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The effect of *Helicobacter pylori* infection on growth velocity in young children from poor urban communities in Ecuador

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Summary

Objective—To characterize the potential effects of *Helicobacter* infections on growth velocity in low socioeconomic status young children in a developing country.

Methods—Children were recruited in poor suburbs of Quito, Ecuador. Normally nourished, mildly and substantially malnourished children (defined using weight-for-age *z*-scores at recruitment) formed equal strata. Six height and weight measurements were collected during one year. Enrollment and exit serum samples were analyzed for anti-*Helicobacter* IgG and exit non-diarrheal feces tested for *Helicobacter* antigen.

Results—Among 124 participants (enrollment age 19±9 months), 76 (61%) excreted fecal antigen at exit (were infected). Of them, 44 were seropositive at least once (chronic infections) and 32 tested seronegative both times (new or acute phase infections). Adjusted linear growth velocity during follow-up in children with new infections was reduced by 9.7 (3.8, 15.6) mm/year compared to uninfected controls and 6.4 (0.0, 12.9) mm/year compared to children with chronic infections. The effects of *Helicobacter* infections on ponderal growth were not significant.

Conclusion—These results suggest that linear growth velocity is reduced in young children during the initial phase of *Helicobacter* infection.

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Ethical approval: All aspects of this study were approved by the ethical committees of Tufts University School of Medicine in Boston, MA and the Corporación Ecuatoriana de Biotecnología in Quito, Ecuador.

Keywords

Helicobacter pylori ; growth velocity; children; fecal antigen; IgG antibody; Ecuador

Introduction

Helicobacter pylori in an important pathogen that can cause gastritis, gastric and duodenal ulcers, and gastric cancer.1 This bacterium is uniquely adapted to chronically infect the human stomach. It elicits a predominantly Th1-type immune response, which may be responsible for gastritis symptoms.2,3 Infections typically spread through person-to-person contacts2 [Suerbaum and Michetti, 2002] but waterborne transmission may also occur.4 In developing countries, most people acquire infection in early childhood.2,5 The acquisition of infection is often followed by transient acute gastritis symptoms and depressed gastric acid secretion.2,6,7 *Helicobacter* has been shown to impair absorption of nutrients and vitamins,8,9 and retard growth in children in developing countries.6,10-13

Helicobacter-infected individuals excrete *Helicobacter* antigen. Fecal antigen tests have excellent sensitivity and specificity as compared to more invasive diagnostic tests in children.14-18 *Helicobacter* infection also induces strong antibody responses.19 Animal experiments have demonstrated that the development of antibody response reduces gastritis symptoms.20 In humans, IgG seroconversion occurs several months post-infection.21,22 This time lag between infection and IgG seroconversion has been utilized to define acute (prior to seroconversion) and chronic (after seroconversion) phases of infection.23-25 Antibodies also persist following spontaneous eradication of *Helicobacter*, which sometimes occurs in children.26 The objective of this study was to evaluate the effects of both chronic and acute *H. pylori* infections on linear and ponderal growth in young Ecuadorian children.

Methods

This prospective study was nested within a larger study of the effects of micronutrient (vitamin A and/or zinc) supplementation in young children (manuscript in review). The parent study was registered at www.ClinicalTrials.gov (NCT 00228254). For that parent study, 6 to 36 month old children were recruited in poor suburbs of Quito, Ecuador at community recruitment sessions in April--May 2002 and 2003. Weight-for-Age and Height-for-Age standard deviation scores or *Z*-scores (WAZ and HAZ) were estimated using the US National Center for Health Statistics reference data. Malnourished children were intentionally over-sampled: substantially malnourished (WAZ < −2), mildly malnourished (−2 < WAZ < −1), and normally nourished children (WAZ > −1) formed one-third each of the study population. Severely malnourished children (weight < 60% of the age-adjusted norm) were excluded from the study and referred to nutritional rehabilitation centers. After recruitment, children were randomly assigned into intervention or placebo groups, and followed for approximately one year starting from June. This manuscript presents data collected from the placebo group.

A household survey was conducted at enrollment to assess demographic, socioeconomic and hygienic characteristics. Study nurses visited all children during follow-up four times

per week to look for respiratory and diarrheal symptoms. Growth parameters of each child were measured at enrollment and approximately every ten weeks thereafter for a total of six measurements per child. Blood samples were collected from each child after overnight fasting at enrollment and exit to measure micronutrient levels.

