows that there is no significant relationship between low serum Ferritin levels in school children in Tehran and positive H.pylori IgG titers and that there is no significant relationship between mean serum Ferritin levels in IgG seropositive and H.pylori seronegative individuals, which is in accordance to the findings of Gasbarrini A,<sup>18</sup> Dubois,<sup>3</sup> and Seo JK,<sup>5</sup> who do not agree with this relationship. Our results are different from those of Cardenas<sup>1</sup> and Baggett,<sup>9</sup> who have found a significant relationship between iron deficiency anaemia with low serum Ferritin levels and H.pylori infection. Also, in a study performed on 6-12 year old Korean children, low serum Ferritin levels were found in IgG H.pylori seropositive subjects. This study showed a clear increase in iron deficiency among seropositive (13.9%) as compared to seronegative individuals (2.8%).<sup>5</sup> It seems that a part of those results are due to the fact that different species of H.pylori may be involved. The species which may be associated with peptic ulcer and gastrointestinal symptoms are those bacteria with Cag (Cagpal) genes but Baysoy showed that some HP species are associated with gastric ulcer or iron deficiency anaemia.<sup>19</sup>

Epidemiologic studies, which have ruled out the relationship between iron reverses and HP, mostly used serum ferritin level estimation, which is an acute phase reactor and its level depends on various factors. Therefore, considering the different factors involved in serum Ferritin level, failure to find a significant relationship does not rule out the association between iron deficiency anaemia and H.pylori infection. Along with other complementary studies, this

preliminary study can be a good means of diagnosing and treating iron deficiency anaemia resistant to therapy.

Based on these findings, we suggest the following in order to reach the goals of our study: The design and availability of iron-fortified food stuff with the help of the Ministry of Health and the Ministry of Education; Identification of the HP bacteria species in Iranian patients.;- Performance of studies to determine the relationship between H.pylori infection and iron deficiency anaemia according to the type of HP bacteria species and Performance of widespread epidemiologic studies on different age groups of asymptomatic healthy children as well as on symptomatic subjects and comparison between the two groups.

### Conclusion

Regarding the overall results of our study as well as those of others, it seems that controversy still exists about the relationship between low serum Ferritin levels, iron deficiency anaemia and H.pylori infection.

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