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Helicobacter pylori, clinical, laboratory, and noninvasive biomarkers suggestive of gastric damage in healthy school-aged children: A case-control study

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ABSTRACT

Background: Helicobacter pylori is acquired largely in early childhood, but its association with symptoms and indirect biomarkers of gastric damage in apparently healthy children remains controversial. We aimed to relate persistent *H. pylori* infection in apparently healthy school-aged children with clinical, laboratory, and noninvasive biomarkers suggestive of gastric damage using a case-control design.
Materials and methods: We followed up 83 children aged 4–5 years with persistent *H. pylori* infection determined by stool antigen detection and/or a urea breath test and 80 noninfected matched controls from a low-income to middle-income, periurban city in Chile for at least 3 years. Monitoring included clinical visits every 4 months and annual assessment by a pediatric gastroenterologist. A blood sample was obtained to determine laboratory parameters potentially associated with gastric damage (hemogram and serum iron and ferritin levels), biomarkers of inflammation (cytokines, pepsinogens I and II, and tissue inhibitor metalloproteinase 1), and expression of cancer-related genes *KLK1*, *BTG3*, and *SLC5A8*.
Results: Persistently infected children had higher frequency of epigastric pain on physical examination (40% versus 16%; $P = 0.001$), especially from 8 to 10 years of age. No differences in anthropometric measurements or iron-deficiency parameters were found. Persistent infection was associated with higher levels of pepsinogen II (median 12.7 ng/mL versus 9.0 ng/mL; $P < 0.001$); no difference was observed in other biomarkers or gene expression profiles.
Conclusions: H. pylori infection in apparently asymptomatic school-aged children is associated with an increase in clinical symptoms and in the level of one significant biomarker, pepsinogen II, suggesting early gastric involvement.

Introduction

Helicobacter pylori is the main cause of peptic ulcer and gastric cancer in adults (Peterson, 1991; Uemura et al., 2001). It is acquired mostly during childhood, but a few studies have prospectively monitored healthy children with persistent infection (Granstrom et al., 1997; Thomas et al., 1999; Passaro et al., 2001; Perez-Perez et al., 2003; Mera et al., 2006; Rupnow et al., 2009; Cervantes et al., 2010; Amberbir et al., 2011; O'Ryan et al., 2013; Akamatsu et al., 2015). An important number of these studies are based on seroprevalence (Kumagai et al., 1998; Malaty et al., 1999, 2000, 2002, 2003; Nakayama et al., 2006), which is not sufficiently reliable for clinical diagnosis in children, as its sensitivity and specificity in children differ widely, and individuals may remain

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positive for specific IgG for years after the infection has resolved (Koletzko et al., 2011; Jones et al., 2017).

We previously reported results from two healthy child cohorts followed up until 3–6 years of age in Colina, a low-income to middle-income, periurban, semirural city in Chile, a country with a mid-level prevalence and high mortality associated with gastric cancer. Both cohorts showed that most infections are acquired during the first 3 years of life, and nearly two-thirds of infected children become persistently infected, according to stool antigen tests, representing 21% of the child population (O'Ryan et al., 2013). Persistent infection was associated with higher serum IgG immune responses against *H. pylori* and differences in gene expression profiles, including some genes potentially associated with cancer development. Infection was also more common in nonsecretor individuals (i.e., those children who do not express H, A, B, or Lewis b antigens in saliva with a *FUT2* genotype concordant with nonsecretor status) (Ueno et al., 2004; O'Ryan et al., 2015; Orellana-Manzano et al., 2016). Importantly, *H. pylori* persistence was not associated with symptoms suggestive of gastric damage in children younger than 5 years (O'Ryan et al., 2015).

In other studies, *H. pylori* infection has been associated with iron-deficiency anemia (Queiroz et al., 2013), malnutrition, and stunting (Dror and Muhsen, 2016). However, we have not found differences in iron status or anthropometric measurements in our cohorts.