All aspects of this nested study were approved by the ethical committees of Tufts University School of Medicine in Boston and the Corporación Ecuatoriana de Biotecnología in Quito. Separate informed consents were obtained from parents or guardians of each participating child for the parent study and nested study.

Leftover serum samples were analyzed for anti-*Helicobacter* IgG responses using commercial ELISA test kits procured from IBL Immuno-Biological Laboratories (Hamburg, Germany). These kits included a microplate coated with a mixture of purified bacterial proteins containing CagA and VacA antigens, secondary horseradish peroxidase-conjugated anti-human antibody solution, and a set of standards. The optical density values for anti-*Helicobacter* IgG responses were converted into arbitrary unit concentrations (U/mL) and dichotomized using microplate-specific cut-off values. At the end of the follow-up (study exit), the participants of this nested study also provided one non-diarrheal fecal sample, which was analyzed for excreted *Helicobacter* antigen using sandwich monoclonal antibody-based ELISA assay (Hp StAR™, DAKO A/S, Glostrup, Denmark). The *Helicobacter* fecal test results were dichotomized at the optical density value of 0.15 per manufacturer's instruction. All laboratory tests were conducted at the Catholic University of Ecuador (Pontificia Universidad Católica del Ecuador) in Quito. Samples were collected from households by study nurses, delivered to the laboratory in refrigerated containers, and stored at −20° C until analysis.

Children were classified as infected or non-infected based on the results of their fecal antigen test. Infected children there further classified based on their serology as having (i) chronic infections (tested seropositive at enrollment and/or exit); or (ii) having new or acute phase infections (tested seronegative both times).

The data were analyzed using SAS v. 9.2 (SAS Institute, Cary, NC). Demographic or socioeconomic risk factors for *Helicobacter* infection were analyzed using logistic regression. Data on growth during follow-up were analyzed using mixed effect regression models using SAS procedure GLIMMIX.

Results

Only children from the placebo group who submitted their non-diarrheal fecal samples at the study exit were included in this analysis (N = 124). Of these, 26 children represented the 2002-2003 cohort of the parent study and 98 children represented the 2003-2004 cohort (20% and 71% respectively of the placebo arm of each cohort). The breakdown of the study population by gender, nutritional status strata and *Helicobacter* infection status is presented in Table 1.

The age at enrollment ranged from 4.6 to 35.7 months, average 19.0 months. The average duration of follow-up (interval between the first and the last anthropometric measurements)

was 388 days, standard deviation 26 days, range from 277 to 413 days. The average age at study exit was 31.8 months. Normally nourished children were, on average, younger than substantially malnourished children while children who were not infected and children who had acute phase infections were, on average, younger than children with chronic phase infections (Table 1). Seventy six (61%) study participants were infected with *H. pylori* (excreted antigen) at study exit (acute and chronic infections combined). A detailed breakdown of the study population by infection status and serology is presented in Table 2. Among 44 children who were classified as chronically infected, 41 (93%) were seropositive at exit. Among 48 children who were not infected with *Helicobacter*, only 4 (8%) were seropositive at exit.

Prevalence rates of *Helicobacter* infection at study exit were 73% in substantially malnourished, 47% in mildly malnourished and 67% in normally nourished children. Although odds of infection in mildly malnourished children were significantly smaller than in normally nourished controls ($p = 0.03$), the inconsistent pattern with greater odds of infection in substantially malnourished children suggests that this observed association was due a random effect. Crowded living conditions (three or more people per room) was the only socioeconomic indicator associated with *Helicobacter* infection (positive fecal test) in logistic regression analysis: odds ratio 2.67 (1.04, 6.91), $p = 0.04$, adjusting for nutritional status strata and age.

Preliminary mixed effect regression analysis at the model development stage demonstrated that adjusted growth velocities during follow-up were similar in all three nutritional status strata. Therefore, interaction terms between nutritional status and time were not included in the final model. Episodes of diarrhea or respiratory illness during follow-up as well as crowded living conditions were not significantly associated with growth velocity. Therefore, these variables were also not included in the final model. Height at enrollment and growth velocity in children who tested seropositive at least once but had negative fecal antigen test at exit (eradicated *Helicobacter* infection) did not differ significantly or substantially from those in children who had all fecal and serological tests negative ($p > 0.5$ for both tests). This confirmed that including all fecal test-negative children in the control group irrespective of their serological status was appropriate.

