Peptic Ulcer Disease in *Helicobacter pylori*–Infected Children: Clinical Findings and Mucosal Immune Response

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ABSTRACT

Objectives: Peptic ulcer disease (PUD) is highly prevalent among adults but less common in children. *Helicobacter pylori* infection, the main cause of PUD, is, however, acquired extremely early in life. The aim of the study was to analyze clinical characteristics of children with PUD in a country with a high prevalence of the disease and to evaluate which host factors could determine this clinical outcome.

Methods: Children referred for upper gastrointestinal (GI) endoscopy with suspicion of peptic diseases were included prospectively during an 8-year period. Antral biopsies were performed to determine *H pylori* presence and mucosal cytokines profile.

Results: A total of 307 children between 3 and 18 years old were enrolled. Of the total, 237 children (46% boys) with complete data were included. *H pylori* infection was confirmed in 133 (56.1%) participants. Duodenal ulcer (DU) was diagnosed in 32 patients (13.5%); among them 29 were infected with *H pylori* (90.6%). Infected children had a nodular appearance of the gastric mucosa more often than noninfected children. Noninfected children had fewer lymphoid follicles and less inflammatory infiltrate than infected children. Only mucosal polymorphonuclear cell infiltration was more intense in DU-infected children as compared with non–DU-infected children. DU-infected children had higher levels of mucosal interferon-γ than noninfected and non–DU-infected patients. Non–DU-infected children had also higher levels of mucosal interleukin-10 than noninfected patients ($P < 0.05$).

Conclusions: PUD in children, especially DU, is strongly associated with *H pylori* infection in developing countries. There is no distinctive clinical presentation of children with PUD. T-helper cytokine balance may influence clinical outcomes in children.

Key Words: *Helicobacter pylori*, peptic ulcer disease, T-helper cytokines

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Peptic ulcer disease (PUD), a highly prevalent gastrointestinal (GI) entity, affects up to 10% of the general population (1,2). Historically, PUD has been classified into primary and secondary ulcers; although in the former no apparent cause is disclosed, in the latter ulcers emerge as a consequence of some evident damage over the gastric mucosa (3). In the past, the cause of primary ulcers was mainly attributed to different harmful factors (eg, emotional stress, tobacco use, consumption of specific food); however, in 1983, Warren and Marshall established the link between the presence of *Helicobacter pylori* and the development of chronic gastritis (CG) (4). This seminal finding led to an explosive growth of related research and ensuing literature that further led to the establishment of the causal relation between *H pylori* and PUD (5), as well as the widely accepted pathogenic role of *H pylori* in CG, gastric carcinoma, and mucosal-associated lymphoma (6,7).

Clinicians have learned that PUD is much less common in children than in adults, but few epidemiological data are available to back up this observation. In developing countries, information is even more scarce. Particularly in Chile, the prevalence of *H pylori* in the adult population was reported as 73% (8). The last study involving children with PUD was performed 28 years ago in the pre–*H pylori* era (2). In that retrospective study, 32 children with PUD from 1974 to 1984 were diagnosed using upper GI radiography and/or upper GI endoscopy (UGE). The authors reported an increase in PUD diagnosis after UGE was incorporated as a diagnostic tool in 1978. Duodenal ulcers (DUs) predominated over gastric ulcers, and they were treated with cimetidine or antacids (2).

Pediatric population provides a unique opportunity to study the role of the host immune response in the development of *H pylori*–associated PUD owing to the elimination of other variables such as chronic exposure to environmental factors, the presence of gastric metaplasia in the duodenum, and longer exposure to bacteria and its virulent factors. Therefore, our goal was to analyze the clinical characteristics of children with PUD in the post–*H pylori* discovery era in a country with a high prevalence of the infection. We also sought to analyze some aspects of the host immune response that may determine adverse clinical outcomes, in this case PUD, in children infected with *H pylori*.