Also, several genes/protein biomarkers have been shown to be upregulated or downregulated in *H. pylori*-infected children, especially among symptomatic patients, as we recently reviewed (George et al., 2020). In symptomatic children, *H. pylori* infection has been reported to be associated with a predominantly anti-inflammatory (regulatory T cell [T_{reg} cell]) gastric immune response (Hernández et al., 2014; Shimizu et al., 2004; Maciorkowska et al., 2005; Serrano et al., 2013), in contrast to adults, in which a predominance of proinflammatory cytokines (T_h17 and T_h1) has been reported (Serrano, 2013). However, Bhuiyan et al. (2014) described in blood samples from asymptomatic infected children the ability to trigger a systemic proinflammatory cytokine profile after in vitro stimulation with *H. pylori*. How this cytokine response correlates with other clinical and molecular indicators of gastric damage over time in apparently healthy children has not been addressed.

Here we present a case-control study including clinical symptoms and blood/serum sample analysis comparing school-aged children with persistent *H. pylori* infection and age- and sex-matched noninfected controls. Our aim was to determine if persistent *H. pylori* infection, documented by stool antigen tests or urea breath tests (UBTs) every 4 months, is associated with an increase in gastric-related symptoms or deterioration of nutritional status and/or the presence of laboratory parameters indicative of potential microscopic gastric bleeding, and/or increase in serum levels of gastric inflammation–related proteins and/or the relative expression of targeted cancer-related genes.

Methods

Overall study design, participants, and procedures

We recruited children from our two previous cohort studies and recruited a new cohort of healthy 4-year-old children (±9 months), living in the same city of Colina, who underwent the same *H. pylori*

Table 1
Baseline demographic factors for the 163 children enrolled in our nested case-control study (additional factors are given in Supplementary Table 3).

Variable	Controls ($N = 80$)	Persistently infected children ($N = 83$)	*P*
Male, *n* (%)	46 (58)	50 (60)	
Age at enrollment[a] (months), median (range)	48 (33–82)	47 (36–80)	
Age at first infection (months), median (range)	–	39 (11–82)	
Age at blood draw (months), median (range)	66 (52–98)	64 (55–99)	
Age at last visit (months), median (range)	88 (60–122)	87 (63–125)	
Number of siblings, median (IQR)	1 (0–2)	1 (1–2)	0.06
Birth order, median (IQR)	2 (1–3)	2 (1–3)	
Attended daycare (<24 months), *n* (%)	24 (30)	38/82 (47)	0.03
Attended preschool (<48 months), *n* (%)	42 (53)	58/82 (72)	0.01
Age child first attended daycare/preschool (months), median (IQR)	18 (8–31.5)	15 (7–24)	
History of			
Gastritis, *n* (%)	14 (18)	16/80 (20)	
Chronic abdominal pain, *n* (%)	14 (18)	15/80 (19)	
Underweight/malnourishment, *n* (%)	6 (8)	7/80 (9)	
Short stature, *n* (%)	13 (16)	14/80 (18)	
Anemia, *n* (%)	5 (6)	3/80 (4)	
Food allergies, *n* (%)	9 (11)	5/80 (6)	
Antibiotic use, *n* (%)	76 (95)	76/80 (95)	
Family history of			
Gastric ulcer, *n* (%)	31 (39)	31/80 (39)	
Helicobacter pylori infection, *n* (%)	16 (20)	12/80 (15)	
Gastric cancer, *n* (%)	21 (26)[b]	14/80 (18)[c]	
Anemia, *n* (%)	22 (28)	28/80 (35)	
Allergic rhinitis, *n* (%)	18 (23)	19/80 (24)	
Asthma, *n* (%)	31 (39)	32/80 (40)	
Allergic dermatitis, *n* (%)	18 (23)	20/80 (25)	
Food allergies, *n* (%)	15 (19)	9/80 (11)	
Digestive symptoms lasting >1 month			
Abdominal distension, *n* (%)	3 (4)	4/80 (5)	
Abdominal pain, *n* (%)	3 (4)	4/80 (5)	
Nocturnal abdominal pain, *n* (%)	3 (4)	3/80 (4)	
Loss of appetite, *n* (%)	8 (10)	11/80 (14)	

IQR, interquartile range.
[a] For this phase of the study.
[b] Eleven great-grandparents, eight grandparents, three uncles.
[c] Nine great-grandparents, four grandparents, one uncle.

screening using ELISA as the previous cohorts (O'Ryan et al., 2013, 2015). Recruitment was halted when we reached 80 persistently infected children (within all three cohorts) and 80 noninfected age- and sex-matched children, who were then followed up for at least 3 years for the nested case-control analysis.

