Iron Status and *Helicobacter pylori* Infection in Symptomatic Children: An International Multi-Centered Study

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### Abstract

**Objective:** Iron deficiency (ID) and iron deficiency anaemia (IDA) are global major public health problems, particularly in developing countries. Whilst an association between *H. pylori* infection and ID/IDA has been proposed in the literature, currently there is no consensus. We studied the effects of *H. pylori* infection on ID/IDA in a cohort of children undergoing upper gastrointestinal endoscopy for upper abdominal pain in two developing and one developed country.

**Methods:** In total 311 children (mean age 10.7 ± 3.2 years) from Latin America - Belo Horizonte/Brazil (n = 125), Santiago/Chile (n = 105) - and London/UK (n = 81), were studied. Gastric and duodenal biopsies were obtained for evaluation of histology and *H. pylori* status and blood samples for parameters of ID/IDA.

**Results:** The prevalence of *H. pylori* infection was 27.7% being significantly higher (p < 0.001) in Latin America (35%) than in UK (7%). Multiple linear regression models revealed *H. pylori* infection as a significant predictor of low ferritin and haemoglobin concentrations in children from Latin America. A negative correlation was observed between MCV (r = −0.26; p = 0.01) and MCH (r = −0.27; p = 0.01) values and the degree of antral chronic inflammation, and between MCH and the degree of corpus chronic (r = −0.29, p = 0.008) and active (r = −0.27, p = 0.002) inflammation.

**Conclusions:** This study demonstrates that *H. pylori* infection in children influences the serum ferritin and haemoglobin concentrations, markers of early depletion of iron stores and anaemia respectively.

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### Introduction

Iron deficiency (ID), the most common nutritional disorder in the world, and iron deficiency anaemia (IDA) affect 500–600 million people globally and represent a major public health problem particularly in developing countries [1,2]. Factors including poor iron intake, low dietary iron bioavailability and gastrointestinal parasite infections contribute to the high frequency of ID/IDA in developing countries. Children are at risk as this age group has high iron requirements for growth [2]. In childhood, iron deficiency has been associated with deficits of immune, cognitive and motor function [2]. Clinically advanced IDA is associated with reduced growth, increased susceptibility to infectious diseases and increased mortality [3].

The high prevalence of combined *H. pylori* infection and ID/IDA in developing countries suggests that infection with this bacterium may be a cause of ID/IDA. Possible mechanisms include increased iron uptake by the *H. pylori* bacterium [4] and
blood loss due to gastric lesions as a consequence of *H. pylori* infection [3]. Reduced iron absorption due to an elevated pH of gastric juice has also been attributed to *H. pylori* [6,7] as there is transient hypochlorhydria of variable duration in the early phase of infection and gastric atrophic changes in the late stages of infection [6,9]. As *H. pylori* infection is primarily acquired in childhood, and iron stores are lower in children than in adults, children are thought to be at a particular increased risk for iron deficiency.

Whereas some epidemiological studies and interventional trials have demonstrated evidence of an association between *H. pylori* infection and ID/IDA in children [10–13], in other studies this link has not been established [14–16]. In addition, studies evaluating children undergoing upper gastrointestinal endoscopy, which allows an accurate diagnosis of *H. pylori* infection as well as the exclusion of other common causes of ID such as coeliac disease and gastrointestinal bleeding, are scarce with few patients having been evaluated [17–18].

Therefore, the aim of this study was to evaluate the effects of *H. pylori* on iron deficiency in a large cohort of children (less than 16 years of age) with symptoms of dyspepsia undergoing upper gastrointestinal endoscopy.

**Patients and Methods**

This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; Pontificia Universidad Catolica de Chile, Santiago, Chile and East London Research Ethics Committee, United Kingdom (UK). The study was also reviewed by the European Union Ethics Committee. The trial was registered with the UK Clinical Research Network (Study Id 5149). Signed informed consent to participate was obtained from the children (whenever possible) and adolescents and their parents.

