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Antral atrophy, intestinal metaplasia, and preneoplastic markers in Mexican children with *Helicobacter pylori*–positive and *Helicobacter pylori*–negative gastritis

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ABSTRACT

Chronic inflammation and infection are major risk factors for gastric carcinogenesis in adults. As chronic gastritis is common in Mexican children, diagnosis of *Helicobacter pylori* and other causes of gastritis are critical for the identification of children who would benefit from closer surveillance. Antral biopsies from 82 Mexican children (mean age, 8.3 ± 4.8 years) with chronic gastritis (36 *H pylori*+, 46 *H pylori*−) were examined for gastritis activity, atrophy, intestinal metaplasia (IM), and immunohistochemical expression of gastric carcinogenesis biomarkers caudal type homeobox 2 (CDX2), ephrin type-B receptor 4 (EphB4), matrix metalloproteinase 3 (MMP3), macrophage migration inhibitory factor (MIF), p53, β-catenin, and E-cadherin. Atrophy was diagnosed in 7 (9%) of 82, and IM, in 5 (6%) of 82 by routine histology, whereas 6 additional children (7%) (3 *H pylori*+) exhibited aberrant CDX2 expression without IM. Significant positive correlations were seen between EphB4, MMP3, and MIF ($P < .0001$). Atrophy and follicular pathology were more frequent in *H pylori*+ biopsies ($P < .0001$), whereas IM and CDX2 expression showed no significant correlation with *H pylori* status. Antral biopsies demonstrating atrophy, IM, and/or aberrant CDX2 expression were seen in 21.95% (18/82) of the children, potentially identifying those who would benefit from closer surveillance and preventive dietary strategies. Biomarkers CDX2, EphB4, MMP3, and MIF may be useful in the workup of pediatric gastritis.

1. Introduction

Chronic inflammation is a risk factor for carcinogenesis in several tissues, including the stomach [1,2]. Inflammation is a well-coordinated response of the innate and adaptive immune systems following infection or injury [1]. Deregulation of the inflammatory response leads to unresolved inflammation and a proneoplastic microenvironment [1]. The tissue damage produced by high levels of phagocyte-generated reactive oxygen, nitrogen, and halogen species can cause mutations and cell death and play a key role in the carcinogenic process [2].

Chronic gastritis in children has multiple etiologies, including gastroesophageal reflux, food allergies, high intake of spicy food, acid peptic disease, nonsteroidal anti-inflammatory drugs, and *Helicobacter pylori* infection. *H pylori* infection increases the production of reactive oxygen and nitrogen species, and the gram-negative microaerophile confers nearly an 11-fold increased risk of gastric cancer (GC) [3]. Infection with *H pylori* is highly prevalent among socially and economically disadvantaged children. Age, overcrowding, number of siblings, and a low maternal education level increase infection risk [4–7].

Globally, GC is the fourth most common cancer and second highest cause of cancer mortality with nearly two-thirds of these deaths occurring in developing nations [3].

Although we seldom see GC in children, these issues are of keen interest in underdeveloped countries where *H pylori* is highly prevalent. Gastric carcinogenesis is hypothesized to be a process involving a number of premalignant genetic and morphologic alterations of gastric mucosa. Busuttil and Boussioutas [3] outline the progression from normal stomach to gastritis and intestinal

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metaplasia (IM). Intestinal metaplasia is considered a preneoplastic lesion, although it should be noted that not all IM advances to dysplasia, the step previous to GC [8,9].

Given the important role of chronic inflammation in carcinogenesis, we sought to determine whether Mexican children with a pathologic diagnosis of chronic antral gastritis exhibited histologic markers associated with adult preneoplastic lesions. Secondly, because caudal type homeobox 2 (CDX2) expression precedes the development of gastric preneoplastic lesions in the setting of IM, we sought to define the *H pylori* status and expression of CDX2 in our cohort of children and compare them with Mexican and American adult cohorts. Finally, we seek a panel of candidate biomarkers to use routinely in gastric biopsies in pediatric populations with a high prevalence of *H pylori* infection [7]. Therefore, we selected an immunohistochemical (IHC) protein profile involved in gastric carcinogenesis and progression: CDX2 [10], ephrin type-B receptor 4 (EphB4) [11,12], matrix metalloproteinase 3 (MMP3) [13,14], macrophage migration inhibitory factor (MIF) [15], p53 (TP53 tumor suppressor gene) [16], β-catenin, and E-cadherin [17,18]. Our ultimate goal is the identification of children with antral lesions who would benefit from closer follow-up surveillance, preventive nutritional strategies, and health promotion activities.

2. Materials and methods

2.1. Patients and samples

This study was conducted with the approval of the Central Military Hospital and the Medical College of Wisconsin Institutional Review Board. Consecutive gastric antral biopsy samples were obtained from 82 Mexican children (Table 1) of middle socioeconomic status attending the Central Military Hospital in Mexico City the first 3 months of 1996 and 2009.

