ARTICULO

Helicobacter pylori and iron status in school children from Valencia, Venezuela

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Abstract

Helicobacter pylori (*H.pylori*) infection is one of the most widespread gastrointestinal diseases worldwide, and in some studies it has been associated with iron-deficiency anemia and other nutritional deficiencies. The purpose of this study was to assess the association of *H.pylori* infection with anemia and iron deficiency in school children from a low-income area of Valencia, Venezuela. 418 children between the ages of 4 and 14 years were evaluated. Socioeconomic status, nutritional status (weight and height), *H.pylori* infection (13C-Urea breath test), iron intake (two 24-hour intake reminders in non-consecutive days), prevalence of anemia (hemoglobin) and iron deficiency according to ferritin (IRMA), and transferrin receptors (ELISA) were determined. Results: 47.8% were boys and 52.2% girls, the majority (96.3%) were from low-income families, and 20.9% had nutritional deficiency) according to BMI. Regarding iron status, 17.2% had an inadequate intake (<77% of RDA), 8.1% (CI95% 5.6-10.6%) had anemia, and 46.4% (CI95% 41.6-51.2%) iron deficiency. Anemia and iron deficiency were negatively associated with age. Prevalence of *H.pylori* infection was 77.8% (CI95% 73.8-81.8%), and was positively associated with age. An association between H.pylori infection and iron deficiency was found. Prevalence of iron deficiency was higher in infection-free children (6.8% versus 12.9%; p = 0.03). Adjusted logistic regression analysis detected no statistically significant association between *H.pylori* infection and anemia (OR 0.74; CI95% 0.32-1.70; p = 0.4) or iron deficiency (OR 0.62; CI95% 0.37-1.04; p = 0.07). This study showed no evidence to support the hypothesis that *H.pylori* cause iron deficiency or anemia in children.

Key words: *H. pylori*, anemia, iron, school children, urea breath test

Resumen

Helicobacter pylori y estado de hierro en preescolares y escolares de la ciudad de Valencia, Venezuela.

La infección por *Helicobacter pylori* (*H.pylori) es* una de las patologías gastrointestinales más ampliamente distribuidas en el mundo, algunos estudios han mostrado asociación entre *H.pylori y* la anemia por deficiencia de hierro y otras deficiencias nutricionales. El objetivo del estudio fue evaluar la asociación de la infección por *H.pylori* con la anemia y la deficiencia de hierro en niños escolarizados. Se evaluaron 418 niños (4 y 14 años de edad). Se determinó el estrato socioeconómico, estado nutricional (peso y talla), infección por *H.pylori* (prueba de aire espirado con Urea-^{13}C), consumo de hierro (dos recordatorios de consumo de 24 horas no consecutivos), prevalencia de anemia (hemoglobina) y deficiencia de hierro (ferritina (IRMA) y receptores de transferrina (ELISA)). El 47,8% eran varones y 52,2% niñas; 96,3% provenían de familias pobres; 20,9% presentaron déficit nutricional según IMC. El 17,2% tenían un consumo inadecuado de hierro (<77% del RDA), 8,1% (IC95% 5,6-10,6%) anémicos, y 46,4% (IC95%

41,6-51,2%) deficientes de hierro. La anemia y la deficiencia de hierro se asociaron de manera inversa con la edad. La prevalencia de infección por *H.pylori* fue de 77,8% (IC95% 73,8-81,8%) y se asoció positivamente con la edad. Hubo asociación entre infección por *H.pylori* y deficiencia de hierro, siendo la prevalencia de deficiencia de hierro más alta entre los niños sin infección (6,8% contra 12,9%; p=0,03). El análisis ajustado por regresión logística no detectó asociación significativa de la infección por *H.pylori* con anemia (OR 0,74; IC95% 0,32-1,70; p=0,4) o deficiencia de hierro (OR 0,62; IC95% 0,37-1,04; p=0,07). Se concluye que no hubo evidencia que soporte la hipótesis de que el *H.pylori* cause deficiencia de hierro o anemia en niños.

Palabras clave: *H. pylori*, anemia, hierro, niños, prueba de aire espirado.