**Patients**

The study cohort comprised 311 children (mean age 10.7 ± 3.2 years, range 3–16 years, 179 girls) who were studied prospectively from June 2007 to September 2011 from three separate cities, Belo Horizonte and Santiago, in Latin America and London, UK. Belo Horizonte is located in Southeast Brazil, where the population shows a mixed ancestry, with well distributed contributions from Caucasian, African and Amerindians descent [19]. In Santiago, Chile, the population is composed by approximately two-thirds with European ancestry. Amerindians or persons of Amerindian admixture constitute most of the remaining third [20]. The children from London were born in England: 48% white; 32% south Asian; 10% black; and 10% mixed ethnic background. All children were symptomatic and underwent upper gastrointestinal endoscopy for investigation of symptoms referable to the upper gastrointestinal tract. The following patients were excluded: those who received antimicrobial drugs, anti-cholinergic and steroid, non-steroidal anti-inflammatory agents and iron supplement for at least 30 days, proton pump inhibitors and H2-receptor antagonist for at least 15 days or antacid for 24 hours before endoscopy; those with peptic ulcer, coeliac disease and intestinal parasites; children with present or past history of gastrointestinal bleeding, oesophageal varices, coagulation disorders, inflammatory diseases, acquired or congenital immunosuppression, renal failure, hematologic disorders, neoplasia or an anatomical obstacle preventing endoscopy. From each female adolescent data was obtained on age of menarche, interval between the menses, duration and amount of monthly flow. Those with heavy menstrual blood loss were not included in the study. The menstrual cycle was considered normal when the interval between flows was 25–31 days and duration between 3 and 5 days [21]. In addition, after endoscopy, additional exclusion criteria were previously undiagnosed coeliac disease or any non-specific inflammation in the absence of duodenal gastric metaplasia. These criteria rigourously excluded possible confounding factors.

A detailed clinical history was obtained from each patient or their parents including presence (none, mild, moderate, severe) and duration (last week, last month, last year) of abdominal pain, acute or chronic diarrhoea, vomiting, heartburn as well as weight loss and fever and use of medications.

Clinical indication of endoscopy was classified according to the referral physician’s indication, as recurrent abdominal pain, gastroesophageal reflux disease, evaluation of vomiting, diarrhea or weight loss.

Biopsy specimens were obtained from antral, corpus and duodenal mucosa for histology and additional antral mucosal biopsies for evaluation of *H. pylori* status by culture and biopsy urease test.

Blood samples were collected from each patient and placed in sterile tubes with ethylenediamine tetraacetic acid to determine the full blood count and in iron-free tubes to determine the levels of serum ferritin, serum iron and serum total iron-binding capacity. IDA was defined as haemoglobin values lower than 110.0 g/L (children from 3 to 5 years of age), lower than 115.0 g/L (6 to 11 years of age) and lower than 120.0 g/L (12 to 16 years of age) and by serum ferritin concentration lower than 12 µg/L for children of 3 to 5 years of age and lower than 15 µg/L for those of 6 to 16 years of age.

Stool samples were obtained for parasitological assessment. Children with parasitic infection were excluded from analysis.

**H. pylori Status**

*H. pylori* status was evaluated by culture, urease biopsy test and carbolufuchsin [22], Gimenez or Giemsa staining of histology sections. Patients were considered *H. pylori*-positive if the culture was positive or two other tests were positive and *H. pylori*-negative when the three tests were negative.

**Histology**

Biopsies from the antral and corpus mucosa were fixed in 10% formalin and embedded in paraffin wax, and 4-µm-thick histological sections were stained with hematoxylin and eosin. The sections were analysed according to the revised Sydney System [23]. Active and chronic inflammation, intestinal metaplasia, atrophy and *H. pylori* density were graded as absent (0), mild (1), moderate (2), or marked (3). Sections of the duodenal mucosa were also assessed.

**Complete Blood Count**

Blood counts were determined using automated electronic counters: Sysmex XT 1800i (Sysmex Corporation, Kobe, Japan) in Brazil, Beckman Coulter, model GenS (Beckman Coulter Inc, CA, USA) in Chile and Sysmex XT 2100i (Sysmex Corporation, Kobe, Japan) in UK.