The final mixed effect model for linear growth included random effects for intercept, age and age squared. Main effects are presented in Table 3. The non-linear effect of age on linear growth was modeled using a cubic polynomial. Indicator variables for gender and nutritional status strata were used to model the effects of these parameters on height at enrollment. Girls were significantly shorter and lighter than boys; substantially malnourished and mildly malnourished children were significantly shorter and lighter than normally nourished children.

The predicted average linear growth velocity in children who were 19 month-old at enrollment (average age of the participants) and remained free of *Helicobacter* infections (all tests negative) was 96 mm/year. To model the effects of *Helicobacter* infections on growth velocity, the regression model contained two interaction terms between indicator variables for new and chronic *Helicobacter* infections and continuous variable for time from

enrollment. Age-adjusted growth velocity during follow-up was reduced in children with new *Helicobacter* infections by 9.7 (3.8, 15.6) mm/year compared to controls while the effect of chronic infections was weak and non-significant. Further analysis (not shown in Table 3) demonstrated that age-adjusted growth velocity in infected seronegative children (new infections) was reduced by 6.4 (0.0, 12.9) mm/year ($p = 0.05$) compared to infected seropositive children (chronic infections). In stratified regression analysis, the adjusted detrimental effects of new *Helicobacter* infections on growth velocity were very similar in boys and girls, 10.5 (−0.9, 21.9) mm/year and 9.0 (−2.2, 20.1) mm/year respectively.

The effects of *Helicobacter* on ponderal growth were analyzed using similar mixed effect regression models (except the effect of age was modeled using a quadratic polynomial). The adjusted detrimental effects of new and chronic *Helicobacter* infections on ponderal growth velocity were small and not significant, 53 (−147, 253) g/year ($p = 0.6$) and 10 (−173, 193) g/year ($p = 0.9$) respectively.

Discussion

The main finding of this study are that new Helicobacter infections defined as positive fecal antigen test and negative serology were associated with reduced linear growth in young children. The estimated deficit in the average growth velocity during one year-long follow-up in children with new infections compared to non-infected controls was 10 mm/year which translates into approximately 10% slower linear growth. There was no evidence of catch-up growth in children with chronic *Helicobacter* infection. These results show that *Helicobacter* infections contributed substantially to the pronounced growth deficit in young Ecuadorian children from poor urban neighborhoods.

The finding of this study is in agreement with previous research in Colombia that also demonstrated that the detrimental effect of *Helicobacter* infection on growth was most pronounced right after the infection.13 It could be speculated that chronic infection is associated with a different pattern of gastric inflammation which does not cause a detrimental effect on growth in children. IgG seroconversion, which occurs several months post-infection in children, can serve as a biomarker of the transition to the chronic phase of infection. Akhiani et al.20 demonstrated that the development of anti-*Helicobacter* antibody response is associated with a reduced gastric inflammation but enhanced colonization in animal models and speculated that anti-*Helicobacter* antibody may facilitate the persistence of this pathogen.

The detrimental effect of new *Helicobacter* infection on linear growth velocity in these Ecuadorian children was almost twice greater than the 5 mm/year effect previously observed in Colombian children.11 This discrepancy may be explained by different source populations and, at least in part, a random sampling effect. The effect of *Helicobacter* on ponderal growth in these Ecuadorian children was small and non-significant, which is consistent with the results of prior research in Colombia.11,13

This study used a monoclonal fecal antigen ELISA test to detect *Helicobacter* infections and serological IgG ELISA tests to further classify infections as new (negative serology)

or chronic (positive serology) using a previously developed infection classification scheme.22-24 It has been demonstrated previously that the HP StAR™ monoclonal antibody ELISA test for fecal *Helicobacter* antigen can be used as a reliable indicator of *Helicobacter* infection in children because it compares very well with invasive diagnostic tests as well as the ^{13}C-urea breath test with sensitivity ranging from 94.4% to 98.2% and specificity from 94.7% to 98.1% depending on the study.14,15,17