METHODS

Study Population

Children age <18 years old were included in this study and were referred for UGE owing to clinical symptoms as diagnosed by their referring physicians. Two centers, the pediatric gastroenterology units from the Pontificia Universidad Católica de Chile Clinical

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Hospital and Dr Sótero del Río Hospital, recruited patients during an 8-year period. A clinical questionnaire, which included age, sex, date of birth, and relevant clinical data, was circulated to collect data. Inclusion criteria considered at least 1 of the following manifestations: hematemesis, chronic epigastric pain or nocturnal awakening with abdominal pain, chronic vomiting associated with eating, suspected PUD relapse, and recurrent abdominal pain in children with a first-degree relative with PUD. Patients treated for PUD, those who received *H pylori* eradication therapy in the last year, or those who received proton pump inhibitors or antibiotics in the last month were excluded. Local institutional review board committees from 2 centers involved approved the study. Informed consent was obtained from the patient's parents or legal guardians before all procedures.

Endoscopy

The endoscopy procedure was performed according to standard techniques; endoscopic findings in the esophagus, stomach, and duodenum were described in a standardized way by the participating endoscopists. The macroscopic appearance of the esophagus, stomach, and duodenum was recorded according to the presence of erythema, antral nodularity, and erosion or ulceration. Nodularity was defined as the presence of multiple, discrete, 0.2 to 0.5 cm in diameter nodules visible on endoscopic examination. Ulceration was defined as a circumscribed lesion of the mucosal surface, with a diameter of 5 mm or more, with apparent depth and covered by exudates. Healed ulcers (scars) were considered part of the ulcer group for the purpose of this study. At least 4 biopsies were performed from the gastric antrum: 1 for histological analysis (including direct visualization of the bacteria), 1 for rapid urease test (He-Py test, BiosChile, Santiago, Chile, and Pronto Dry, Ecifarma, Santiago, Chile), and 2 for analysis of gastric mucosal cytokines.

Histology and *H pylori* Status

Histological analyses were performed at the Department of Pathology of the Pontificia Universidad Católica de Chile. Serial sections of formalin-fixed and paraffin-embedded (Paraplast; Leica Biosystems, Santiago, Chile) biopsies were stained with hematoxylin and eosin. All biopsy specimens were codified and evaluated independently twice by 2 pathologists in a blinded fashion. *H pylori* presence, activity (polymorphonuclear cell infiltration), inflammation (mononuclear cell infiltration), gastric atrophy, and intestinal metaplasia were recorded according to the Sydney grading system (9). Thereafter, a histology score reflecting the level of inflammation was determined by adding the score of each parameter. A colonization score was calculated based on *H pylori* density (0 = absent, 1 = mild, 2 = moderate, 3 = intense). A patient was considered infected with *H pylori* if 1 of 2 antral biopsy-dependent tests (*H pylori* stain or rapid urease test) was positive.

Cytokine Determination

Proinflammatory cytokines interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α; T helper (T_H) cytokines IL-12, interferon (IFN)-γ, IL-4, IL-10, and tumor growth factor (TGF)-β were measured by enzyme-linked immunosorbent assay (ELISA) from gastric biopsies. For cytokine determination, antral biopsy specimens were homogenized (OMNI International, Kennesaw, GA) separately in 750 μL of normal saline. Supernatants obtained by centrifugation in a mini Eppendorf centrifuge (12,000*g* for 5 minutes at 4°C) were frozen at −80°C in sterile vials until used for an ELISA. Total protein content was measured in biopsy homogenates using a modified bicinchoninic acid method (Pierce, Rockford, IL) and was expressed as milligrams per milliliter. The specific protein content for each cytokine was determined by ELISA using human recombinant cytokines as positive controls for the development of standard curves, according to the manufacturer's instructions. The results were expressed in picograms per milliliter. Nevertheless, the final concentrations in homogenized biopsies were expressed in picograms per milligram of total protein. IL-10, IL-12, IFN-γ, and IL-4 (BD Biosciences Pharmingen, San Diego, CA) limits of detection were 2.0, 4.0, 1.0, and 2.0 pg/mL, respectively. TNF-α, TGF-β, IL-1β, IL-6, and IL-8 (R&D Systems, Minneapolis, MN) limits of detection were 2.4, 7.0, 1.0, 0.7, and 3.5 pg/mL, respectively.