INTRODUCTION

Infection with *Helicobacter pylori (H. pylori)*, one of the most widespread gastrointestinal diseases worldwide, has been associated with iron deficiency anemia and other nutritional deficiencies (1). Several studies have shown this association: Barabino et al (2) observed that eradication of *H. pylori* was associated with recovery from iron deficiency anemia in patients not treated with iron salts. Kostaki et al (3) reported the case of three children with chronic active gastritis that presented anemia due to iron deficiency, in whom iron supplementation therapy was effective only after eradication of *H. pylori* infection. Bagget et al (4), in a study done with 688 Alaska Native children, found out that the probability of identifying iron deficiency was higher in children infected with *H. pylori* than in those not infected. A recent meta-analysis that included 15 observational studies on the prevalence of Iron Deficiency Anemia (IDA) in *H pylori*–positive and –negative subjects demonstrated a correlation between infection and IDA. This effect was significant in children and adolescents but not in adults (5).

Iron deficiency is considered one of the major nutritional problems affecting millions of people worldwide. In a significant number of cases, the deficiency is severe enough to cause anemia. The non-hematological implications of iron deficiency are diverse, including effects on gastrointestinal function and structure, immunity and infection, and physical and neurological function. In relation to neurological function, poor school performance, as well as chronic fatigue and other non specific symptoms have been attributed to iron deficiency.

Some studies have established an association between iron deficiency, with or without anemia, and attention deficit disorders that affect children's learning and problem-solving abilities (6)

H. pylori Infection may cause iron deficiency anemia (IDA) through different mechanisms. One of them is the alteration of acid secretion due to inflammation of the gastric mucosa caused by the bacteria, which affects the normal absorption of iron (1). Another possible mechanism would be a competition of the bacteria with the host for the iron coming from the diet. In case of a severe *H. pylori* infection, its affinity for iron fixation and uptake could increase the host's demands for iron (7). In addition, the consequences of an infection of the gastric mucosa from *H. pylori* will depend on the extent of the damage. If the resulting inflammation extends to the oxyntic mucosa, acid secretion of the mucous membrane will decrease due to inhibition of parietal cells, with the ensuing occurrence of gastric atrophy and pH increase (8). In this sense, the IDA associated with *H. pylori* is a consequence of pH increase in the gastric environment to values above 3, and a decrease of intragastric concentrations of ascorbic acid, which is the greatest promoter of non- haem iron absorption (9-10).

The purpose of this study was to investigate the association between *H. pylori* infection and the presence of anemia and iron deficiency in schoolchildren from a low-income area in Valencia, Venezuela.

MATERIALS AND METHODS

This was an observational, cross-sectional study of school children aged between 4 and 14 with no apparent disease. The children were randomly selected from two schools located in the Miguel Peña Municipality, a low-income area in the city of Valencia, Carabobo State, located in Venezuela's north-central region. 10% of anemia prevalence, 50% of *H. pylori* colonization, and 95% of confidence level were considered for the determination of the sample size. The selected sample consisted of 477 children. In each school the number of children selected was proportional to the number of students enrolled. Assessment was done on 418 children who met the inclusion criteria and whose parents gave a written consent after being informed of what the study was about, the benefits and potential risks, as well as all steps for the assessment. Inclusion criteria were: not having ingested antibiotics, anti-acids or proton pump inhibitors medications during three months prior to the assessment. For data collection, a medical history was designed in which the identification of the child, its socioeconomic status, and anthropometric data were registered. Food intake of children was also registered through interviews with the mother.

Socio-economic and anthropometric assessment: The Graffar Méndez-Castellano methodology was used to determine the socioeconomic status. This methodology classifies families in five strata: upper class (stratum I), upper middle class (stratum II), middle class (stratum III), relative poverty (stratum IV) and critical poverty (stratum V) (11). Weight and height values were registered according to the International Biological Program for anthropometric evaluation, using a Health-o-meter scale with height rod, which was previously calibrated (12). Nutritional diagnosis was made using Height/Age (H/A) and Body Mass Index (BMI) indicators.

The Z-score value was used for the processing and interpretation of anthropometric indicators, and it was compared with the growth reference group of the National Center for Health Statistics (13). The Z-score calculations were performed using the EPI Info program, version 3.2.2, with the following cutoff points: moderate or severe deficit (<-2 Z-score), mild deficit (between -2 and – 1 Z-score), normal (between - 1 and +1 Z-score), and above normal values (≥+1 Z-score).