**Determination of Serum Ferritin, Serum Iron, Serum Total Iron-binding Capacity and Serum Transferrin Saturation**

The serum concentration of ferritin was determined by a chemiluminescence method employing the ADVIA Centaur® Immunoassay CP System (Siemens Healthcare, Erlangen, Germany) in the samples from Brazil and Chile and an electrochemiluminescence immunoassay (Cobas Analyzer, Roche Diagnostics Ltd., Switzerland) in the samples from UK. The serum iron
and the serum total iron binding capacity were determined by colorimetric methods (Brazil, Chile and UK). Serum transferrin saturation was obtained by dividing the serum iron concentration by the total iron binding capacity.

Statistical Analysis

Data were analysed with SPSS statistical software package version 17.0 (SPSS Inc., Chicago, IL). In addition to the visual examination of the histograms and box plots, the Kolmogorov-Smirnov goodness-of-fit was used to assess the normality of the data. When significant departures from normality were detected, the data were log transformed. The degree of gastric active and chronic inflammation was compared among the groups by the two-tailed Mann Whitney U test. For other comparisons, the two-tailed Chi-square test, Fisher’s test or Students t test, Pearsons or Spearman’s correlations were employed as indicated. The level of significance was set at \( p \leq 0.05 \). Multiple linear regression analyses (“enter option”) were used in order to quantify the simultaneous and mutually independent contribution of selected relevant predictor candidates, e.g. \( H. pylori \) infection, for low ferritin and haemoglobin blood concentration (dependent variables) while controlling for confounders such as gender and age. Variables with \( p \) values \( \leq 0.20 \) in the univariate analyses were selected for the multivariate analyses. The optimum sample size, based on a significant level of 0.05 and a statistical power of 0.80 (type II error 0.02) for a multiple regression analyses with 4 predictor variables is at least 200 cases.

Results

Demographic and Clinical Characteristics of the Included Children

The demographic and clinical characteristics of the included children are described in Table 1. There was no statistically difference in regard to the age, gender and clinical indications of endoscopy among children from Belo Horizonte, Santiago and London (Table 1).

H. pylori Infection

The overall prevalence of \( H. pylori \) infection was 27.7% (86 children were \( H. pylori \)-positive and 225 \( H. pylori \)-negative). \( H. pylori \)-positive status was defined by positive culture in 71 (82.5%) children and by positive urease test and histology in 15 (17.5%) children.

The prevalence of infection was significantly higher in Santiago and Belo Horizonte than in London (\( p < 0.001 \) for both) (Table 1), but no difference (\( p = 0.40 \)) was observed when comparing Belo Horizonte and Santiago.

\( H. pylori \)-infected children (11.5\( \pm \)3.0 years) were older (\( p = 0.005 \)) than those not infected (10.4\( \pm \)2.2 years), but no difference (\( p = 1.0 \)) was observed between girls (27.4\%, 49/179), and boys (28.0\%, 37/132).

\( H. pylori \) infection was associated with vomiting (46.5\% vs. 34.6\% for infected and non-infected children, respectively; \( p = 0.05 \)), but not with abdominal pain (39.5\% vs. 83.3\%; \( p = 0.29 \)), chronic (5.8\% vs. 10.2\%; \( p = 0.32 \)) or acute (9.3\% vs. 15.1\%, \( p = 0.25 \)) diarrhoea, weight loss (18.6\% vs. 22.2\%; \( p = 0.59 \)) and fever (9.3\% vs. 8.0\%; \( p = 0.89 \)). When the population of each country was individually analysed, no association could be detected among the clinical symptoms and \( H. pylori \) infection in Belo Horizonte (\( p = 0.18 \)) and London (\( p = 0.58 \)). However, in Santiago, \( H. pylori \) infection was associated with vomiting (41.9\% \( H. pylori \)-positive vs. 24.2\% \( H. pylori \)-negative; \( p = 0.05 \)).