Routine healthy-child visits and stool sample collection for *H. pylori* antigen detection were scheduled every 4 months for 4 years. Stool samples were obtained by the parent/guardian within the 24-h period before the scheduled visit and stored in an ad hoc recipient in their home refrigerator. For older children who refused to provide stool samples, the alternative of a UBT was offered. Ad hoc questionnaires for gastrointestinal findings were used during each visit (available in supplementary material). Children were evaluated yearly by a pediatric gastroenterologist (YL) blinded to the *H. pylori* status of the participant. Blood samples were collected in 2014 from all children; those samples of infected children that were taken before infection or before at least 1 year of infection were excluded from the analysis. All samples were stored at −20 °C until they were tested.

H. pylori stool detection and UBT

Stool samples were tested for *H. pylori* by ELISA (Premier Platinum HpSA®, Meridian Diagnostics, Cincinnati, OH, USA) according to the manufacturer's instructions. The UBT was performed by collection of breath samples before and after ingestion of [^{13}C]urea (50 mg), and the samples were analyzed with an IR-Force infrared spectrometer.

Anthropometric, clinical, and laboratory evaluations

The study nurse recorded the weight and height at all visits. All children underwent yearly clinical examination by a pediatric gastroenterologist (YL) blinded to the infection status for clinical evaluation, including alarm symptoms suggestive of organic upper abdominal pain as defined by the Rome III consensus (Rasquin et al., 2006). Children with upper abdominal pain and at least one alarm symptom were offered the opportunity to undergo endoscopy (or for out-of-protocol reasons, as determined by the specialist).

Blood samples in EDTA tubes were processed for hemogram and serum iron and serum ferritin. Two milliliters of blood was collected in an EDTA tube and transported at room temperature to the hematology laboratory of Hospital Clínico de la Universidad de Chile for hemogram automatic processing by an ADVIA 2120i analyzer (Siemens, Erlangen, Germany), specifically for red blood cell count, morphology, and hemoglobin quantification. An additional 5 mL of blood was collected in a metal-free and anticoagulant-free tube and transported at room temperature for serum iron determination by a photometric colorimetric test with a Shimadzu UVmini 1240 spectrophotometer and serum ferritin determination by a chemiluminescent microparticle immunoassay.

Molecular biomarkers

We selected six cytokines related to inflammation (interferon-γ [IFN-γ], IL-12, and TNF for the T_h1 pathway, IL-17 for the T_h17 pathway, and the T_{reg} cell cytokines IL-10 and transforming growth factor β [TGF-β]), two gastric-related proteins (pepsinogens [PGs] I and II), tissue inhibitor metalloproteinase 1 (TIMP-1), and three genes related to gastric cancer (*BTG3* [B-cell translocation gene 3], *KLK1*, and *SLC5A8*), on the basis of previous preliminary results (O'Ryan et al., 2015) and a literature review (Supplementary Table 1).

For determination of cytokines, an aliquot of serum extracted by centrifugation from the whole blood sample was processed by a Luminex® assay. PGI and PGII levels were assessed in serum with use of GastroPanel® (Biohit Oyj, Helsinki, Finland). TIMP-1 was measured by ELISA with a Quantikine® human TIMP-1 immunoassay (R&D Systems Inc., Minneapolis, MN, USA).

For cancer-associated genes, RNA was extracted from blood with a QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany). Briefly, for the synthesis of complementary DNA (cDNA), reverse transcription was performed with 0.5 μg total RNA using a mix of oligonucleotide (dT) and random primers with MMLV-RT enzyme (Promega, USA) at 37 °C for 60 min. The PrimeTime® Std quantitative PCR assay (FAM/ZEN/IBFQ; Integrated DNA Technology, Coralville, IA, USA) was used for the analysis of expression of *BTG3* (assay ID Hs.PT·58·3357167.g), *KLK1* (assay ID Hs.PT·58·20,142,515) and *SLC5A8* (assay ID Hs.PT·58·2,702,823) using the housekeeping β-actin gene *ACTB* (assay ID Hs.PT·39a·22,214,847). Quantitative PCR assays were performed with an AriaMx system (Agilent Technologies Inc., Santa Clara, CA, USA) with 2 μL of cDNA according to the manufacturer's instructions.

