Iron, Zinc, and Copper Nutritional Status in Children Infected With *Helicobacter pylori*

*Mariana A. Janjetic, *Cinthia G. Goldman, †Norma E. Balcarce, †Eduardo Cueto Rua, *Andrea Beltrán González, ‡Julián A. Fuda, ‡Emiliano I. Meseri, ‡Horacio E. Torti, *Julieta Barrado, *Marcela B. Zubillaga, §Laura B. López, and *José R. Boccio

ABSTRACT

Objective: *Helicobacter pylori* colonizes the gastric mucosa of about half of the world's population and it has been related to extragastrointestinal diseases. The present study sought to evaluate the association between *H pylori* infection and iron, zinc, and copper nutritional status in symptomatic children.

Patients and Methods: A cross-sectional study was carried out in 395 children (4–16 years) with upper gastrointestinal symptoms, who were tested for *H pylori* infection by the ^{13}C-urea breath test. Iron status was determined by hemoglobin, serum ferritin, and serum transferrin receptors. Copper and zinc serum concentrations were also evaluated. Epidemiological data, dietary assessment, and anthropometric indicators were analyzed as potential confounding factors.

Results: Prevalence of *H pylori* infection was 24.3%. Anemia and iron deficiency (ID) were found in 12.0% and 14.3% of the *H pylori*–positive and 8.9% and 11.0% of the *H pylori*–negative children, respectively. There was no association between *H pylori* infection and anemia (odds ratio = 1.54 [95% confidence interval [CI] 0.73%–3.24%]) or ID (odds ratio = 1.35 [95% CI 0.67–2.70]). Crude β coefficients showed that *H pylori* has no significant effect on hemoglobin, serum ferritin, serum transferrin receptors, copper, and zinc concentrations. However, adjusted results suggested that *H pylori*–infected children had an increase of 9.74 μg/dL (95% CI 2.12–17.37 μg/dL) in copper concentrations.

Conclusions: This study revealed that *H pylori* infection was not associated with iron deficiency, anemia, or zinc concentrations; however, a positive relation with copper status was found after adjusting for confounding factors. The contribution of *H pylori* infection to higher copper concentrations needs to be confirmed by additional studies.

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From the *Laboratory of Stable Isotopes Applied to Biology and Medicine, Department of Physics, School of Pharmacy and Biochemistry, the †Gastroenterology Unit, Children's Hospital ''Superiora Sor Maria Ludovica,'' La Plata, the ‡Laboratory of Atomic Absorption Spectrometry, Department of Physics, School of Pharmacy and Biochemistry, and the §Nutrition Department, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina.

Address correspondence and reprint requests to Mariana A. Janjetic, Stable Isotopes Laboratory Applied to Biology and Medicine, School of Pharmacy and Biochemistry, University of Buenos Aires; Junin 956 (1113), Buenos Aires, Argentina (e-mail: mjanje@ffyb.uba.ar).

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Helicobacter pylori infection is the most common chronic bacterial infection in humans. It is recognized as a major etiologic factor in the development of chronic gastritis and peptic ulcer disease in adults and children (1). The infection is mainly acquired during childhood, and its prevalence tends to be higher in developing countries than in developed ones (2). Nevertheless, previous epidemiologic studies conducted in Argentina showed 40% infection prevalence in symptomatic children (3) and 15.7% prevalence in the asymptomatic population (4), results that are similar to those reported in developed countries (5).

Several studies have related *H pylori* infection to extragastrointestinal diseases, including iron, B_{12}, and folic acid deficiencies, among others (6,7). Different hypotheses have been proposed to explain the association between *H pylori* infection and iron deficiency (ID) or iron-deficiency anemia (IDA). One of them suggests that *H pylori*, when affecting the gastric body, induces gastric acid hyposecretion (8). In addition, it has been demonstrated that *H pylori* gastritis induces a decrease in intragastric ascorbic acid concentrations with a consequent impairment in the absorption of many nutrients, compromising the nutritional status of infected individuals (9); however, the pathophysiologic mechanisms involved in this phenomenon have not been confirmed. All of the factors described above lead to 1 important question: why do only a few number of infected individuals develop ID or IDA, despite the worldwide distribution of *H pylori* infection and micronutrient deficiencies?