***H. pylori* Detection:** The ^{13}C labeled urea breath test was used for the assessment of *H. pylori* infection. This technique is based on the ability of the *H. pylori* to produce the urease enzyme and to degrade the urea. A basal sample of exhaled breath under fasting conditions was taken. Later the individual was provided with a dose of 50 mg of ^{13}C-labeled urea with an acid drink (to inhibit gastric emptiness). Samples of breath 30 and 45 minutes after the administration of labeled urea were taken to measure the enrichment of exhaled air with $^{13}CO_2$. The samples were sent for analysis to the Stable Isotope Laboratory at the Pharmacy and Biochemistry Faculty of the Buenos Aires University. Each sample of exhaled air was measured in a Gas Chromatograph-Mass Spectrometer (Finnigan BreathMAT GMBH The Corp., Bremen, Germany). An increase 3.5 % over the baseline (Delta Over Base, DOB) was considered as a positive result (14-15).

Food Iron Intake: The 24 hour recall method, developed especially for iron and zinc by

Gibson and Ferguson in 1999 was used to assess food iron intake. This tool was applied in two non-consecutive days of a week, asking the mother or care-taker about the food consumed by the child. This method allows to obtain the usual intake of iron at an individual level and to estimate the amount of ingested inhibitors and facilitators that influence mineral absorption (16). Once the food intake was registered, the program Food Processor for Windows Version 8.7.0 (17) was used to calculate the average daily consumption of iron of each child, a value which was adjusted by taking into account the quality of the diet, according to the phytate/iron ratio. Finally, adequate consumption percentage of this nutrient was estimated by comparison of obtained intake values to requirements for each age group.

Anemia and Iron Deficiency: For hematological and biochemical determinations, a blood sample of 4 ml was taken under fasting conditions, which was placed in two test tubes, one with EDTA as an anticoagulant for the hematology, and another one without an anticoagulant to obtain serum for biochemistry tests.

Hemoglobin concentration was determined by an automated method, using the Beckman Coulter hematology analyzer, model Ac.T 5diff. The diagnosis of anemia was made according to the cutoff points established by the World Health Organization (W.H.O.) for each gender and age group (18).

The biochemical assessment consisted of the determination of serum levels of ferritin, C-reactive protein (CRP) and soluble transferrin receptors (sTfR). Serum ferritin levels were determined using the enzyme immunoassay method (ELISA) with a DRG International commercial kit, and readings done in an ELISA Labsystems Reader. CRP was measured by liquid-phase immunoprecipitation assay with nephelometric detection using a commercial kit in a Turbox analyzer from Orion Diagnostica (19). The sTfR were determined by immunoenzymatic method (Quantikine IVD from R & D System).

Due to limited information regarding normal levels of sTfR, the reference value taken was the one recommended by the manufacturer. In that sense, sTfR values above 2.3 mg / L were defined as iron-deficient erythropoiesis.

An algorithm was constructed for the diagnosis of iron deficiency (Figure 1), using the determinations of serum ferritin, sTfR, and CRP and leukocyte count, according to cutoff points recommended by the literature (18).

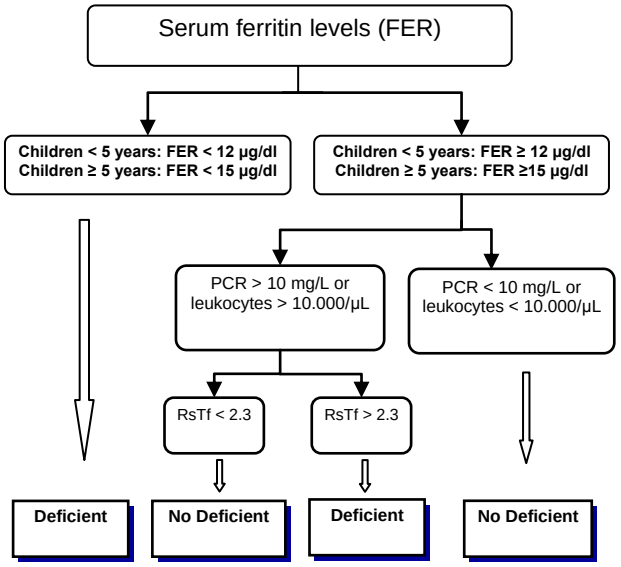

Figure 1. Algorithm for the diagnosis of iron deficiency

Statistical Analysis

The statistical program SPSS for Windows, version 13.0, was used for data analysis. Prevalences and confidence intervals of 95% (CI95%) were estimated for iron deficiency, iron deficiency anemia and *H. pylori* infection. The χ^2 test was used to measure the association between *H. pylori* and haematological and biochemical results. A logistic regression

analysis was performed to determine the effect of *H. pylori* infection on the occurrence of iron deficiency and anemia. The adjusted variables associated with *H. pylori*, and with each hematological and biochemical outcome, with a level of significance $p \leq 0.20$, were included in the regression model. Subsequently, these variables were excluded one by one until achieving a final model whose variables had a statistical level of significance $p < 0.05$.