All samples were analyzed for *BTG3* in three independent experiments; in each experiment we ran the samples in duplicate. The average of the threshold cycle (Ct) values from the three experiments was used for analysis. The relative expression levels were reported with use of the $2^{-\Delta\Delta Ct}$ quantification method. A fold change of more than 1 was considered increased expression. Samples with *ACTB* Ct values less than 19 (0 samples) or greater than 23 (40 samples) were removed from the analysis; negative controls with one *H. pylori*-positive sample were also removed (6 samples), and one child who spontaneously cleared *H. pylori* was removed from the analysis.

Because we were not able to amplify *KLK1* and *SLC5A8* in any of the samples using the gBlock gene fragment (Integrated DNA Technology, Coralville, IA, USA) as a positive control, we further synthesized cDNA using specific primers (see Supplementary Table 2 for a list of primers used). In addition, we repeated the protocol previously described (Ueno et al., 2004) using TaqMan® probes (Applied Biosystems, Foster City, CA, USA) for *SLC5A8* (Hs00377618_m1) and *ACTB* (Hs01060665_g1).

Definitions and study end points

H. pylori infection was considered present when a child had more than one ELISA-positive stool sample, and persistent if a minimum of three consecutive stool samples tested positive. Spontaneous clearance corresponded to a persistently infected child with three or more successive negative samples. Noninfected controls were those who had a maximum of one sample positive for *H. pylori*.

The end points established before study initiation were as follows:

- Gastric damage–associated clinical end points: (i) alarm symptoms and signs suggestive of gastritis/peptic ulcer disease by the Rome III consensus (Rasquin et al., 2006); (ii) growth retardation (*z*score of height for age less than − 2SD by WHO growth charts) and undernutrition (*z*score of weight for height less than −2SD in children younger than 6 years and BMI less than fifth percentile in older children).
- Gastric damage–associated laboratory end points: (i) iron deficiency (low serum iron and ferritin levels according to age and sex) and/or (ii) hypochromic-microcytic anemia (by age and sex).

Gastric damage–associated molecular end points: (i) serum expression of *BTG3*, *KLK1*, and *SLC5A8* and (ii) serum levels of PGI

Table 2
Clinical evaluation by a pediatric gastroenterologist. Listed for each symptom is the overall occurrence (79 controls, 82 persistently infected children), followed by the occurrence at 4–7 years of age (78 controls, 82 persistently infected children) and at 8–10 years of age (60 controls, 62 persistently infected children).

Clinical assessment	Variable	Controls, *n* (%)	Persistently infected children, *n* (%)	*P*
History	Upper abdominal pain (parent or child verbal/report)			
	Overall	8 (10)	17 (21)	0.06
	4–7 years	5 (6)	8 (10)	
	8–10 years	6 (10)	12 (19)	
	Upper abdominal pain causing night waking			
	Overall	8 (10)	12 (15)	
	4–7 years	5 (6)	6 (7)	
	8–10 years	4 (7)	6 (10)	
	Heartburn[a]			
	8–10 years of age	2 (3)	2 (3)	
	Persistent vomiting			
	Overall	2 (3)	7 (9)	
	4–7 years	1 (1)	5 (6)	
	8–10 years	1 (2)	3 (5)	
	Nausea[a]			
	Overall	1 (1)	0 (0)	
	8–10 years	1 (2)	0 (0)	
	Weight loss			
	Overall	2 (3)	1 (1)	
	4–7 years	1 (1)	1 (1)	
	8–10 years	1 (1)	0 (0)	
	Bloody stools			
	Overall	2 (3)	0 (0)	
	4–7 years	2 (3)	0 (0)	
	8–10 years	0 (0)	0 (0)	
	Difficulty swallowing			
	Overall	3 (4)	1 (1)	
	4–7 years	1 (1)	1 (1)	
	8–10 years	2 (3)	0 (0)	
	Loss of appetite			
	Overall	13 (16)	23 (28)	0.08
	4–7 years	12 (15)	20 (24)	
	8–10 years	3 (5)	5 (8)	
Physical examination	Pallor[a]			
	8–10 years	0 (0)	1 (2)	
	Malnutrition[b]			
	Overall	5 (6)	2 (2)	
	4–7 years	3 (4)	2 (2)	
	8–10 years	2 (3)	0 (0)	
	Short stature			
	Overall	2 (3)	1 (1)	
	4–7 years	2 (3)	0 (0)	
	8–10 years	2 (3)	1 (2)	
	Epigastric pain on palpation			
	Overall	13 (16)	33 (40)	0.001
	4–7 years	3 (4)	5 (6)	
	8–10 years	9 (15)	29 (47)	<0.001
	Upper abdominal pain with alarm symptoms			
	Overall	6 (8)	8 (10)	
	4–7 years	3 (4)	3 (4)	
	8–10 years	4 (7)	6 (10)	