It is well known that ID, and specifically IDA, remain 1 of the most severe and important nutritional deficiencies in the world (10). According to the last National Nutritional and Health Survey published in 2007 by the Argentine Ministry of Health, the prevalence of anemia evaluated in infants 6 to 24 months and children 2 to 5 years of age was 34.9% and 10.6%, respectively (11). Iron exhibits important interactions with other essential mineral elements such as copper and zinc, showing competitive inhibitions in their transport and bioavailability (12,13). However, zinc and copper have not been evaluated in the national survey. Copper serves as an important catalytic cofactor in a number of critical enzymes that are required in biological functions for growth and development (13), and zinc plays multiple roles in physical growth, immunocompetence, reproductive function, and others (14).

All of the evidence described above supports the importance of establishing whether *H pylori* is associated with micronutrient deficiencies in children. The aim of our study was to evaluate the relation between *H pylori* infection and iron, zinc, and copper

nutritional status in symptomatic children from Buenos Aires, Argentina.

PATIENTS AND METHODS

Patients

This cross-sectional study was carried out in children with ages ranging from 4 to 16 years, who were referred to the gastroenterology unit of the Children Hospital "Superiora Sor Maria Ludovica," La Plata, Argentina, for evaluation of upper gastrointestinal signs and symptoms (gastroesophageal reflux, symptoms of esophagitis, dyspepsia, and abdominal pain). The hospital is a tertiary-level health care referral institution with the highest clinical complexity for attending children in the Buenos Aires province. To determine the number of individuals to be included in this study, the following parameters were set: α and β errors of 0.05 and 0.20, respectively, a relative risk $\geq$2.0, an anemia prevalence of 13% among unexposed (*H pylori* negative) children, and a prevalence of *H pylori* infection obtained for the same population in a previous study (3). A value of 20% was added to account for expected refusals and subject dropouts, and to allow adequate controlling of confounding factors. The final sample size of 390 children was estimated.

Ethics

The study was approved by the ethics committee of the hospital, and the parents or legal guardians were instructed to carefully read the protocol information and sign a written consent form according to the Declaration of Helsinki. Subjects taking antibiotics or acid suppressants during the previous month as well as mineral supplements (including iron, zinc, and copper) during the previous 3 months were excluded from the study. Data collection was carried out from April 2006 to April 2008. All of the results on blood parameters and *H pylori* infection status were sent to the responsible health facilities, where children received the appropriate treatment.

^{13}C-urea Breath Test

Children were instructed to fast for at least 6 hours before the diagnostic test was performed. ^{13}C-urea breath test (UBT) consisted of the following: 2 samples of exhaled air were taken previous to the ingestion of the labeled solution to determine basal $^{13}C/^{12}C$ ratios. Then, 150 mL of reconstituted powdered nonfatty milk containing 50 mg of ^{13}C-urea (Cambridge Isotope Laboratories Inc, Andover, MA) was taken by each patient. Breath samples were collected at 30 and 45 minutes after the ingestion of the labeled solution in hermetically sealed containers (Labco Ltd, Buckinghamshire, UK). Each sample of exhaled air was measured in a mass spectrometer coupled to a gas chromatographer (FinniganMAT GmbH, ThermoQuest Corp, Bremen, Germany). A change of $>$3.5‰ in the delta over baseline values was considered positive. The ^{13}C-UBT is a highly accurate diagnostic test, with values of sensitivity and specificity $>$95% (15,16).

Biochemical Analysis

Venous blood samples were obtained in the morning before performance of the ^{13}C-UBT. Iron status was evaluated by determination of hemoglobin, serum ferritin (SF), and serum transferrin receptors (sTfR) concentrations. Hemoglobin was measured using an electronic counter by the cyanmethemoglobin method. SF was determined by an immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX) and sTfR by means of an enzyme immunoassay (TF-94 Ramco Laboratories, Stafford, TX). Copper and zinc serum concentrations were measured by an atomic absorption spectrophotometer (Buck Scientific, 200A, East Norwalk, CT). Serum samples were kept at -70°C until assay. Anemia was defined with hemoglobin values $<$115 g/L for children younger than 12 years, and $<$120 g/L for children older than that age. SF cutoff was 12 μg/L for children younger than 5 years and 15 μg/L for children older than that age. Serum sTfR concentrations were measured as an additional marker of functional ID, with a normal range of 2.9 to 8.5 mg/L. ID was defined as SF concentration lower than the cutoff value. IDA was defined when both ID and anemia were determined (10). Plasma zinc and copper concentrations were measured following the addition calibration technique (17). Zinc deficiency was defined with zinc values $<$74 μg/dL for males older than 10 years of age and $<$70 μg/dL for the rest of the children (14). Copper deficiency was defined when copper concentration was $<$70 μg/dL for boys and $<$80 μg/dL for girls (14,18).