Endoscopy and Histology

With respect to the gastric endoscopy findings, \( H. pylori \) infection was associated with the presence of antral (26.7\% \( H. pylori \)-positive vs. 11.5\% \( H. pylori \)-negative; \( p = 0.002 \)) and corpus (8.1\% \( H. pylori \)-positive vs. 0.9\% \( H. pylori \)-negative; \( p = 0.002 \)) erythema and antral nodularity (41.8\% \( H. pylori \)-positive vs. 2.6\% \( H. pylori \)-negative; \( p < 0.001 \)).

The degree of antral and corpus active and chronic inflammation was significantly higher (\( p < 0.001 \) for all) in the \( H. pylori \)-positive than in \( H. pylori \)-negative children (Table 2). Antral lymphoid follicles were also more frequently observed (\( p < 0.001 \)) in infected (68.2\%) than in the non-infected children (10.4\%). Only two children presented with a degree of corpus atrophy. Neither antral atrophy nor antral and corpus intestinal metaplasia was observed.

IDA

IDA was detected in three \( H. pylori \)-positive children: a 13 year old girl from Belo Horizonte, with values of haemoglobin and serum ferritin of 111.0 g/L and 13.7 \( \mu g/L \), respectively, and two girls from Santiago 14 and 12 years of age with haemoglobin and ferritin values of 119.0 g/L and 5.4 \( \mu g/L \) and 117.0 g/L and 10.0 \( \mu g/L \), respectively, but no child uninfected with \( H. pylori \) had IDA (\( p = 0.02 \)).

Blood Iron Parameters

As significant departures from normality were detected for ferritin, concentrations were log-transformed and thus became normally distributed. The data obtained in the anaemia and iron parameters of the children from all countries according to \( H. pylori \) infection are presented in Table 3.

By analyzing all countries together by linear regression, \( H. pylori \) infection was not a predictor of low haemoglobin and ferritin, the best markers of iron deficiency and anaemia, respectively (Table 4).

We then evaluated by linear regression each country separately (Table 5). \( H. pylori \) infection was not associated with low haemoglobin and low ferritin concentrations in London, but in Chile and in Brazil \( H. pylori \) infection was a predictor of low ferritin concentration. Furthermore, a tendency of association between the infection and low haemoglobin concentration was also observed in both countries.

Table 1. Demographic and clinical characteristics of the included children according to the country of birth.

| Variables                  | UK   | Chile | Brazil | P value |
|---------------------------|------|-------|--------|---------|
| (n = 81)                  | (n = 105) | (n = 125) |       |         |
| Mean age (SD)             | 10.1 (3.6) | 10.6 (3.1) | 11.1 (2.9) | 0.58    |
| Female/Male               | 46/35 | 59/46 | 74/51  | 0.88    |
| Indications for endoscopy |       |       |        |         |
| RAP (%)                   | 65 (80.2) | 84 (80.0) | 102 (81.6) | 0.95    |
| GERD (%)                  | 8 (9.9)  | 11 (10.5) | 13 (10.4)  | 0.99    |
| Vomiting (%)              | 3 (3.7)  | 9 (8.6)  | 4 (3.2)   | 0.14    |
| Others* (%)               | 5 (6.2)  | 1 (0.9)  | 6 (4.8)   | 0.14    |
| \( H. pylori \)-positivity (%) | 6 (7.4) | 33 (31.4) | 47 (37.6) | <0.001  |