RESULTS

To study the association between iron status and *H. pylori* colonization, a total of 477 children were initially selected, but 418 remained in the sample, from whom a written consent of parents was obtained, and who complied with all the assessments. In terms of the sociodemographic characteristics of the sample, a similar representation of both genders was found (47.8% male and 52.2% female), with an average age of 8.9±2.3 years and a greater representation of children aged between 7 and 14 (81.5%) than children under the age of 7. 96.3% of the assessed families were in poverty (77.7% in relative poverty and 18.6% in poverty).

Table 1 describes the sample and prevalence of anemia and iron deficiency according to independent variables. There was no difference in the prevalence of anemia or iron deficiency in relation to gender. The prevalence of anemia in boys was 9% and of iron deficiency 43.7%. For girls, the corresponding values were 7.3% and 49.5%.

There was a strong association between children's age and prevalence of both anemia and iron deficiency. The association of anemia with age was linear, being four times more prevalent in children under the age of 7 than in older children ($p = 0.003$). Iron deficiency was also more prevalent among children below 7 years (65.3%) compared to other age groups ($p = 0.002$). There was no association between social stratum and anemia or iron deficiency.

In terms of nutritional status, it was found that 20.9% of children were malnourished according to their BMI and 9.2% had a deficit or were at risk of presenting a deficit in height for their age according to Z-score. No association was established between nutritional status according to BMI or height/age indicator, and anemia or iron deficiency. However, the prevalence of iron deficiency was higher among children with or at risk of a height/age deficiency than among those within a normal range (59.5% and 45.2% respectively) ($p = 0.09$).

Table 1. Sample characteristics and prevalence of anemia and iron deficiency according to independent variables. Valencia, Venezuela, 2006. (n = 418)

Variable	Categories	n (%)	Prevalence of anemia (%)	p	Prevalence of iron deficiency	P
Gender	Boys	200 (47.8)	9.0	0.5	43.7	**0.2**
	Girls	218 (52.2)	7.3		49.5	
Age (years)	≤ 6	77 (18.4)	19.5	0.003*	65.3	**0.002**
	7-8	110 (26.3)	5.5		38.2	
	9-10	114 (27.2)	6.1		41.2	
	> 10	117 (28.0)	5.1		48.3	

Cont… Table 1.

Variable	Categories	n (%)	Prevalence of anemia (%)	p	Prevalence of iron deficiency	P
Socieconomic Status	Middle Class	11 (3.4)	9.1	0.9	60.0	0.3
	Relative poverty	255 (77.7)	9.8		46.9	
	Critical poverty	61 (18.6)	6.6		39.3	
BMI (deficit)	Yes	6 (20.9)	7.0	0.6	40.0	0.1
	No	325 (79.1)	8.6		48.0	
Height /Age (Low or at Risk)	Yes	38 (9.2)	2.6	0.1	59.5	0.09
	No	374 (90.8)	8.8		45.2	
Iron Intake	Low	71 (17.2)	4.2	0.2	49.3	0.6
	Normal	342 (82.8)	9.1		45.9	
H. pylori	Infected	325 (77.8)	6.8	0.06	44.0	0.03
	No infected	93 (22.2)	12.9		56.5	

*Linear tendency test

The average intake of iron was 14.6 ± 5.3 mg/day and it was above the requirements for this age group. However, when analyzing the diet, a characteristic intake pattern of a diet with an intermediate availability of iron was observed, with a mineral supply primarily from foods of vegetable origin, high in fiber, and other inhibitors of absorption (16). When compared with requirements, it was found that 17.2% of the group had an inadequate iron intake (less than 77% of daily requirements). Iron intake was not associated with the presence of anemia or iron deficiency.