Statistical Analysis

The χ^2 test was used to compare categorical variables. The Kruskal-Wallis test was used for comparison between continuous variables. The Dunn posttest was used to make comparisons between any 2 groups if any statistical difference was found. Statistical significance was defined as $P < 0.05$.

RESULTS

General Characteristics of Patients

A total of 307 (44.6% boys) children between 3 and 18 years old (mean age 12.5 ± 2.9 years) were enrolled. *H pylori* infection was confirmed in 176 (57.3%) children. 40 patients developed PUD, 35 of them (87.5%) had DU, while 3 (7.5%) had gastric ulcer (GU). Two (5%) patients showed ulcers present in both locations. Because of the low number of patients with GU, we analyzed only patients with DU. In addition, 70 patients were excluded because of incomplete datasets (*http://links.lww.com/MPG/A356*). Therefore, 237 (46% boys) children were included. *H pylori* infection was confirmed in 133 (56.1%) children. In 32 patients (13.5%) DU was diagnosed, of which 29 (90.6%) were infected with *H pylori*; this represents the 12.2% of the total study population. Three patients had non–*H pylori*-associated DU.

The patients were divided into 4 groups: group I, children without *H pylori* infection and DU (*Hp*− and DU−); group II, children infected with *H pylori* without DU (*Hp*+ and DU−); group III, children infected with *H pylori* with DU (*Hp*+ and DU+); and group IV, children noninfected with *H pylori* with DU (*Hp*− and DU+). Table 1 summarizes clinical and demographic characteristics of the different groups. As expected, children in group I were younger than patients in groups II and III ($P < 0.05$). Interestingly, there was a higher proportion of boys in group III in comparison with the other groups ($P < 0.05$). Regarding symptoms that warranted the UGE for each group, there was no significant difference comparing all groups and indications. The most common symptom for referral in our population was chronic vomiting (53.6%). This was also the most frequent indication for referral in all of the analyzed groups individually. In addition, group III children had a higher frequency of history of upper GI bleeding (6.9%) than groups I, II, and IV children (1%, 1%, and 0%, respectively).

Endoscopic Findings

For this analysis only 130 patients had protocol-based information (Table 2). Endoscopic findings were classified according to type and location of the different lesions. There was no significant difference in esophageal findings. As expected, children in group I exhibited normal endoscopic findings in the stomach and duodenum, which were found more often than other groups. Group III children showed a higher frequency of antral and duodenal erythema and, by definition, ulcers. Also, in children

TABLE 1. Clinical characteristics of patients by group

General characteristics	I *Hp*−, DU−	II *Hp*+, DU−	III *Hp*+, DU+	IV *Hp*−, DU+
No. patients (%)	101 (42.6)	104 (43.9)	29 (12.2)	3 (1.3)
Age, y, mean ± standard deviation*	11.8 ± 3.6	13.2 ± 2.3	13.9 ± 2.2	11 ± 5.3
Sex, no. males (%)**	40 (40)	47 (45)	20 (69)	2 (67)
Clinical indication for endoscopy, no. (%)				
RAP†/dyspepsia	27 (26.7)	17 (16.4)	8 (27.6)	1 (33.3)
Chronic vomiting***	53 (52.5)	64 (61.5)	9 (31)	1 (33.3)
RAP/dyspepsia	9 (8.9)	9 (8.7)	3 (10.3)	0 (0)
Suspected PUD relapse	4 (3.9)	7 (6.7)	3 (10.3)	0 (0)
Other‡	8 (7.9)	7 (6.7)	6 (20.7)	1 (33.3)