[a] Added later to the questionnaire.
[b] Defined as the *z* score for height/weight less than −2SD for children younger that 6 years and for BMI less than −2SD for children aged 6 years or older.

and PGII (and PGII/PGII ratio), TIMP-1, IFN-γ, IL-12, TNF, IL-17, IL-10, and TGF-β. As gastric damage was not routinely assessed through endoscopy in our children, the definitions of gastric damage–associated clinical and laboratory end points were operational, guided by clinical and laboratory findings suggestive of a pathogenic process related to *H. pylori* gastric infection and not necessarily endoscopic or histological gastritis.

Statistics

Statistical differences by *H. pylori* detection status were tested by Pearson's chi-squared test (or Fisher's exact test) for categorical variables and ANOVA for continuous demographic variables. Stepwise multivariate logistic regression models were used to determine whether any of the study variables were associated with persistent infection. Sample size calculations determined we needed 80 persistently infected children and 80 noninfected children for our case-control analysis. Participants providing at least six stool samples were included for analysis. Statistical analysis was performed with R version 3.0.0 (R Core Team, 2018). *P*values less than 0.1 are shown in the tables; however only values of 0.05 of less were considered statistically significant. As blood samples were taken at a fixed date, those children who had infection for less than 12 months at the time of blood draw were excluded from the analysis ($n = 5$). Luminex analysis was run in two plates, and results from the two sets of analysis were compared by the *t*test when data were normally distributed and ANOVA when they were not normally distributed. Values that were below the detection level were listed as 0. To account for dilution, TGF-β values were multiplied by 30.

Study approval

Consent and assent were obtained from guardians and from children aged 8 years or older, respectively. The Ethical Committee of the Faculty of Medicine, University of Chile, approved all aspects of this study.

Results

Eighty-three persistently infected children and 80 noninfected controls, from three child cohorts, were included in the nested case-control analysis (Figure 1). (Details of these three cohorts can be found in Supplementary Tables 3 and 4 and Supplementary Fig. 1). The duration of infection ranged from 8 to 106 months, with a median of 47 months at the end of follow-up (these numbers are minimum estimates, as 28 of 83 children entered the study infected, and thus we do not know their true age at first infection; the median length of infection for those with a known first infection was 63 months). Persistently infected children were more likely to be from larger families, as defined by the number of siblings, and to have attended daycare centers (less 2 years of age) and preschool (less then 4 years of age) (Table 1). The prevalence of *H. pylori* infection within our three consecutive cohorts was remarkably stable at approximately 24% for children aged 3–10 years in Colina. Infection onset dominantly occurred between 1 and 3 years of age (Supplementary Fig. 2).

Clinical and laboratory findings suggestive of gastric damage

Although no statistically significant differences were found in symptoms reported by parents, there was a trend toward higher frequency of upper abdominal pain (21% vs 10%; $P = 0.06$) and loss of appetite (28% vs 16%; $P = 0.08$) in persistently infected children compared with controls (Table 2). There were no differences in the occurrence of upper abdominal pain with alarm symptoms between groups (Table 2). Epigastric pain on physical examination was more common in persistently infected children overall (40% versus 16%; $P = 0.001$), and was more common in children aged 8–10 years. Growth retardation, malnutrition, and parameters related to iron deficiency did not differ by infection status (Supplementary Table 6). All four persistently infected children and one of four noninfected children referred for endoscopy had endoscopic and histological evidence of gastritis (Supplementary Table 5).