H. pylori Infection in the sample was 77.8% (CI 95% 73.8 - 81.8%) (Table1). In this study, *H.pylori* infection was linearly associated with age and iron intake with prevalence ranging from a 25% at age 4, to 97% at age 14. 45.5% of children under the age of 7 were infected, whereas among the 7-8, 9-10 and >10 years old groups, the frequencies were 74.5%, 87.7% and 9.3% (p<0.001), respectively. Among those with adequate iron, the prevalence of *H.pylori* infection was 80.1%, while in children with inadequate intake was 66.2% (p= 0.01).

Anemia prevalence among children infected with H. *pylori* was 6.8% and among those non-infected, 12.9% (p=0.06). Iron deficiency was less common among infected children (44%) than among non-infected ones (56.5%), this difference being statistically significant (p = 0.03).

Average concentration of hemoglobin was 12.7 ± 0.89 g/dL with a prevalence of anemia of 8.1% (CI 95% 5.6 - 10.6%). Serum ferritin levels were 29.7 ± 25.6μg / dL, with 28.7% of children with serum levels below the cutoff points recommended by W.H.O. for their age

group. However, elevated levels were found when assessing serum concentrations of CRP (>10 mg/L), suggesting the presence of infectious or inflammatory processes in 30.5% of children. Therefore, for diagnosing iron deficiency it was necessary to incorporate additional criteria to serum ferritin levels. The prevalence of iron deficiency was recalculated based on the algorithm of Figure 1, developed by the authors in accordance to the cutoff points recommended by W.H.O. in 2001, showing it to be of 46.4% (CI 95% 41.6 – 51.2%).

The independent variables associated with *H. pylori* infection, as well as the haematological and biochemical results, were included in the multivariate model with a value of $p \leq 0.20$. The logistic regression model to assess the effect of *H. pylori* infection on the occurrence of anemia included potential confounding variables such as age, iron intake, and the nutritional indicator height-for-age. The adjusted analysis showed that the infection was not associated with anemia. The value of the adjusted odds ratio indicated that infected children had 26% less chances to have anemia in comparison with non infected ones. This result was not statistically significant because the confidence interval of 95% included the unit, and p is higher than 0.05.

Table 2. Net and adjusted analysis for anemia (2a) and iron deficiency (2b) between children infected and non infected with *H. pylori.* Valencia, Venezuela, 2006

Table 2a: Net and adjusted analysis for anemia	**OR (CI 95%)**	**p**
Model 1: *H. pylori* positive	0.49 (0.23-1.03)	**0.06**
Model 2: Model 1 + age, iron intake and height/age indicator	0.74 (0.32-1.70)	**0.4**

Table 2b: Net and adjusted analysis for iron deficiency	**OR (CI 95%)**	**p**
Model 1: *H. pylori* positive	0.60 (0.38-0.96)	**0.03**
Model 2: Model 1 + age, BMI and height/age	0.62 (0.37-1.04)	**0.07**

The effect of *H. pylori* infection on iron deficiency had borderline statistical significance with an odd ratio of 0.60 (CI 95% 0.38 - 0.96, p = 0.03), showing that those infected with *H. pylori* had a lower probability of having iron deficiency in comparison with the non infected ones. The multivariate model included age, BMI and the nutritional indicator height-for-age. With the adjustment, the oddsratio changed to 0.62 (95 % CI 0.37 - 1.04; p=0.07), thus disappearing the statistical significance.

DISCUSSION

From the total of selected children (477), 12% did not complete the study because of no attendance to the appointment, leaving the sample with 418 children. This loss can be explained by the inconvenience that children had to attend two medical appointments. On the first appointment, anthropometric assessments and socioeconomic and intake surveys were made, and blood samples taken. On the second one, the 13C-Urea Breath Test to diagnose *H. pylori* was carried out.

The results of the exhaled breath test showed that *H. pylori* infection is present at a very early age (25% at 4 years of age) and reaches a high level (around 90%) at the age of 10. This pattern of infection is similar to that observed in other developing countries, while in developed countries; infection occurs later with much lower prevalence rates in adulthood, close to 30% (20). The infection rate found in this study was higher than the one reported by Cavazza et al (21), in a seroprevalence study conducted on 1,041 Venezuelan children and adults, symptomatic and asymptomatic, from different states. The authors found a positive seroprevalence in children ranging between 30% and 60%, and in adults between 68% and 93%, depending on the geographic area studied. The differences that were found could be explained partly by the high rate of poverty that was observed in the present study, since over 95% of the families were living in poverty, and it has been reported that poor sanitary conditions are a factor associated with the infection (22-23).

