Helicobacter pylori and Iron-deficiency Anemia in Adolescents in Brazil

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ABSTRACT

Aim: The aim of the study was to evaluate the association between *Helicobacter pylori* infection and iron deficiency (ID) in adolescents attending a public school.

Patients and Methods: From March to June 2001, a cross-sectional study was conducted among adolescents (10–16 years) enrolled in a single public school in São Paulo, Brazil. Of 400 eligible students, 195 agreed to participate, but 1 was excluded due to sickle cell disease. A blood sample was collected from each subject to measure hemoglobin and ferritin. *H pylori* status was investigated with the ^{13}C-urea breath test. All of the subjects with either anemia or ID were given iron therapy.

Results: *H pylori* prevalence was 40.7% (79/194), being higher in male subjects (45/90 vs 34/104, $P = 0.014$). There was no relation between infection and nutritional status. Abnormally low serum ferritin was observed in 12 subjects, half of whom were positive for *H pylori* (odds ratio [OR] 1.49, 95% confidence interval [CI] 0.38–5.81). The median serum ferritin was 33.6 ng/mL (interquartile range 23.9–50.9) in infected subjects and 35.1 ng/mL (interquartile range 23.7–53.9) in uninfected subjects. Anemia was detected in 2% (4/194) of the students, half of whom were infected (OR 1.47, 95% CI 0.1–20.6). The mean hemoglobin value in infected subjects was 13.83 g/dL ± 1.02 versus 14 g/dL ± 1.06 in uninfected subjects.

Conclusions: The study was not able to find a relation between *H pylori* infection and ID or anemia.

Key Words: anemia, *Helicobacter pylori*, iron deficiency, urea breath test

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Helicobacter pylori infection is extremely prevalent in developing countries. The infection is most often acquired in childhood, and spontaneous eradication is rare (1). *H pylori* is recognized as the most common etiological agent of chronic gastritis, but the infection is asymptomatic in most patients (2,3). However, the infection is associated with gastric and duodenal peptic ulcers, gastric cancer, and mucosa-associated lymphoid tissue lymphoma in a minority of patients, mainly in adulthood (4).

Recently, the *H pylori* infection has been associated with iron deficiency (ID) and ID anemia. Dufour et al (5) reported a 7-year-old patient with ID anemia who was successfully treated by eradicating the infection. Thereafter, other studies have reported on extensively investigated patients with ID anemia refractory to conventional therapy, who improved only after eradicating the infection (6–10). In addition, further evidence on the association between *H pylori* infection and ID comes from a recent controlled, open-label, randomized clinical trial conducted among Alaskan schoolchildren. In the study, all of the children with ID and *H pylori* infection were treated with a 6-week course of iron sulfate, and the intervention group also received treatment for the *H pylori* infection. In the first report, on 14-month evaluation, there was no difference between the control group and the intervention group regarding ferritin level, hemoglobin level, prevalence of ID, and prevalence of anemia (11); however, a significantly higher prevalence of ID anemia was observed in the control group after 40 months of follow-up (12).

Epidemiological studies have also evaluated the association between *H pylori* infection and ID anemia. In Denmark, an epidemiological study showed reduced iron storage in anti-*H pylori*–positive individuals compared with seronegative controls (13). Another epidemiological study reported that *H pylori* infection is a significant risk factor for ID (prevalence ratio [PR] 1.6), ID anemia (PR 4), and anemia without ID (PR 3.7). Interestingly, the association was stronger in individuals older than 9 years, which may signal that long-lasting infection increases the risk of developing ID anemia in infected subjects (14).

H pylori infection and iron-deficiency anemia are prevalent in Brazil. The aim of the present study was to evaluate the association between *H pylori* infection and sideropenic anemia in children and adolescents attending a public school in São Paulo, Brazil.

PATIENTS AND METHODS

A cross-sectional study was conducted during a 4-month period (March–June 2001) in a public school in São Paulo, Brazil. The school (Escola Estadual Prof Napoleão de Carvalho Freire) is located in a middle-class neighborhood from São Paulo's south side, and it provides elementary and high school education.

The purpose and the design of the study were explained in a meeting with parents and teachers, and all of the adolescent (10–16 years old) students were invited to participate (n = 400). Exclusion criteria were menstrual flow alterations, use of steroids or nonsteroidal anti-inflammatory drugs (NSAIDs) for more than 7 days, recent use (previous month) of antibiotics, and chronic disease. One hundred ninety-five students (and their parents) agreed to participate in the study and provided written consent, but 1 was excluded due to sickle cell disease.