RAP, recurrent abdominal pain; GERD, gastroesophageal reflux disease; *diarrhea, weight loss.
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H. pylori and Iron Status in Children
**Table 2.** Histological comparison of antral (n = 85) and corpus (n = 83) gastric mucosa of *H. pylori* (HP)-positive children and antral (n = 202) and corpus (n = 214) gastric mucosa of *H. pylori*-negative children.*

| Inflammation                  | Absent n (%) | Mild n (%) | Moderate n (%) | Marked n (%) | P value |
|------------------------------|--------------|------------|---------------|--------------|---------|
| Antrum chronic inflammation  |              |            |               |              |         |
| HP-positive                  | 2 (2.4)      | 32 (37.6)  | 50 (58.8)     | 1 (1.2)      |         |
| HP-negative                  | 154 (76.2)   | 47 (23.4)  | 1 (0.4)       | 0            | <0.001  |
| Antrum active inflammation   |              |            |               |              |         |
| HP-positive                  | 13 (15.3)    | 44 (51.8)  | 27 (31.8)     | 1 (1.1)      |         |
| HP-negative                  | 187 (92.6)   | 15 (7.4)   | 0             | 0            | <0.001  |
| Corpus chronic inflammation  |              |            |               |              |         |
| HP-positive                  | 22 (26.5)    | 53 (63.9)  | 8 (9.6)       | 0            |         |
| HP-negative                  | 156 (72.9)   | 57 (26.6)  | 1 (0.5)       | 0            | <0.001  |
| Corpus active inflammation   |              |            |               |              |         |
| HP-positive                  | 40 (48.2)    | 39 (46.9)  | 4 (4.9)       | 0            |         |
| HP-negative                  | 191 (89.3)   | 22 (10.3)  | 1 (0.4)       | 0            | <0.001  |

*Twenty-four antral and 13 corpus gastric biopsy specimens were deemed to be inadequate for histology assessment. n, number.

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Taking into account the association observed between the iron parameters and *H. pylori* infection in the Latin-American countries in addition to the similar degree of socioeconomic development between the two populations, the results from Brazil and Chile were analysed together. In this combined population, *H. pylori* infection was an independent and significant predictor of low ferritin and haemoglobin concentrations. As expected, female gender was also a predictor of low ferritin and haemoglobin concentrations and increasing age was associated with increasing haemoglobin concentration (Table 6).

In these populations, but not in London children, *H. pylori* infection was also independently associated in the multivariate analysis with low values of haematocrit, MCV and MCH (Table S1 and Table S2).

**Gastric Pathology and Blood Iron Parameters**

Inflammatory changes in the gastric mucosa induced by *H. pylori* are thought to contribute to changes in the gastric physiology which in turn influence iron absorption. We therefore evaluated the association between factors associated with reduced blood iron and gastric inflammatory parameters in the *H. pylori* infected and non-infected children.

In the *H. pylori*-positive children, a negative correlation was observed between MCV ($r = -0.26; p = 0.01$) and MCH ($r = -0.27; p = 0.01$) values and the degree of antral chronic inflammation; as well as between MCH and the degree of corpus chronic inflammation ($r = -0.29, p = 0.008$) and active inflammation ($r = -0.27, p = 0.002$). No other correlation was observed ($p \geq 0.19$) in this group.

No correlations among the gastric inflammatory and the ID/IDA parameters were observed in the non-infected children ($p>0.15$).

**Table 3.** Comparison of the iron deficiency/iron deficiency anaemia parameters between *H. pylori*-positive (n = 86) and negative (n = 225) children from Brazil, Chile and United Kingdom.

| Variables | *H. pylori* status | P value |
|-----------|-------------------|---------|
|           | Negative (mean±SD)| Positive (mean±SD)|
| Serum ferritin (µg/L)| 41.6 (29.9) | 36.3 (23.5)| 0.06 |
| Transferrin saturation (%)| 29.2 (11.0) | 28.3 (11.1) | 0.52 |
| Haemoglobin (g/L)| 130.6 (9.6) | 129.9 (10.9) | 0.57 |
| Haematocrit (L/L)| 0.39 (0.03) | 0.39 (0.03) | 0.57 |
| MCV (FL)| 83.8 (4.7) | 83.3 (5.0) | 0.48 |
| MCH (µg)| 28.2 (1.8) | 27.8 (1.9) | 0.09 |

*obtained by dividing the serum iron concentration by the total iron binding capacity; SD, standard deviation; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin.
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**Discussion**

Iron deficiency is the most common nutritional disorder in the world, particularly affecting children in developing countries. In addition to the high iron requirement in childhood, dietary iron bioavailability and gastrointestinal parasite infections are frequent in developing countries also contributing to iron deficiency in this age group.