Serum protein and blood genetic biomarkers

There were no differences in TIMP-1 expression or the levels of T_h1, T_h17, and T_{reg} serum cytokines between infected and noninfected children (Table 3). The level of PGII but not that of PGI was higher in infected children versus noninfected children (median 12.7 ng/mL, interquartile range [IQR] 9.4–17.5 ng/ml, versus 9.0 ng/mL, IQR 6.3–11.4 ng/ml; $P < 0.001$) with a diminished PGI/PGII ratio (median 6.1, IQR 4.9–7.6, versus 8.2, IQR 6.4–10.8; $P < 0.001$) (Figure 2, Table 3). There was no relationship between PGI and PGII values and the length of infection or the child's age.

BTG3 expression values did not differ between infected children (median fold change 1.44, IQR 0.71–2.17, $n = 56$) and noninfected children (median fold change 1.4, IQR 0.60–1.96, $n = 58$; $P = 0.330$). There was no difference in increased expression (fold change greater than 1) between infected children and noninfected children (Table 3). *KLK1* and *SLC5A8* could not be amplified after repeated attempts (details in supplementary material).

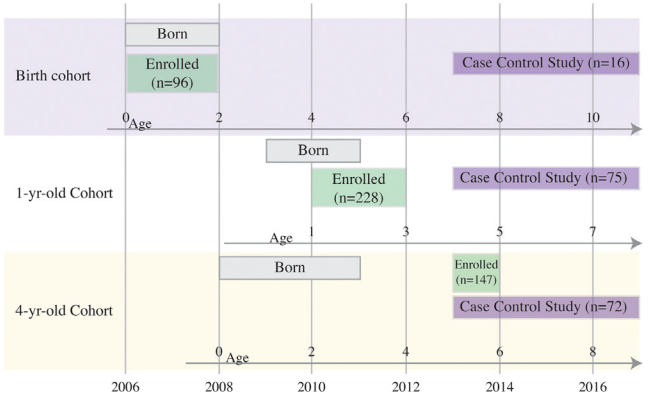

Figure 1. The three cohorts included this case-control study.

Logistic regression

We found that if a child attended daycare/preschool (odds ratio [OR] 2.6), had epigastric pain on physical examination (OR 2.5), or had higher PGII levels in blood (OR 1.075), there was a greater probability that the child was persistently infected with *H. pylori* (Table 4).

Discussion

H. pylori persistently infected 21–24% of children from three different low-income to middle-income cohorts in a periurban Chilean city on the basis of noninvasive detection of *H. pylori* (antigen stool test and/or UBT). Infection was acquired largely before 4 years of age. Using a nested case-control design, we found epigastric pain on physical examination was significantly more common among infected children aged 8–10 years; however, this was not spontaneously reported/perceived by parents, and only approximately 5% of children were flagged for endoscopy. Infection was not associated with alterations in iron status, anemia, or nutritional status. Infected children, irrespective of symptoms, had higher serum PGII levels compared with noninfected age- and sex-matched controls. The levels of other targeted molecules did not differ between groups.

These results strongly suggest that by the time children reach school age, infection with *H. pylori* may be compromising the gastric mucosal level; however, for most children this has not yet translated into a significant increase in clinical symptoms. Similar findings have been reported in Japan, where infection rates are significantly lower (3%) (Nakayama et al., 2017).

In our population, The level of PGII but not that of PGI was increased among infected children compared with noninfected children, possibly indicating more prolonged infection, similarly to what was described by Kassem et al. (2017) in Israeli children. PGI and PGII serum levels have been reported to be increased among *H. pylori*-infected children compared with noninfected children and positively correlated with histological antral inflammation in symptomatic children, with PGII as the main predictor of gastric inflammation (de Angelis et al., 2007; Guariso et al., 2009). In a Japanese study the 2017 Helicobacter study by Nakayama et al., 12 of 14 adolescents infected with *H. pylori* were subjected to endoscopy, and all were found to have nodular gastritis and/or atrophy. In our study, endoscopy was performed in only a few symptomatic children with alarm signs, with all infected children showing gastritis. It is likely that a significant proportion of infected, nonsymptomatic children in our Chilean population would also have gastric abnormalities if endoscopy had been performed.

Our study has limitations. Although the infection rates were remarkably similar between the three consecutive cohorts, they were all from the same location. Infection rates can differ

Table 3
Biomolecular factors potentially associated with gastric damage in Chilean children (case-control study).