DU = duodenal ulcer.
† RAP: recurrent abdominal pain + family history of PUD (peptic ulcer disease).
‡ Other: include GERD (gastroesophageal reflux disease) and UGB (upper gastrointestinal bleeding).
* $P < 0.05$, children from group I were significantly younger than those in groups II and III (Kruskal-Wallis test, Bonferroni posttest).
** $P < 0.05$, group III had a significantly higher proportion of male patients than the other groups.
*** $P < 0.05$, children from groups I and II had a higher proportion with patients who presented chronic vomiting than those in group III (Kruskal-Wallis test, Bonferroni posttest).

from groups II and III a nodular appearance of the gastric mucosa was found more often than in group I. In children from group III, all of the ulcers were duodenal, with the exception of 1 patient, who had ulcers in both the gastric antrum and duodenum.

Histological Findings

Table 3 describes the histological findings in the 237 children with complete histology analysis based on a full Sydney score description. Noninfected children (group I) had fewer lymphoid follicles, a lower intensity of CG, and less mononuclear and polymorphonuclear infiltration than infected children (groups II and III) ($P < 0.05$). To determine whether the macroscopic finding nodularity had any histologic correlates, we compared histological findings and *H pylori* infection in patients with and without nodules. Patients with nodules had higher levels of lymphoid follicles, and mononuclear and polymorphonuclear cell infiltration, although infection with *H pylori* was present in 83% of patients with nodules (data not shown). We next analyzed histological markers in *H pylori*–infected children, and no differences were found between *H pylori*–infected children without DU (group II) or with DU (group III) in any histological variable. In addition, we determined the mean histological and colonization score for each group (Fig. 1). Bacterial colonization was similar in both infected groups, but mean histological score was significantly higher for infected patients regardless of whether DU was present.

TABLE 2. Endoscopic findings by group

	I *Hp*−, DU−	II *Hp*+, DU−	III *Hp*+, DU+	IV Hp−, DU+
No. patients (%)	63 (48.5)	49 (37.7)	15 (11.5)	3 (2.3)
Esophagus				
Esophagitis	4 (8.7)	6 (12.2)	1 (6.7)	0 (0)
Stomach				
Erythema*	10 (15.9)	3 (6.1)	5 (33.3)	2 (6.7)
Ulcer	0 (0)	0 (0)	1 (6.7)‡	0 (0)
Nodules*	6 (9.5)	24 (49)	10 (66.7)	1 (33.3)
Other lesion*,†	1 (1.6)	2 (4.1)	0 (0)	0 (0)
Duodenum				
Erythema*	5 (7.9)	5 (10.2)	5 (33.3)	1 (33.3)
Ulcer	0 (0)	0 (0)	15 (100)‡	3 (100)
Nodules	0 (0)	3 (6.1)	0 (0)	0 (0)
Other lesion†	3 (4.8)	4 (8.2)	0 (0)	1 (33.3)

DU = duodenal ulcer.
† Nonspecific signs such as minimal erosions and mucosal friability.
‡ One child had both gastric and DUs.
* $P \leq 0.05$ using the χ^2 test when all groups were compared for individual endoscopy finding.

Cytokine Profile in the Gastric Mucosa

We determined proinflammatory cytokine levels (IL-1β, IL-6, IL-8, and TNF-α) and T_H cytokines (IL-12, IFN-γ, IL-4,