This study, by analyzing children undergoing endoscopy for upper gastrointestinal symptoms that allowed better evaluation of *H. pylori* status and the exclusion of ID causes such as celiac disease, ulcer and erosions, demonstrates an inverse association between *H. pylori* infection and ferritin and haemoglobin concentrations, markers of iron deficiency and anaemia, respectively, in two Latin American countries. As ferritin is an acute

**Table 4.** Multiple linear regression models including ferritin or haemoglobin as dependent variables and age, gender, country of birth and *H. pylori* infection as independent variables in children from United Kingdom, Chile and Brazil (n = 311).

| Variables | Univariate analysis | Multivariate analysis |
|-----------|---------------------|-----------------------|
|           | Beta | P value | Beta | P value |
| FERRITIN |       |         |      |         |
| age | $-0.12$ | 0.84 | $-0.14$ | $<0.01$ |
| gender | $-0.203$ | $<0.001$ | $-0.204$ | $<0.001$ |
| country of birth | $-0.020$ | 0.73 | $-0.194$ | $<0.001$ |
| *H. pylori* infection | $-0.108$ | 0.06 | $-0.110$ | 0.05 |
| HAEMOGLOBIN |       |         |      |         |
| age | $0.303$ | 0.000 | $0.327$ | $<0.001$ |
| gender | $-0.141$ | 0.01 | $-0.194$ | $<0.001$ |
| country of birth | $-0.163$ | 0.004 | $-0.141$ | 0.009 |
| *H. pylori* infection | $-0.033$ | 0.57 | $-0.194$ | $<0.001$ |

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The lower ferritin values we observed in the infected Latin American children points to the role of H. pylori in depletion of iron stores. Reinforcing experimentally these data, in an animal model, gastric depletion of iron stores. Of note, in the Brazilian cohort, the concentration of gastric serum iron and transferrin and hypochlorhydria was observed H. pylori infection associated with different genera of children undergoing upper gastrointestinal endoscopy and biopsied gastric and duodenal mucosa. This allowed the selection of patients were adopted. Firstly, we studied only ethnic groups in the United Kingdom, Chile and Brazil. We observed. Due to inadequate diet, children from developing countries could have a small iron reserve that contributes to the development of iron deficiency and IDA in the course of H. pylori infection.

Several studies describing associations between H. pylori infection and extra-gastric disease have been published. Among them, cross-sectional studies [13,24] and clinical trials [12,14] point to the role of H. pylori infection in the development of iron deficiency/IDA in children. However, there is considerable variation in the results of such studies largely due to methodologic variation. Differences in the study design, inclusion criteria, number of included children, H. pylori diagnosis criterion and ethnicity could explain the discrepancies amongst the studies. It has to be emphasized that, in the present study, rigorous criteria in the selection of patients were adopted. Firstly, we studied only symptomatic children undergoing upper gastrointestinal endoscopy and biopsied gastric and duodenal mucosa. This allowed the exclusion of common causes of iron deficiency such as gastrointestinal bleeding, peptic ulcer disease, extensive erosions and celiac disease. Furthermore, in contrast to other studies [16,30], female adolescents with heavy menstrual blood loss were not included.