Anemia was present in 8.1% of cases, and this prevalence is very similar to that reported in previous studies in Venezuela. In the 1992 a national survey, reported 13% of anemia (24). In year 2005, a study of 2,013 children and adolescents from the metropolitan area of Caracas, reported a prevalence for preschoolers, school children and female adolescents of 9.8%, 9.4% and 8.9%, respectively (25).

Regarding iron status, while only 17% of the studied group had an inadequate iron intake, 46.4% had low body iron deposits. The difference observed between iron intake and body condition suggestss that there are other factors different from iron intake that are affecting absorption and bioavailability of iron provided by the diet. The literature reports that although the most important reason for iron deficiency in humans is a poor nutritional intake, there are other factors related to diet, body status of this mineral, and physiological and health status of the individual, that can reduce the bioavailability of iron provided by food (26). On the other hand, it must be taken into consideration that the assessment of iron intake estimates the contribution of iron to the body the week prior to evaluation, while the body iron status is the result of a long term situation.

The dietary assessment showed a protein intake derived mainly from cereals and legumes, a high content of fiber, and iron provided primarily by foods of vegetable origin (non- haem). The absorption of non- haem iron is highly dependent on gastric acidity and the presence of other facilitating factors such as ascorbic acid intake.

Intestinal parasitosis is another variable associated with reduced bioavailability of iron in the diet. Parasites such as hookworms cause rectal bleeding and, therefore, loss of iron in feces. An analysis of feces in a sub-sample of 181 children was performed, finding that 54% of them were infected (data not shown), but none of the samples had hookworms or other hematophagous parasites, which have been associated with an increased iron deficiency (27).

In this study, despite the high rate of *H. pylori* infection, there was no evidence that the infection was associated with anemia or iron deficiency. On the contrary, the verified net association between infection and iron deficiency was protective.

Most studies where *H. pylori* infection has been associated with anemia or iron deficiency are studies of clinical cases in which the infection is accompanied by clinical manifestations such as

chronic gastritis or severe gastritis, or there is some evidence of refractory anemia (2, 3, 28).

A recent study by Chen and Luo (29) of 86 patients with chronic gastritis showed that treatment for *H. pylori* infection increased the effectiveness of therapy with ferrous succinate to treat iron deficiency anemia. However, the results of epidemiological studies have not always shown an association between anemia and infection.

Data from a regional study with the support of the International Atomic Energy Agency, which investigated the association between H. *pylori* infection and anemia in six Latin American countries (Argentina, 307 children, Bolivia, 424 children, Brazil, 1007 adults, Cuba, 996 school children, and Mexico, 71 pregnant women), did not show such association (30).

One possible explanation for the conflicting results from different studies is the fact that the decrease of acidity due to infection, which could explain a lower absorption of iron and an increased deficiency of this mineral, will depend on the intensity and location of the inflammation produced by the bacteria.

When it is severe and extends towards the antral region of the stomach, the degree of achlorhydria is much higher; ascorbic acid secretion is also affected and, consequently, the absorption of iron decreases (8-9). In this study, as in others, endoscopy was not performed to assess the intensity or location of bacteria, which can constitute a limitation for the interpretation and comparison of results with those from other authors.

Another factor that could explain the lack of association observed in this study on the adjusted analysis between infection and iron deficiency is the size of the sample. Due to the unknown prevalence of infection in this population, we assumed a prevalence of 50% for sample estimation. However, we found an infection rate much higher, reaching 70% and increasing up to a 100% in 14 year olds. If sample size estimation had been based on actual prevalence, 1,400 children would have been required to determine the existence of association or lack of it between the variables at a 95% confidence level.

It is surprising that, even though the direction of the effect of infection on the risk of iron deficiency was not statistically significant, the effect observed was protective. Compared with uninfected children, those infected with *H. pylori* had an odds ratio below one. It can be concluded that in this research it was not possible to demonstrate that the *H. pylori* infection constitutes a risk factor for anemia or iron deficiency, even though the prevalence of this infection was high. Age, iron intake and nutritional status were the variables that best explained the pattern of anemia, whereas for iron deficiency it was only age and nutritional status.

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