Dietary Assessment

A 24-hour dietary recall was administered to the mothers or guardians of each child to obtain dietary information. A book of picture charts was used to aid respondents in portion size estimation (19). To adjust mean mineral intakes to estimate the usual intake distribution according to age, a second 24-hour dietary recall was applied in a subsample of 100 children. Prevalences of iron, zinc, and copper inadequacy were defined when nutrient intakes were below the estimated average requirement, according to age and sex (20). Data analysis was performed using the food composition database compiled in 2007 by the Argentine Ministry of Health (21) and the food composition table of the US Department of Agriculture (22). Nutrient intakes were analyzed according to the guidelines of the US National Research Council, National Academy of Sciences (20).

Epidemiological Questionnaire

An epidemiological questionnaire was administered to the parents or guardians of the participant children to obtain information about possible predictive variables for *H pylori* positivity. The questionnaire was focused on ethnicity (white or south Amerindian) and sociodemographic factors such as family crowding (number of siblings, rooms in the house, family members in the household), children's educational level (kindergarden, basic general education, polimodal), the educational level of mothers and heads of the household (only primary school or less, finished secondary school, finished college or university), and sanitary standards (type of flooring [wooden, cement, soil], type of toilet [sewer, septic tank, pit latrine], and source of water [tap water, well-shaft not treated, treated system]). Unsatisfied basic needs were defined according to the guidelines of the Argentine Bureau of Statistics and Census (Instituto Nacional de Estadísticas y Censos) (23).

Anthropometric Indicators

Height was recorded using a stadiometer (Stanley, Morangis, France); weight was measured by a portable mechanical scale (CAM, Buenos Aires, Argentina). Height and weight were expressed (as z scores) relative to the Centers for Disease Control and Prevention 2000 age- and sex-appropriate standards. Underweight and stunting were defined as weight-for-age and height-for-age z score below -2 standard deviations from the median of the

standard reference population. Overweight and obesity were defined according to body mass index centiles ≥85 and ≥95, respectively (24). All of the measurements were made in triplicate by the same interviewer. Anthropometric techniques were previously standardized (25). Percentiles and *z* scores were obtained using Epi Info, version 3.2 software (Centers for Disease Control and Prevention, Atlanta, GA).

Statistical Analysis

The Fisher exact test was used to analyze dependency between *H pylori* positivity and other categorical variables, and the chi-square test was applied to variables with more than 2 categories. To analyze whether variances of quantitative variables were homogeneous for both *H pylori*–positive and –negative groups, the Levene test was applied. Student *t* test was used when it was proven that variances were homogeneous; if not, the nonparametric Mann-Whitney *U* test was applied. A binary logistic regression was performed to estimate the impact of *H pylori* status alone and adjusted for confounders as predictive variables for anemia and ID. Plasma ferritin concentrations were transformed logarithmically before the analysis, allowing the expression of the results as geometric means (±1 SD). Linear regression was used to evaluate the effect of *H pylori* infection status alone and adjusted for confounders on hemoglobin, SF, sTfR, copper, and zinc serum concentrations. In all of the analyses, variables were considered to be possible confounders of the effect of *H pylori* on the outcome if they were associated with both the outcome and *H pylori* infection with *P* values <0.20. Crude and adjusted odds ratios (OR) and β coefficients were obtained with 95% confidence intervals (95% CI). Significance levels were set at $\alpha < 0.05$. Statistical analyses were performed using StatCalc from Epi Info 3.2 and SPSS 11.5 (Chicago, IL) software.

RESULTS

The 395 participating children had a mean age of 9.86 years. A total of 96 patients were found to be *H pylori* positive by means of the ^{13}C-UBT, with a prevalence of 24.3% (95% CI 20.2%–28.9%) in this symptomatic population. *H pylori* infection was associated with low socioeconomic status, poor sanitary conditions, a high number of siblings and family members, ethnicity, and low educational level of the parents. These results are consistent with epidemiological data previously described by our group in the same population (3) and others (26). In the present study, no significant differences were found between anthropometric indicators and *H pylori* status (Table 1).

Measurement of biochemical parameters related to micronutrients status could not be performed in the whole population. Incomplete data rates were 12.2% and 3.2% for hemoglobin and SF, respectively. In addition, serum zinc and copper concentrations could not be obtained in 11.4% of the samples. We found a zinc deficiency of 0.5% (95% CI 0.0%–1.8%) and a copper deficiency of 1.7% (95% CI 0.7%–3.9%) in the whole population. Low prevalence of micronutrient intake inadequacy was found in our population (Table 1), with no significant differences in the mean intakes of iron, zinc, and copper between the *H pylori*–positive and –negative groups. Evaluation of sTfR was performed in a subsample of 295 children, which had no statistical differences in *H pylori* prevalence and socioeconomic status compared with the whole population.