Patients presented with 1 or more of the following symptoms: chronic epigastric or abdominal pain, pyrosis, or gastrointestinal bleeding. Gastroesophageal junction, antrum, and duodenum biopsies were examined by an attending hospital pathologist. Our cases did not include autoimmune gastritis, chemical gastritis, primary bile reflux gastritis, inadvertent sampling of the gastroduodenal junction, or postoperative gastritis, and none had received *H pylori* eradication therapy. The adult biopsies were used as controls to compare CDX2 and the gastritis criteria with the children's biopsy results. Thirty-five adult antral specimens were obtained from either Froedtert Hospital in Milwaukee, WI (n = 14), or the Mexican Institute of Social Security (n = 21) (Table 1).

2.2. Immunohistochemical staining

Biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. Four-micrometer-thick sections were deparaffinized in xylene, hydrated in descending dilutions of ethanol, and exposed to heat-induced epitope retrieval. See Table 2 for details.

Pediatric CDX2 and p53 IHC staining was performed manually. Immunohistochemistry for β-catenin, E-cadherin, adult CDX2, EphB4, MIF, MMP3, and *H pylori* was performed using reagents from the Dako Envision FLEX High pH kit and the Dako Autostainer Plus (Dako, Carpinteria, CA). Following pretreatment with target retrieval solution (pH 9.0), tissue was blocked with peroxidase-blocking reagent for 5 minutes, treated with phosphate-buffered saline/bovine serum albumin (EphB4, MIF, MMP3) for 30 minutes, incubated with primary antibody for 10 minutes (CDX2) or 30 minutes (β-catenin, E-cadherin, EphB4, MIF, MMP3) at room temperature, followed by 20-minute horseradish peroxidase, 10-minute 3,3′-Diaminobenzidine (DAB), and EnVision FLEX hematoxylin (Dako) counterstain.

Table 1
Patient age, sex, and *H pylori* status

Variables	Children (n = 82)	Adults (n = 35)
Age (y), mean (range)	8.1 (0.3-17)	66.3 (50-81)
Sex (female, male)	47, 35	18, 17
H pylori (+, −)	36, 46	21, 14

Table 2
Antibodies and pretreatment used

Antigen	Clone	Source	HIER	Dilution
β-Catenin	β-Catenin-1	Dako	TRS pH 9.0	RTU
CDX2 (children)	AMT28	Leica Microsystems	Citrate buffer pH 6.0	1/100
CDX2 (adults)	DAK-CDX2	Dako	TRIS pH 9	RTU
E-cadherin	NCH-38	Dako	TRIS pH 9	RTU
EphB4	3D7G8	Invitrogen	TRIS pH 9	1/50
H pylori	Rabbit	Dako	Citrate pH 6	RTU
MIF	D-2	Santa Cruz Biotech	TRIS pH 9	1:200
MMP3	10D6	R&D Systems	TRIS pH 9	1:50
p53	Pab 1801	Leica Microsystems	Citrate pH 6	1:500

Abbreviations: *HIER*, heat-induced epitope retrieval; *RTU*, ready to use; *TRS*, target retrieval solution.

2.3. Immunohistochemistry analysis

The percentage of total gastric gland epithelial cells staining positive was determined (EphB4, MIF, MMP3). The samples were analyzed by a pathologist (ACM and/or ALG) blind to gastritis parameter scores or *H pylori* infection status. To validate IHC, we stained various adult tissue specimens. These staining patterns were used as reference intensities for the gastric staining and scoring. β-Catenin, E-cadherin, and CDX2 were scored as either 1 (low) or 2 (high) staining intensity. β-Catenin expression was evaluated in membranous or nuclear location. Staining intensity for EphB4, MIF, and MMP3 was determined as 0 (none), 1 (mild), 2 (moderate), and 3 (strong). The most intense stain covering at least 10% of the biopsy was used as the intensity score. p53 was evaluated based on presence of nuclear positivity. Positive control tissues included breast cancer (p53), colon (β-catenin, E-cadherin, EphB4), duodenum (CDX2), and liver (MIF, MMP3).

Four criteria of chronic gastritis—gastritis activity, atrophy, follicular pathology, and IM—were evaluated by a pathologist (ALG). Histologic classification of all biopsies' hematoxylin and eosin (H&E) stains was done according to the Updated Sidney System [19].

Warthin-Starry stain was used for the detection of *H pylori* in the US adult samples. A modified Giemsa stain was used to detect *H pylori* in the specimens from the Mexican children and adults. All pediatric samples that were *H pylori* negative were confirmed with *H pylori* IHC.

2.4. Statistical analysis

Statistical analyses were performed using SAS 9.2 Statistical software (SAS Institute Inc., Cary, NC, USA). The correlation between the antibodies and gastritis parameters was measured by Spearman rank-order test. The association between *H pylori* status and gastritis criteria was investigated by Pearson χ^2 test. The association of antibody staining intensities with *H pylori* infection status as well as the association of CDX2 with *H pylori* infection status for children and adults was tested by Fisher exact test. Significance was set at $P < .05$.

3. Results

The distribution of selected gastritis histopathology and IHC variables in the pediatric cohort is shown in Table 3. Antral atrophy