Gastric-related cytokines, median (IQR)	Controls *N* = 50	Persistently infected children *N* = 50	*P*
IFN-γ, pg/mL	7.0 (3.4–12.7)	6.8 (2.9–18.2)	
IL-12p70, run 1, pg/mL	1.3 (0.9–2.7)	3.2 (1–6)	
IL-12p70, run 2, pg/mL	25.1 (1.3–36.4)	3.4 (1.8–24.3)	
TNF, run 1, pg/mL	5.1 (3.9–7.3)	6.2 (3.3–8.7)	
TNF, run 2, pg/mL	22 (17.1–27.3)	25.1 (20.5–29.1)	
IL-17, run 1, pg/mL	2.5 (1.4–3.7)	3 (1.4–6.3)	
IL-17, run 2, pg/mL	7.7 (4.5–15.9)	4.7 (1.6–24.8)	
IL-10, pg/mL	3.5 (2.4–7.1)	4.9 (2.2–11.6)	
TGF-β1, run 1, pg/mL, mean (range)	93 (61–132)	97 (61–148)	
TGF-β1, run 2, pg/mL	56 (51–69)	51 (47–68)	
PGI	*N* = 74	N = 72	
Concentration, ng/mL, median (IQR)	76.9 (56.8–101.8)	79.8 (57.7–97)	
>1 SD over control mean, *n* (%)	9 (12.2)	6 (8.3)	
>2 SD over control mean, *n* (%)	4 (5.4)	3 (4.2)	
PGII	*N* = 74	N = 72	
Concentration, ng/mL, median (IQR)	9.0 (6.3–11.4)	12.7 (9.4–17.5)	<0.001
>1 SD over control mean, *n* (%)	6 (8.1)	12 (16.7)	
>2 SD over control mean, *n* (%)	3 (4.1)	3 (4.2)	
PGI/PGII ratio, median (IQR)	8.2 (6.4–10.8)	6.1 (4.9-–7.6)	<0.001
BTG3	*N* = 58	*N* = 56	
BTG3 fold change, median (IQR)	1.4 (0.60–1.96)	1.44 (0.71–2.17)	
Increased expression (fold change >1), *n* (%)	34 (60.7)	34 (58.6)	
>1 SD over control mean, *n* (%)	6 (10.3)	11 (19.6)	
>2 SD over control mean, *n* (%)	2 (3.5)	4 (7.1)	
TIMP-1	*N* = 73	*N* = 72	
Concentration, ng/mL, median (IQR)	184.4 (156.5–209.3)	183.4 (153.7–188.2)	

IFN, interferon; IQR, interquartile range; PG, pepsinogen; TGF, transforming growth factor; TIMP-1, tissue inhibitor metalloproteinase 1.

Table 4
Logistic regression. Outcome, persistent infection (versus noninfection).

	OR	95% CI	*P*
Attended daycare or preschool[a]	2.596	1.263–5.489	0.011
Epigastric pain	2.533	1.154–5.758	0.023
PGII (ng/mL)	1.075	1.02–1.144	0.013

Receiver operating characteristic curve 73%. Hosmer and Lemeshow test, $P = 0.641$.
CI, confidence interval; OR, odds ratio; PG, pepsinogen.
[a] Before 4 years of age.

geographically (Zabala Torres et al., 2017), and thus our findings should be evaluated in other localities. We selected a relatively low number of biomarkers from those reported to be potentially altered in association with childhood *H. pylori* infection (George et al., 2020). Increasing the number of biomarkers could further demonstrate the early effect of *H. pylori* infection during childhood. Conversely, biomarkers indicative of systemic responses, such as serum cytokine levels, did not differ by infection status, which may be a reflection of a localized effect at the gastric level only, where we would expect a T_h1 and/or T_h17 cytokine bias in those infected children with other signs of gastric damage and a T_{reg} cytokine predominance in infected children with no signs of gastric damage. In the case of TIMP-1, which was previously reported to be underexpressed in serum samples of symptomatic *H. pylori*-infected children, the fact that there were no differences in our children according to infection status may be related to population differences. Just as Rautelin et al. (2010) evaluated children and teenagers referred for endoscopy, and endoscopic gastritis was found in all those infected with *H. pylori*, it is possible that our younger asymptomatic children may be in an earlier stage of the pathogenic process. We hypothesize that significant results would