TABLE 1. Anthropometric and nutritional characteristics of the study participants

	H pylori (−)	*H pylori* (+)	*P*
n	299 (75.7)*	96 (24.3)	
Age, y†	9.86 ± 3.13	10.18 ± 3.24	0.38
Sex			
Female	158 (52.8)	54 (56.2)	0.56
Male	141 (47.2)	42 (43.8)	
Ethnicity			
White	280 (93.6)	83 (86.5)	0.03
South Amerindian	19 (6.4)	13 (13.5)	
Anthropometric indicators			
Stunted	15 (5.0)	6 (6.2)	0.59
Underweight	17 (5.7)	8 (8.3)	0.36
Overweight and obesity	60 (20.0)	15 (15.6)	0.32
Nutrient intake inadequacy			
Iron	9 (3.0)	4 (4.1)	0.74
Zinc	15 (5.0)	9 (9.4)	0.14
Copper	62 (20.7)	22 (22.7)	0.66

* n and % in parentheses, unless otherwise specified.
† Mean ± SD.

Table 2 shows mean values of the biochemical markers evaluated for iron, zinc, and copper status according to *H pylori* infection. No significant differences were found for *H pylori*–positive and –negative patients in any of the biochemical markers evaluated. Moreover, calculated crude β coefficients showed that the presence of *H pylori* infection has no significant effect on hemoglobin, SF, sTfR, zinc, and copper serum concentrations (Table 3). However, after adjusting β coefficients for confounding variables, the results suggested that *H pylori*–infected children had an increase of 9.74 μg/dL (95% CI 2.12–17.37 μg/dL) in copper concentrations compared with noninfected children. In contrast, the adjusted β coefficients for the other biochemical markers were not associated with *H pylori* infection (Table 3).

Prevalence of anemia was 12.0% (95% CI 7.2%–22.6%) for the *H pylori*–positive group and 8.9% (95% CI 6.0%–13.6%) for the *H pylori*–negative group. ID was found in 14.3% (95% CI

TABLE 2. Comparison of iron, zinc, and copper biochemical markers for *H pylori*–positive and –negative patients

	H pylori (+) mean ± SD	*H pylori* (−) mean ± SD	*P*
Hemoglobin, g/L	126 ± 11	127 ± 11	0.42
Serum ferritin, μg/L	30.9 (16.3–58.6)*	35.3 (17.8–70.1)*	0.10
Soluble transferrin receptors, mg/L	4.2 ± 1.6	4.4 ± 1.6	0.38
Serum zinc, μg/dL	140.5 ± 42.2	137.5 ± 40.2	0.48
Serum copper, μg/dL	133.1 ± 29.5	129.5 ± 28.8	0.12

SD = standard deviation.
* Geometric mean (+1 SD, −1 SD).

TABLE 3. Crude and adjusted β coefficients for iron, zinc, and copper status indicators according to *H pylori* infection

	β coefficient$_{crude}$ (95% CI)	*P*	β coefficient$_{adjusted}$ (95% CI)	*P*
Hemoglobin, g/L	−0.11 (−0.37 to 0.15)	0.42	−0.05 (−0.34 to 0.23)*	0.70
Serum ferritin, μg/L	−6.79 (−14.11 to 0.52)	0.07	−5.70 (−13.27 to 1.85)†	0.14
Soluble transferrin receptors, mg/L	−0.23 (−0.78 to 0.30)	0.38	−0.18 (−0.73 to 0.36)‡	0.51
Serum zinc, μg/dL	3.01 (−7.02 to 13.05)	0.55	3.02 (−7.33 to 13.38)§	0.56
Serum copper, μg/dL	6.99 (−0.47 to 14.45)	0.07	9.74 (2.12–17.37)¶	0.01

* Adjusted for serum copper, children's educational level, and type of flooring.
† Adjusted for source of water, head of the household's educational level, overcrowded living conditions, and type of flooring.
‡ Adjusted for source of water and unsatisfied basic needs.
§ Adjusted for source of water, serum copper, ethnicity, serum ferritin, and overcrowded living conditions.
¶ Adjusted for source of water, children's educational level, number of siblings, and weight for age.

7.8%–23.2%) and 11.0% (95% CI 7.6%–15.2%) of the *H pylori*–positive and –negative patients, respectively. A low rate of IDA was observed in the studied population, with a prevalence of 2.4% (95% CI 1.1%–4.8%).