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The study was approved by the ethics committee of the Universidade Federal de São Paulo, Escola Paulista de Medicina, and it was authorized by the school board. Informed consent was gathered from the adolescents and their parents or legal guardians. The students and their parents filled out a clinical questionnaire, including data on menstrual flow alterations, the presence of chronic pathologies, the use of antibiotics or NSAIDs in the previous 3 months, gastrointestinal symptoms, previous anemia, a family history of peptic disease or anemia, and a brief nutritional record. Then, a blood sample (8 mL) was collected from each adolescent after overnight fast for hemogram and serum ferritin measurement. Next, a ^{13}C-urea breath test was performed. The subjects with either anemia or ID were given iron therapy (ferrous sulfate $5\,mg \cdot kg^{-1} \cdot day^{-1}$) for 3 months and reevaluated after 4 weeks.

^{13}C-urea Breath Test

Breath samples were collected from each subject at baseline and 30 minutes after drinking 200 mL of whole orange juice containing 75 mg of ^{13}C-urea. An additional breath sample was collected 30 minutes after the ingestion of the tracer. The test was performed with a nondispersive infrared spectrometer (Wagner Analyser, Bremen, Germany), and the results were expressed as surplus of isotopic ratio over baseline isotopic ratio (delta over baseline [DOB]). DOB value >4% was considered positive, according to instructions from the manufacturer. This test was previously validated in our setting, where it provided a sensitivity of 96.8% and a specificity of 93.2% (15).

Laboratory

Hemogram was done by an automated method using the Cell Dyn 3700 or 4000 hematometer (Abbott Diagnostics) at the laboratory facility of Hospital São Paulo. Serum ferritin was measured by immunoenzymatic ferritin analysis method (AIA-PACK FER, Tosoh Corp, Tokyo) at another laboratory facility of the UNIFESP/EPM (Laboratory of Psychopharmacology).

Definitions

H pylori infection was defined by a positive ^{13}C-urea breath test. Anemia was defined according to the cutoff values proposed by Dallman (16): hemoglobin <11.5 g/dL for patients between 5 and 10 years of age; hemoglobin <12.0 g/dL for patients between 11 and 14 years of age; hemoglobin <12.0 g/dL for female subjects older than 15 years; and hemoglobin <13.0 g/dL for male subjects older than 15 years. ID was defined according to serum ferritin levels, which were regarded as abnormal when <10 ng/mL (12 if older than 15 years) (16).

Statistical Analysis

Qualitative variables were described by their proportions, and continuous variables (age, hemoglobin, ferritin, body mass index [BMI]) were summarized either by their mean and standard deviation (SD) or by median and interquartile range, according to its distribution. Group comparison on contingency table was done with the Pearson χ^2 test or the Fisher exact test when indicated. Odds ratios (ORs) and their exact 95% confidence intervals (CIs) were provided for the association between anemia, sideropenia, and *H pylori*. Dependent variables evaluated were ferritin and hemoglobin. The correlation between DOB values and ferritin and hemoglobin was performed among infected students with the Spearman correlation test. The influence of previous use of NSAIDs on ferritin and hemoglobin levels was tested using the Mann-Whitney test. The serum ferritin levels in the studied population did not exhibit a normal distribution, as tested graphically and with the Shapiro-Wilk *W* test. Because the data were not normalized even after logarithmic transformation, the median (50th percentile) value of serum ferritin of *H pylori*–infected and noninfected students was compared with quantile regression, unadjusted and adjusted for sex, age, and BMI. In addition, the hemoglobin level of *H pylori*–infected and noninfected students was compared with linear regression, unadjusted and adjusted for sex, age, and BMI. The level of significance adopted was 0.05. Statistical analysis was performed using the computing environment R (version 2.6.2 for Linux, R Development Core Team, 2005) and the statistical package Stata (version 9 for Windows, StataCorp, College Station, TX).

RESULTS

One hundred ninety-four subjects were evaluated (age range 10–16 years, mean 13 years, SD 1.5 years), 105 (54.1%) being girls. *H pylori* infection was detected in 40.7% (79/194), and its prevalence was higher in male subjects (50% vs 32.7%, $P = 0.014$). In addition, *H pylori* prevalence increased from 33.3% (11/33) in the younger age group (10–11 years) to 41.6% (37/88) among subjects ages 12 to 13 years, and finally 42.5% (31/73) in the oldest age group (14–16 years), but this difference was not significant ($P = 0.636$). The BMI ranged from 13.78 to 38.74 (median 19.68, interquartile [IQ] range 18–22). Previous use of NSAIDs was reported by 37 (19.1%), being slightly less common in *H pylori*–infected subjects (27% vs 44%, $P = 0.059$).