**Table 5.** Multiple linear regression models including ferritin or haemoglobin as dependent variables and age, gender and H. pylori (HP) infection as independent variables in children from United Kingdom (n = 81), Chile (n = 105) and Brazil (n = 125).

| Variable | United Kingdom | Chile | Brazil |
|----------|---------------|-------|--------|
|          | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
|          | Beta | P value | Beta | P value | Beta | P value | Beta | P value | Beta | P value |
| Ferritin | | | | | | | | | | |
| Age      | -0.135 | 0.25 | -0.046 | 0.64 | -0.048 | 0.59 | - | - |
| Gender   | -0.157 | 0.02 | -0.165 | 0.09 | -0.169 | 0.08 | -0.286 | 0.001 | -0.276 | 0.02 |
| HP infection | -0.043 | 0.71 | -0.211 | 0.03 | -0.214 | 0.03 | -0.155 | 0.08 | -0.197 | 0.05 |
| Haemoglobin | | | | | | | | | | |
| Age      | 0.451 | <0.001 | 0.46 | <0.001 | 0.187 | 0.06 | 0.294 | 0.005 | 0.280 | 0.002 | 0.304 | 0.001 |
| Gender   | -0.161 | 0.17 | -0.199 | 0.07 | -0.127 | 0.19 | -0.248 | 0.01 | -0.151 | 0.09 | -0.140 | 0.10 |
| HP infection | 0.216 | 0.06 | 0.069 | 0.53 | -0.182 | 0.06 | -0.197 | 0.03 | -0.164 | 0.09 | -0.149 | 0.09 |

**Table 6.** Multiple linear regression models including ferritin or haemoglobin as dependent variables and age, gender, country of birth and H. pylori infection as independent variables in children from Chile (n = 105) and Brazil (n = 125).

| Variable  | Chile | Brazil |
|-----------|-------|--------|
|           | Univariate analysis | Multivariate analysis |
|           | Beta | P value | Beta | P value |
| Ferritin  | | | | |
| Age       | 0.014 | 0.8 | - | - |
| Gender    | -0.222 | 0.001 | -0.222 | 0.001 |
| Birth in Brazil | -0.155 | 0.02 | -0.176 | 0.006 |
| HP infection | -0.164 | 0.01 | -0.172 | 0.007 |
| Haemoglobin | | | | |
| Age       | 0.236 | <0.001 | 0.291 | <0.001 |
| Gender    | -0.141 | 0.03 | -0.191 | 0.003 |
| Birth in Brazil | -0.19 | 0.76 | - | - |
| HP infection | -0.130 | 0.04 | -0.159 | 0.01 |

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because menstrual iron loss is an important determinant of iron status in young women. Intestinal parasitic infections that lead to blood loss were also an exclusion criterion. In addition, we included a large number of children and employed direct methods to diagnosis *H. pylori* infection. Most earlier studies used only one indirect test for diagnosis of *H. pylori* [14,16], and these indirect tests, such as serology have a low accuracy rate for the diagnosis of *H. pylori* in children [31].

In conclusion, the results of this study demonstrate that *H. pylori* infection is a predictor of decreasing serum ferritin and haemoglobin concentrations. The gastric inflammation induced by the infection also negatively influences some haematological parameters. The last Maastricht Florence Consensus Report (Maastricht IV) recommends treating *H. pylori*-positive patients with IDA after the exclusion of the other common causes of the disease [32]. Based on the results of this study, children infected with *H. pylori* with decreased serum ferritin, even without anaemia, may well benefit from therapy for this microorganism.

**Supporting Information**

**Table S1** Multiple linear regression models including haematocrit, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) as dependent variables and age, gender, country of birth and *H. pylori* infection as independent variables in children from Chile (n = 105) and Brazil (n = 125).

**Table S2** Multiple linear regression models including haematocrit, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) as dependent variables and age, gender and *H. pylori* infection as independent variables in children from London (n = 81).

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**Author Contributions**

Conceived and designed the experiments: DMMQ PRH IRS HJW DK JEC. Performed the experiments: MMW AMCR GAR SFSB LPFC AV CS. Analyzed the data: DMMQ AMCR JEC. Contributed reagents/materials/analysis tools: HJW. Wrote the paper: DMMQ AMCR JEC. Revision of the manuscript: DK IRS MMW.

**References**

1. WHO/UNICEF/UNU (2001) Iron deficiency anaemia assessment, prevention, and control. Geneva: World Health Organization. Available: http://www.who.int/nutrition/publications/en/ida_assessment_prevention_control.pdf. Accessed 2013 April 22.