TABLE 3. Histological findings by group

	I *Hp*−, DU−	II *Hp*+, DU−	III *Hp*+, DU+	IV *Hp*−, DU+
No. patients (%)	101 (42.6)	104 (43.9)	29 (12.2)	3 (1.3)
CG*				
None	87 (86)	15 (14)	1 (3.5)	2 (66.7)
Present	14 (14)	89 (86)	28 (96.5)	1 (33.3)
Lymphoid follicles*				
None	87 (86)	66 (63)	19 (65.5)	2 (66.7)
Mild	14 (14)	37 (35)	8 (27.5)	1 (33.3)
Moderate	0 (0)	1 (0.9)	2 (6.9)	0 (0)
Mononuclear cell infiltration*				
None	88 (87.1)	15 (14.4.)	1 (3.5)	2 (66.7)
Mild	13 (12.9)	85 (81.7)	26 (89.7)	1 (33.3)
Moderate	0 (0)	4 (3.9)	2(6.9)	0 (0)
Polymorphonuclear cell infiltration*				
None	96 (95.1)	52 (50)	0 (34.5)	2 (66.7)
Mild	4 (3.9)	41 (39.4)	13 (44.8)	1 (33.3)
Moderate	1 (1)	11 (10.6)	5 (17.2)	0 (0)
Severe	0 (0)	0 (0)	1 (3.5)	0 (0)
Atrophy				
None	94 (93.1)	97 (93.3)	27 (93.1)	3 (100)
Present	7 (6.9)	7 (6.7)	2 (6.9)	0 (0)
Intestinal metaplasia				
None	100 (99)	104 (100)	29 (100)	3 (100)
Present	1 (1)	0 (0)	0 (0)	0 (0)

CG = chronic gastritis; DU = duodenal ulcer.
* $P < 0.05$ for analysis between all groups (the χ^2 test).

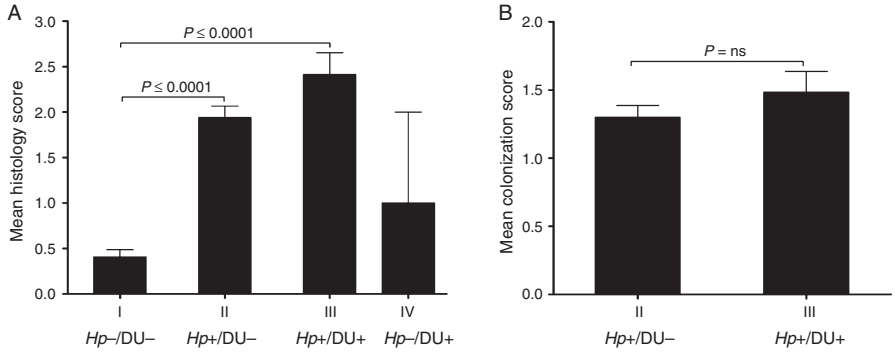

FIGURE 1. Levels of gastric inflammation and *H pylori* colonization in the different study groups. A, *H pylori* induced significant inflammation in the gastric mucosa of infected children regardless of the presence of ulceration. Inflammation was less severe in noninfected children with ulcer disease. B, Levels of *H pylori* colonization in gastric mucosa were similar in infected children with or without ulcerations. Levels of gastric inflammation and colonization are presented as the histology score and colonization score (mean ± standard deviation), respectively (see Methods). DU = duodenal ulcer.

IL-10, and TGF-β) in 107 and 130 patients, respectively. Because of the low number of children in group IV (noninfected children with DU), they were excluded from the cytokine analysis. Considerable variability in cytokine values was observed. No differences in proinflammatory cytokine levels (IL-1β, IL-6, IL-8, and TNF-α) were observed among study groups (Fig. 2); however, infected children without ulcers showed significantly increased levels of IL-10 than noninfected children ($P < 0.05$). In addition, children with DU (group III) presented higher levels of IFN-γ than the other study groups ($P < 0.05$, Fig. 3).

DISCUSSION

In this study, we evaluated 307 children referred for UGE because of varied GI symptoms. DU was diagnosed in 13.5% of these patients, and it was associated with *H pylori* infection in 90.6% of them.