The median serum ferritin was 34.3 ng/dL (range 2.4–165 ng/dL, IQ range 23.9–52.4 ng/dL). Abnormally reduced levels of serum ferritin were detected in 12 (5.7%) of the patients, 10.5% (11/105) of the girls and 1.1% (1/89) of the boys ($P = 0.016$). However, anemia was detected in only 1 of them, who was negative for *H pylori*. The median serum ferritin was 33.6 ng/mL (IQ range 23.9–50.9) in infected subjects and 35.1 ng/mL (IQ range 23.7–53.9) in noninfected subjects. ID was detected in 7.59% (6/79) *H pylori*–infected subjects and 5.22% noninfected subjects (OR 1.49, 95% CI 0.38–5.81). Previous use of NSAIDs did not influence the ferritin level ($P = 0.747$). There was no significant correlation between values of the breath test and ferritin in infected subjects. Although the median ferritin of *H pylori*–infected subjects was 1.5 ng/dL lower than that of noninfected subjects, this difference was not significant (Fig. 1, Table 1).

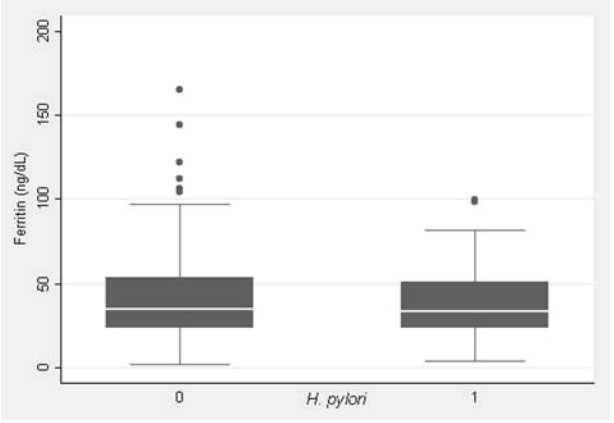

FIGURE 1. Box plot of serum ferritin levels according to *Helicobacter pylori* status (0 = negative, 1 = positive).

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TABLE 1. Influence of age, sex, BMI, and *H pylori* infection on serum ferritin levels: univariate and multivariate quantile regression

Variable	Coef (95% CI)	*P*	Coef adj (95% CI)*	*P*
Constant			26.2 (−2.01 to 54.42)	0.069
Age	0.62 (−1.53 to 2.78)	0.569	−0.24 (−2.33 to 1.84)	0.623
Male sex	13.2 (7.9–18.5)	<0.001	13.87 (8–19.74)	<0.001
BMI	0.69 (−0.15 to 1.53)	0.110	0.37 (−0.43 to 1.17)	0.361
H pylori	−1.5 (−8.6 to 5.6)	0.677	−1 (−7.13 to 5.13)	0.748

adj = adjusted for sex and age; BMI = body mass index; CI = confidence interval; Coef = coefficient.
* Adjusted for sex, age, and BMI.

The mean hemoglobin value was 13.9 g/dL (SD 1.0), being 14 g/dL (SD 1.0) among boys and 13.7 g/dL (SD 1.0) among girls. There were 4 (2%) subjects with anemia, all of whom were girls. However, their hemoglobin values were close to cutoff value, that is, 11.8, 11.8, 11.9, and 11.9 g/dL. Anemia was detected in 2 of 79 *H pylori*–infected subjects (2.53%) and 2 of 115 noninfected subjects (1.74%) (OR 1.47, 95% CI 0.1–20.6). The average hemoglobin was 13.83 g/dL (SD 1.02) in infected students and 14 g/dL (SD 1.06) in noninfected students. Previous use of NSAIDs did not influence the hemoglobin ($P = 0.689$). DOB values and hemoglobin did not significantly correlate in *H pylori*–positive students. There was no statistical relation between *H pylori* status and hemoglobin in univariate and multivariate analyses (Table 2).

Neither anemia nor ID was detected in 15 subjects (7.7%), 10.12% among *H pylori*–infected and 6.08% among the remaining subjects (OR 1.74, 95% CI 0.52–5.89). The presence of 1 of these abnormalities was significantly associated with female sex (14/104 vs 1/90, Fisher exact test $P = 0.001$). All of the patients were given oral iron supply: 5 were lost to follow-up, 1 did not comply, and 9 presented normal values of hemoglobin and ferritin 4 weeks after beginning the treatment.