The 61% prevalence rate of *Helicobacter* infections in these Ecuadorian children as determined by the HP StAR™ fecal antigen test is similar to the 53% prevalence rate in two-year-old Colombian children as determined by the ^{13}C-urea breath test5 but higher than the 23% prevalence rate reported in another group of Colombian children who were, on average, approximately four years of age.11

A limitation of this study was the lack of fecal *Helicobacter* antigen testing at enrollment. This did not allow a more accurate assessment of the timing of *Helicobacter* infection and duration of its effects on growth. Moreover, severe episodes of diarrhea in study participants were also treated with antibiotics altering the natural history of the episode. Therefore, the lack of association between episodes of diarrhea and growth may not be generalizable to the source population of low socioeconomic status Ecuadorian children. Strengths of this study include the collection of detailed growth data and the use of a socio-economically homogenous source population. The results of this study provide further evidence of detrimental developmental effects of *Helicobacter* in young children living in poor economic conditions.

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Table 1

Descriptive statistics of the study population

Factor	Category	Number (percent) of participants	Mean age at enrollment (STD), months	Mean height at enrollment (STD),mm	Mean height-for-age Z-score at enrollment (STD)	Mean linear growth velocity (STD), mm/year	Mean ponderal growth velocity (STD), kg/year
All participants	na	124 (100)	19.0 (8.5)	758 (82)	−1.77 (1.04)	97 (22)	2.17 (0.88)
Gender	Boys	73 (58.9)	19.3 (8.4)	766 (78)	−1.82 (1.13)	94 (22)	2.13 (1.09)
	Girls	51 (41.1)	18.7 (8.6)	747 (86)	−1.71 (0.90)	99 (27)	2.18 (0.53)
Nutritional status	Substantially malnourished	37 (29.8)	20.3 (8.1)	750 (68)	−2.44 (0.69)	92 (21)	2.15 (0.70)
	Mildly malnourished	45 (36.3)	19.9 (7.6)	767 (70)	−1.87 (0.84)	92 (21)	2.19 (1.23)
	Normally nourished	42 (33.9)	16.9 (9.4)	756 (103)	−1.08 (1.06)	103 (28)	2.10 (0.59)
H. pylori infection	New infection	32 (25.8)	18.3 (8.2)	753 (84)	−1.87 (1.19)	92 (24)	2.08 (0.53)
	Chronic infection	44 (35.5)	20.6 (8.8)	766 (76)	−1.90 (1.00)	94 (25)	2.09 (0.49)
	Not infected	48 (38.7)	18.1 (8.3)	755 (86)	−1.59 (0.95)	100 (24)	2.25 (1.30)

Table 2

Detailed breakdown of the study population by infection status and serology

Infection status category	Fecal antigen test	Serum test at enrollment	Serum test at exit	N	Percent of children in infection status category
Acute phase	Positive	Negative	Negative	32	100%
Chronic phase	Positive	Positive	Negative	3	7%
	Positive	Negative	Positive	19	43%
	Positive	Positive	Positive	22	50%
	Subtotal for category			44	100%
Not infected	Negative	Negative	Negative	36	75%
	Negative	Positive	Negative	8	17%
	Negative	Negative	Positive	3	6%
	Negative	Positive	Positive	1	2%
	Subtotal for category			48	100%

Table 3

Results of linear mixed effect regression analysis of the effects of *H. pylori* infections on linear growth in Ecuadorian children

Variable	Category	Effect estimate (95% CI)	*P*-value
Age (years)		187.0 (168.0, 206.0)	<0.0001
Age^2		−32.2 (−41.5, −22.9)	<0.0001
Age^3		3.2 (1.8, 4.7)	<0.0001
Gender	Male	13.4 (4.4, 22.3)	0.004
	Female	0	na
Nutritional status strata	Substantially malnourished	−44.1 (−55.2, −33.0)	<0.0001
	Mildly malnourished	−27.4 (−38.0, −16.9)	<0.0001
	Normally malnourished	0	na
H. pylori infection * time from enrollment	New infection	−9.7 (−15.6, −3.8)	0.001
	Chronic infection	−3.3 (−8.7, 2.2)	0.2
	Not infected	0	na