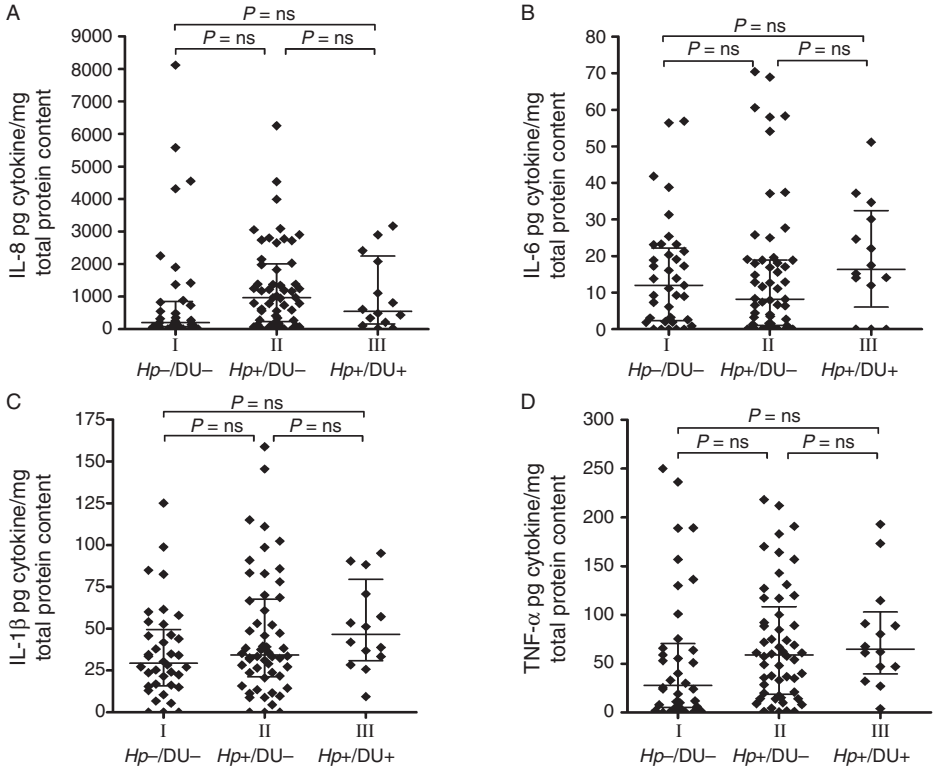

FIGURE 2. Levels of proinflammatory cytokines in the gastric mucosa of the different study groups. (A) IL-8, (B) IL-6, (C) IL-1β, and (D) TNF-α showed similar levels of protein expression in gastric mucosa regardless of *H pylori* infection and ulcer status. Cytokine expression was determined in gastric biopsies by enzyme-linked immunosorbent assay and normalized to each biopsy total protein content. Data are presented as individual points along with median and interquartile range. DU = duodenal ulcer; IL = interleukin; TNF = tumor necrosis factor.

Endoscopy findings such as antral nodularity were observed more often in children with DU, which was in agreement with the previous reports (10–13). Nodularity in children from developing countries (14) has been related to high-grade bacterial colonization and severe gastritis. In our study, nodularity was present mostly in infected patients, and the association between increased levels of lymphoid follicles, and mononuclear and polymorphonuclear cell infiltration correlated with the presence of the bacteria rather than the nodules by themselves.

Histological findings in *H pylori*–infected adults and children have been extensively described elsewhere (9,10,15,16). We have reported that *H pylori*–infected children, regardless of the clinical outcome, displayed reduced levels of gastric polymorphonuclear and mononuclear cell infiltration, lymphoid follicle accumulation, intestinal metaplasia, mucosal atrophy, and ulceration compared with infected adults (17,18). In this report histopathological findings correlated mostly with the presence of *H pylori*. Low frequency of lymphoid follicules and mild levels of mono- and polymorphonuclear infiltration were observed with similar frequencies in infected children regardless of the presence of DU.