DISCUSSION

The study failed to demonstrate a relation between *H pylori* infection and sideropenic anemia in children and adolescents. This is the first pediatric study on this subject in our country, but recently, a collaborative Latin American study (17) with children and adults from 6 countries, including adults from Brazil, reported similar data. Interestingly, the prevalence of anemia herein reported was low, lower than the figure observed in adults (20.6%) in Brazil.

The study presents 2 major limitations. The first is selection bias, because only 1 school was evaluated. This school was chosen by convenience, and it is not a representative sample of adolescents in São Paulo. Indeed, this is clearly demonstrated by the low prevalence of anemia reported in the present study. The second limitation is that the study is underpowered. The sample size was small to address the proposed target, mainly due to the small observed prevalence of anemia.

The relation between refractory ID anemia and *H pylori* infection was initially described in some case reports, in which patients with anemia had *H pylori* infection and improved after *H pylori* eradication therapy. Mostly, these patients were male children (15/19) with severely depleted iron stores, different from the subjects herein reported (5–7,9,10). In the present study there was no patient presenting with anemia who was refractory to the conventional treatment. However, it may be explained by the rareness of this condition, difficult to detect in a population setting.

The study cannot exclude *H pylori* as a risk factor for ID (OR 1.49, 95% CI 0.38–5.81). However, other authors reported a significant relation between *H pylori* infection and ID, anemia, and anemia without ID among children in Alaska, in a region with high prevalence of both anemia and *H pylori* infection (14). Additionally, a recent systematic review summarized the available evidence, concluding that *H pylori* infection is a significant risk factor for ID (OR 1.36, 95% CI 1.16–1.65) and ID anemia (OR 2.0, 95% CI 1.5–2.9) (18). However, this issue is still a matter of controversy, because recently an important multicentric Latin American study did not find a significant relation between iron status and this infection (17). In the present study, the serum ferritin level was slightly lower in infected subjects, but the difference was not significant. Although a type II error may have prevented reaching statistical significance, the clinical and epidemiological relevance of such a small difference (1.5 ng/dL in the median values) is uncertain.

The clinical response to *H pylori* treatment was not evaluated in the present study because all of the complying patients improved with iron therapy. Kurekci et al (19) reported 25.7% of ID and 12.9% of anemia in *H pylori*–infected children. Moreover, after *H pylori* eradication, the levels of ferritin and hemoglobin rose significantly. Choe et al (20), in Korea, conducted a randomized double-blind study with 43 adolescents, 33 of whom were girls, with 25 (58.1%) infected patients. Patients received 3 types of treatments: ferrous sulfate and anti-*H pylori* treatment (bismuth,

TABLE 2. Influence of age, sex, BMI, and *H pylori* infection on hemoglobin levels: univariate and multivariate linear regression

Variable	Coef (95% CI)	*P*	Coef adj (95% CI)*	*P*
Constant			13.34 (12.43–14.25)	<0.001
Age	0.004 (−0.02 to 0.03)	0.730	0.002 (−0.02 to 0.02)	0.623
Male sex	0.32 (0.03–0.61)	0.035	0.36 (0.06–0.65)	0.020
BMI	0.03 (−0.01 to 0.06)	0.187	0.02 (−0.01 to 0.06)	0.229
H pylori	−0.17 (−0.47 to 0.13)	0.260	−0.21 (−0.51 to 0.1)	0.178

adj = adjusted for sex and age; BMI = body mass index; CI = confidence interval; Coef = coefficient.
* Adjusted for sex, age, and BMI.

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amoxicillin, and metronidazole); placebo and anti-*H pylori* treatment; and ferrous sulfate and placebo treatment. The last group presented slower resolution of anemia, even slower than the group that had received only eradication therapy. Furthermore, a similar open-label randomized clinical trial demonstrated that a late effect may also be important, because after 40 months of *H pylori* treatment, children who remained negative for the infection exhibited a lower prevalence of ID than infected children (12). However, the small number of subjects treated in the present study and the short follow-up period impair drawing inferences on the effect of *H pylori* infection on the treatment of ID.

The prevalence of anemia in the present study (2%) is lower than that reported in other studies. ID anemia is a major worldwide public health issue, and its prevalence in Brazil ranges from 2.8% to 24.5% among adolescents and is around 50% among infants and toddlers (21–23). Even in European schoolchildren, a recent World Health Organization document reported a higher prevalence (9.2%) of anemia (24). The possible explanation for this unexpected low prevalence of anemia is that the school investigated in the recent study is located in a wealthy neighborhood.

In conclusion, although no relation was found between *H pylori* infection and ID in the population under analysis, it cannot be excluded because anemia was unexpectedly infrequent in the study population.

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