A binary logistic regression was performed to estimate the impact of *H pylori* status alone and adjusted for confounders as a predictive variable for anemia and ID. Under this analysis, the crude OR for anemia was 1.54 (95% CI 0.73–3.24), and after adjusting this value by source of water and type of flooring, an OR of 1.11 (95% CI 0.49–2.50) was obtained. The crude OR for ID was 1.35 (95% CI 0.67–2.70) and the adjusted OR (by type of toilet, mother's educational level, ethnic group, and overcrowded) was 1.45 (95% CI 0.69–3.04). These results showed that there was no association between *H pylori* infection and anemia or ID.

DISCUSSION

The relation between *H pylori* infection and ID or IDA has been studied by several groups during the last 2 decades; however, a uniform and clear conclusion has not been reached. The Maastricht III Consensus Report in the management of *H pylori* infection (27) recommended that *H pylori* infection should be sought for and treated in children and adolescents with refractory IDA. Nevertheless, the American College of Gastroenterology Guideline (28) established that further properly designed trials would be needed to assess whether *H pylori* eradication offers benefit to patients with unexplained IDA.

Some cross-sectional studies performed in children and youth described an association between *H pylori* infection and IDA (29,30), although no significant differences were found in mean hemoglobin values between infected and uninfected children (29,30). Several reports demonstrated a lack of association between *H pylori* either with IDA (31) or with anemia (9,32).

Relatively few intervention studies have sought to establish a cause-and-effect relation between *H pylori*, ID, and anemia. Two randomized controlled trials in youth and children suggested a role for *H pylori* in causing anemia and ID (33,34), but an even larger open-label study, which involved 219 children, failed to demonstrate any improvement in hemoglobin and SF concentrations attributable to *H pylori* eradication (35). Moreover, the results of 1 of the latest randomized controlled trial performed in 200 Bangladeshi children concluded that *H pylori* infection is neither a cause of IDA/ID nor a reason for treatment failure of iron supplementation in young children (36).

In the present study we have not found an association of *H pylori* infection with ID or with anemia, although these results could not be generalized to the whole population because our study was conducted among a population of patients who, according to the inclusion criteria, have an increased prevalence of gastrointestinal disease, and may not be representative with respect to *H pylori* or micronutrient deficiencies.

Mean SF value was lower in the *H pylori*–positive group; however, the difference was not statistically significant (Table 2). The global prevalence of anemia, ID, and IDA found in the studied population was 10.1%, 14.0%, and 2.4%, respectively. Unfortunately, we could not evaluate the association between *H pylori* infection and IDA because of its low prevalence.

Our results did not show an association between *H pylori* infection and copper serum concentrations when a crude analysis was performed. These results were also described by Toyonaga et al (37), who reported a lack of statistical association in unadjusted serum copper concentrations between *H pylori*–positive and –negative adults residing in Japan. However, after adjusting β coefficients for confounding variables, our results showed that *H pylori*–infected children had a significant increase in copper concentrations compared with noninfected children (Table 3). Although an α statistical error because of confounding factors among others could not be discarded, these findings could also be related to the alteration of copper metabolism in *H pylori*–positive individuals, because it was described for other conditions of inflammation or infectious diseases in animals and humans that led to an increase in serum copper concentrations (13,38,39). If this is so, the evaluation of a marker of raised serum copper concentrations should be useful in considering the biological impact of this condition. Future studies would help to clarify this issue.

The relation between *H pylori* infection and zinc has not been extensively investigated. Our results are in accordance to those reported for children by Akcam et al (40). We have not found significant differences either for serum zinc mean concentrations or for zinc mean intakes between *H pylori*–positive and –negative children, with low zinc intake inadequacies for both groups (Table 1).

To our knowledge, this is the first cross-sectional study performed in symptomatic children seeking the association between *H pylori* infection and iron, zinc, and copper status. This study revealed that *H pylori* infection was not associated with ID, anemia, or zinc concentrations; however, a positive relation was found with copper status after adjusting for confounders. Our results are coincident with those reported in a coordinated series of cross-sectional studies performed in Latin American countries (41), where the lack of association between *H pylori* and anemia argues against the causative role of this bacterium in the development of anemia in Latin America. *H pylori* genotype and bacterial virulence factors are related to the severity of gastric disease (42); however, their role in micronutrient deficiencies has not been established. Because controversial evidence is still being reported, further

studies should evaluate the possible mechanisms by which *H pylori* may affect mineral status. The contribution of *H pylori* infection to higher copper concentrations needs to be confirmed by additional studies.

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