H pylori infection is highly prevalent among the adult general population in Chile, as reported in the 2003 National Health Survey (8), and this fact is mirrored in children. The extent and magnitude of the immune response to *H pylori* infection have been widely accepted as the most important contributors to mucosal damage (19). Interestingly, *H pylori*–infected children have substantially less ulceration than infected adults (10,20,21). Robinson et al (22) reported that *H pylori* induces both an effector $T_H1/2$ and regulatory Treg response in the gastric mucosa of infected patients. PUD in this series is characterized by an imbalance of effector and regulatory T cells, with ulcer patients showing decreased expression of regulatory cytokines and Treg cells. Interestingly, *H pylori*–infected children have substantially less ulceration than infected adults (10,20,21). This finding is consistent with the increased Treg response observed in the gastric mucosa of infected children in comparison with infected adults (17).

We found that infected children exhibited higher levels of mucosal IL-10, compared with noninfected children as previously described (17,23,24). IL-10 plays a crucial role in immunoregulation in *H pylori* infection as described by Matsumoto et al (25). In the murine model of *H pylori* infection, the lack of IL-10 increases the mucosal infiltration of immune cell, resulting in clearance of the bacteria and increased mucosal damage. Although we did not find significant differences in IL-10 or other immunoregulatory cytokines between infected children with or without DU, this may be obscured by the fact that children in general tend to have an augmented Treg response in the presence of *H pylori*. In contrast, IFN-γ was present in higher levels in the stomach of those *H pylori*–infected children who were found to have developed DU than in those who had not, suggesting a relation between a T_H type 1–mediated inflammation and DU, similar to what has