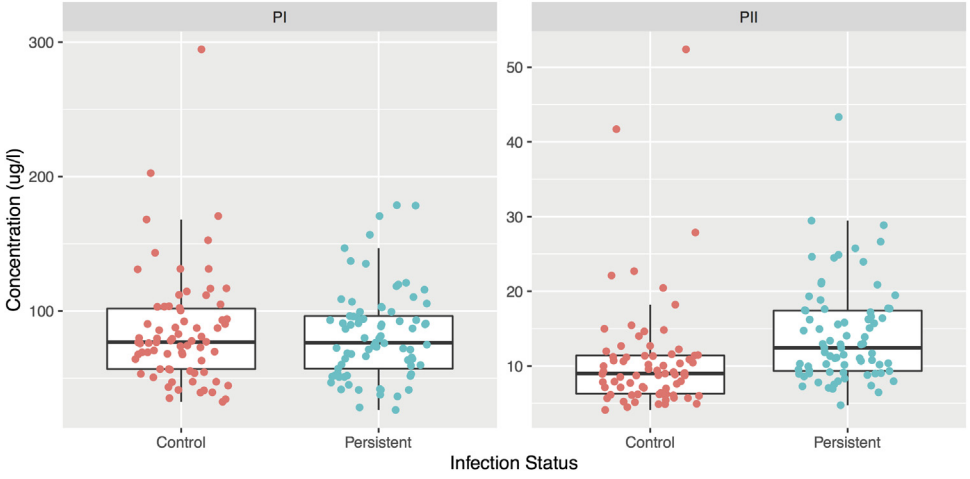

Figure 2. Concentrations of (A) serum pepsinogen I (PGI) and (B) serum pepsinogen II (PGII) in persistently infected children and noninfected children.

have been detected if these biomarkers had been assessed at older ages with a longer time of persistent infection. Importantly, we were unable to detect *SLC5A8* expression levels in blood of infected or noninfected children despite numerous methodological adjustments. The inability to replicate results from a previous report associating infection with decreased expression of this gene in young children (Orellana-Manzano et al., 2016) significantly downplays the potential role of infection over the expression of this biomarker. Overall, we were able to amplify only one of the three targeted mRNAs in blood, reflecting a rather poor capacity of these biomarkers to reflect *H. pylori*-associated effects. Our study design resulted in differences in the length of infection at the time of patient evaluation (clinical evaluations, blood sampling), which may affect the interpretation of observed differences. Ideally, comparative analysis should have been performed at a similar time interval after infection, considering several years of nonsymptomatic infections, paired with noninfected age-matched controls, but the logistics of such a study would be extremely difficult, as exact determination of infection can be assured only with birth cohorts. It is likely that observed differences, as well as differences in other potentially relevant biomarkers, would increase with such a design.

Our findings have several implications for future approaches toward *H. pylori* infection in children. First, infection is not innocuous in 5–10-year-old children, despite a generalized lack of symptoms. This finding should lead us to potentially reconsider current recommendations for the management of nonsymptomatic *H. pylori*-infected children. Currently, treatment recommendations based on a "screen and treat" strategy have proven to be effective in adults to prevent gastric cancer in countries with high mortality associated with this neoplasia and are being proposed for high school students in Japan (Akamatsu et al., 2015; Okuda et al., 2017). Our results support advancing "screen and treat" strategies to children in middle school or high school in countries similar to Chile.

The fact that one in every four to five school-aged children residing in a middle-income, periurban area of a middle-income to high-income country have a well-documented persistent *H. pylori* infection should trigger interest in obtaining further knowledge of childhood infection prevalence in different localities/regions. As infection is largely acquired during the first 5 years of life (O'Ryan et al., 2013, 2015), screening school-aged children from different regions could provide robust data on regional differences in infection prevalence. These results should reinvigorate interest in infection prevention through vaccination (Jones et al., 2017), a strategy promoted during the early years of the first decade of the twenty-first century but largely abandoned because of nonconclusive results, with only one candidate reaching a clinical trial in the past 5 years (Zeng et al., 2015), which has since been halted.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.11.202.

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