2. Zimmermann MB, Hurrell RF (2007) Nutritional iron deficiency. Lancet 370: 511–520.

3. Stolzhaus R (2001) Iron-deficiency anaemia: re-examining the nature and magnitude of the public health problem. Am J Nutr 131: 697S–701S.

4. Yokota S, Kono M, Mino E, Sato K, Takahashi M, et al. (2008) Enhanced Fe ion-uptake activity in *Helicobacter pylori* strains isolated from patients with iron-deficiency anaemia. Clin Inf Dis 46: e31–33.

5. Yap R, Limburg PJ, Aliqust DA, Carpenter HA, O’Neill A, et al. (1997) Pervasive occult gastrointestinal bleeding in an Alaska native population with prevalent iron deficiency. JAMA 277: 1135–1139.

6. Annibale B, Capurso G, Lahner E, Passi S, Ricci R, et al. (2003) Concomitant iron-uptake activity in *Helicobacter pylori* strains isolated from patients with iron-deficiency anaemia. J Ped Gastroenterol Nutr 36: 701–705.

7. Harris PR, Serrano CA, Villagran A, Walker MM, Thomson M, et al. (2013) *Helicobacter pylori* infection induces deficiency in the INS-GAS mouse. PloS One 7: e50194.

8. Parra HC, Amado RC, Lamberti JC, Rocha J, Antunes CM, et al. (2003) Color and genomic ancestry in Brazilians. Proc Natl Acad Sci USA 100: 177–182.

9. Czuczo R, Moreno RS (1994) Genetic epidemiology of single gene defects in Chile. J Med Genet 31: 702–706.

10. Vannella L, Aloz Spiriti MA, Cozza G, Tardella L, Monaca B, et al. (2008) Benefit of concomitant gastrointestinal and gynaecological evaluation in premenopausal women with iron deficiency anaemia. Aliment Pharmacol Ther 28: 422–430.

11. Baggett H, Parkinson AJ, Muth PT, Gold BD, Gessner B (2006) Endemic iron deficiency in Alaska. J Ped Gastroenterol Nut 51: 85–89.

12. Fagan RP, Dunaway CE, Bruden DL, Parkinson AJ, Gessner BD (2009) *Helicobacter pylori* infection on iron deficiency among children in rural Alaska. J Clin Dis 66: 343–347.

13. Cardenas VM, Molla ZD, Ortiz M, Graham DY (2006) Iron deficiency and *Helicobacter pylori* infection in the United States. Am J Epidemiol 163: 127–34.

14. Choi JW (2003) Does *Helicobacter pylori* infection relate to iron deficiency anaemia in prepubescent children under 12 years of age? Acta Paediatr 92: 970–972.

15. Sarker SA, Davidson L, Mahumud H, Walezyk T, Hurrell RF, et al. (2004) *Helicobacter pylori* infection, iron absorption, and gastric acid secretion in Bangladeshi children. Am J Clin Nutr 28: 149–153.

16. Harris PR, Serrano CA, Villagran A, Walker MM, Thomson M, et al. (2013) *Helicobacter pylori*-associated hypoclorhydria in children, and development of iron deficiency. J Clin Pathol 66: 343–347.

17. Parra HC, Amado RC, Lamberti JC, Rocha J, Antunes CM, et al. (2003) Color and genomic ancestry in Brazilians. Proc Natl Acad Sci USA 100: 177–182.

18. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

19. Parra HC, Amado RC, Lamberti JC, Rocha J, Antunes CM, et al. (2003) Color and genomic ancestry in Brazilians. Proc Natl Acad Sci USA 100: 177–182.

20. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

21. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

22. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

23. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

24. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

25. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

26. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

27. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

28. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

29. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

30. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

31. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.

32. Queiroz DMM, Guerra JB, Rocha GA, Roche AM, Santos A, et al. (2004) IL10 and IL1R /polymorphic genes and *Helicobacter pylori* cagA strains decrease the risk of reflux esophagitis. Gastroenterology 127: 75–79.