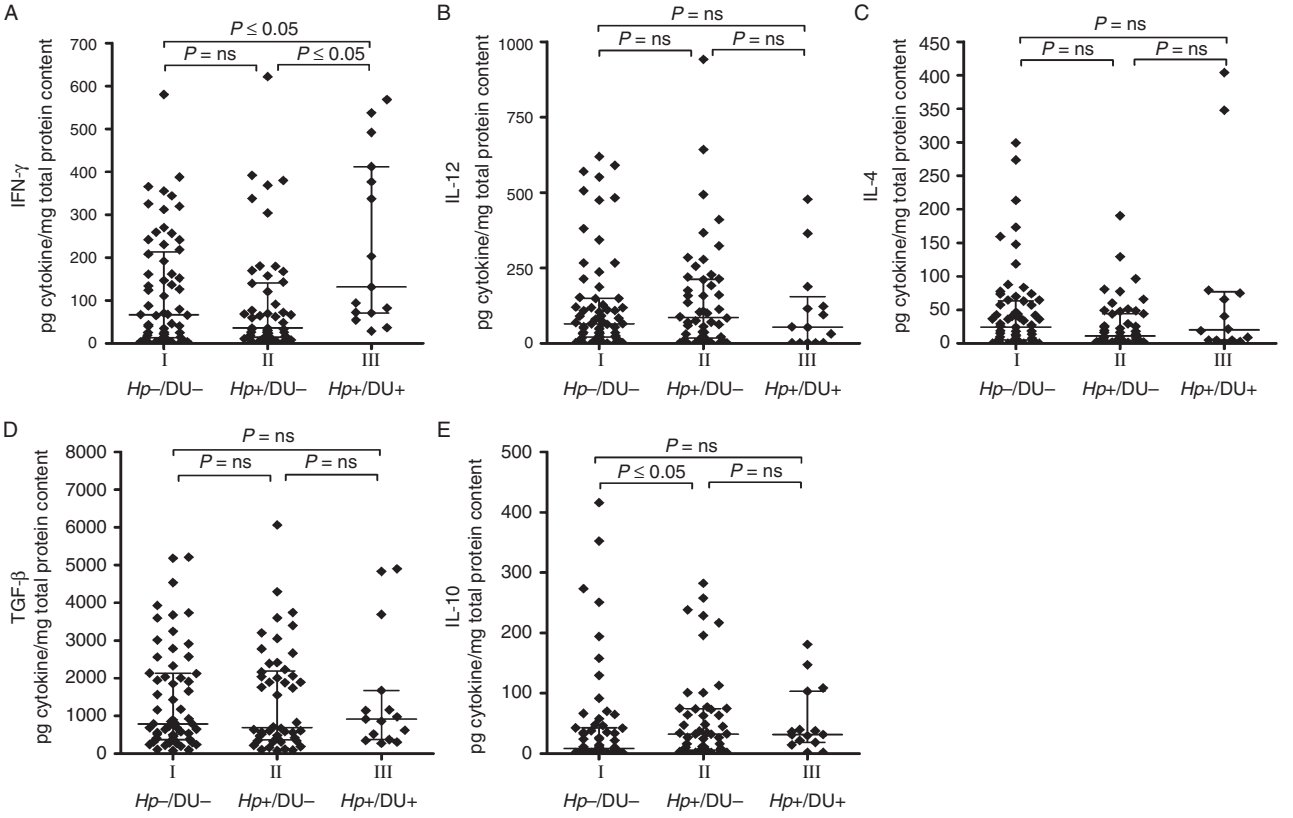

FIGURE 3. Levels of T helper cells cytokines in the gastric mucosa of the different study groups. A, IFN-γ showed significantly increased levels in infected children with DU in comparison with infected non-DU children and noninfected non-DU controls. (B) IL-12, (C) IL-4, and (D) TGF-β showed similar levels of protein expression in gastric mucosa regardless of *H pylori* infection and DU status. E, IL-10, however, showed significantly increased levels in infected children in comparison with noninfected non-DU controls. Cytokine expression was determined in biopsies by enzyme-linked immunosorbent assay and normalized to each biopsy total protein content. Data are presented as individual points along with median and interquartile range DU = duodenal ulcer; *Hp* = *Helicobacter pylori*; IFN-γ = interferon-γ; IL = interleukin; ns = not significant; TGF = transforming growth factor.

been shown in adults and animal models (26,27). *H pylori*–specific gastric mucosal T cells that exhibit a T_H1 phenotype are characterized by an upregulation of IFN-γ that promotes gastric inflammation and contribute to the disturbed epithelial barrier function, causing tissue damage (27). This response has been found exacerbated in adult patients who eventually develop DU, just as in our series. In addition to the well-documented T_H1 response observed in adult ulcer patients, T_H17 cells have also been involved in *H pylori*–generated mucosal damage (28,29). Our group and others have shown that children have reduced IL-17 levels and IL-17–producing cells (18,30). In this cohort of patients, we did not observe differences in the levels of gastric mucosa IL-17 between the 3 groups analyzed (*Hp*− and DU−, *Hp*+ and DU−, *Hp*+ and DU+; data not shown); however, only a small number of children in the group *H pylori*–infected with DU had tissue available for the protein determination, limiting the strength of the results. Nevertheless, most of the evidence regarding *H pylori*–induced T_H responses reported to date indicate that children have a predominant Treg response instead of a T_H1/T_H17 response (18,30).

H pylori has long been considered as a major cause of antral gastritis and PUD in children (31). We suggest the possibility that early events in *H pylori*–infected gastric mucosa play an important role in the progression of *H pylori* inflammatory disease in young hosts, limiting more severe clinical outcomes such as ulceration. How *H pylori* induces Treg cells in early infection and how T_H1/T_H17 cells appear to eventually override the Treg cell influence on *H pylori*–induced inflammation warrant critical investigation. In addition to T_H cell balance, proinflammatory cytokines are increased in *H pylori*–infected mucosa in both adults and children.

Although a large portion of the world's population remains infected with *H pylori*, the majority of individuals are asymptomatic. Pathological conditions associated with this bacterium are, however, severe, so the identification of potential clinical and laboratory markers to identify patients with a higher risk of PUD development is important for both clinical practice and patients' well-being.

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