Journal of Cytology & Histology

Rodríguez-Velasco et al., J Cytol Histol 2014, 5:4
DOI: 10.4172/2157-7099.1000250

Research Article Open Access

Histopathological Findings in Duodenal Biopsy of a Mexican Children Population with Gastritis by *Helicobacter Pylori*

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Rec date: Feb 28, 2014, **Acc date:** May 23, 2014, **Pub date:** May 25, 2014

Abstract

Introduction: Chronic inflammation and duodenal gastric metaplasia (DGM) increase with the presence of *Helicobacter pylori* (Hp). DGM is considered the earliest change in the development of duodenal ulcer in children with Hp gastritis. Until now, histopathological duodenal changes in children with Hp associated gastritis had not been studied in Mexico, where there is a high gastric Hp prevalence.

Objective: This study aims to describe the histopathological findings of duodenal biopsy in a Mexican children population with Hp-associated gastritis.

Materials and Methods: This cross-sectional, retrospective and analytical study examined, using light microscopy, the gastric and duodenal biopsies from a consecutive series of cases of children with and without Hp gastritis, received from 2000 to 2007. In the duodenal biopsy, active or non-active chronic duodenitis and DGM were observed. The DGM was highlighted with alcian blue/PAS stain.

Results: 306 patients with a histopathological diagnosis of chronic gastritis, 95 with Hp and 211 without Hp, are included in this study. The frequency of chronic duodenitis in patients with chronic gastritis by Hp is 100%. We found the highest gastric Hp frequency ($p<0.001$) in the group of oldest patients (11 to 16 years). The frequency of DGM was higher (15.7% vs 7.5%) when there was gastric Hp ($p<0.019$). It was demonstrated that there is no correlation ($p>0.05$) between Hp-associated chronic gastritis and the following: alteration of villus height/crypt depth ratios, damage to surface enterocytes, loss of brush border, and duodenal ulcer.

Conclusions: In the Mexican children population with and without Hp chronic gastritis studied, chronic duodenitis is a very common histopathological finding. The DGM is related to the presence and density of gastric Hp. To confirm DGM, an ancillary method could be used in the endoscopic duodenal biopsy.

Keywords: Helicobacter pylori; Duodenal biopsy; Gastritis; Histopathological

Introduction

The prevalence of the *Helicobacter pylori* (Hp) infection in different children populations varies mainly with socioeconomic conditions, with less than 10% in industrialized countries to more than 80% in developing countries [1,2]. Studies indicate that in México, national Hp seroprevalence increases with age: from 20% in children as young as 1 year old up to 50% by the age of 10, and approximately 70% of the population is infected by the age of 20 [3,4]. The microorganism is the most prevalent gastric microbial pathogen. In a group of individuals infected with Hp, approximately 10% to 20% develop gastroduodenal disease, while in a group of children with chronic nodular gastritis, depending on the country, up to a 100 % might present Hp infection [3,4]. The Hp associated gastritis frequently appears with nausea, vomiting, and epigastric pain, lasting from weeks to months [4,5]. One of the primary indications for upper gastrointestinal (GI) endoscopy in children is the presence of chronic abdominal pain, and the endoscopic mucosal gastric biopsy, to date, remains as the gold standard for the diagnosis of Hp gastritis, because noninvasive methods do not help predict active inflammation or assess the success of eradication therapy [5]. The histopathological findings of Hp-associated gastritis may be seen in children as early as 2 to 3 years old. The prevalence and density of lymphocyte aggregates and follicles, the most common histopathological findings, strongly correlate with the severity of the inflammation of the antral mucosa and the presence of neutrophils with activity. According with some authors, antral chronic inflammation causes duodenal gastric metaplasia (DGM), with the consequent development of duodenal inflammation and possible ulceration [6]. The prevalence of DGM has been observed in 13% to 42% of children infected with Hp [5-7], however, there is no definitive correlation between the influence of chronic duodenitis and duodenal ulceration with the presence DGM [8]. DGM is common in the proximal duodenum and in persons over 7 years of age [9-11]. Duodenal ulcer prevalence increases with age, and its higher incidence is related with the chronic gastritis associated with Hp infection. Even though Hp causes duodenal ulcers, the organism is not found in duodenal mucosa except in instances of

DGM. The microscopic diagnosis of chronic duodenitis is mainly established by an increase in lamina propria of inflammatory cells. The activity of the duodenitis depends on the infiltration of neutrophils. The aim of this study is to investigate the duodenal changes in children with Hp gastritis, because endoscopic gastric and duodenal biopsies are frequent and there is no information related to histopathological duodenal changes for Mexican children with Hp gastritis. The study was carried out in a tertiary-care hospital where all the gastric and duodenal mucosal biopsies included were done to children for chronic abdominal pain, persistent after a non-specified pharmacological therapy given elsewhere.

Materials and Methods

At the "Hospital de Pediatría, Centro Médico Nacional Siglo XXI, del Instituto Mexicano del Seguro Social (HP CMN S. XXI IMSS)", a tertiary-care medical center, children with chronic abdominal pain undergo upper GI endoscopy. Gastroenterologists routinely take biopsies of the gastric and duodenal bulb mucosa for routine pathologic examination. We performed a retrospective cross-sectional and a comparative analytical study related with the histopathological findings in the duodenal biopsies between groups of children with and without Hp gastritis. All the slides collected in a period of eight years (January 2000 to December 2007) of gastric (corpus and antrum) endoscopic biopsies from children that range from one month to 17 years old, of any sex with the histopathological diagnosis of non-specific gastritis and Hp gastritis, accompanied with endoscopic mucosal biopsy from the duodenal bulb, were eligible for inclusion. The slides were retrieved and reviewed by two pathologists. Only biopsies that were well oriented were used. The age and sex were obtained from the study request.

Because the prevalence of non-specific vs. Hp gastritis of cases reviewed at the service is approximately 3:1, 70 biopsy specimens with Hp infection (cases) and 220 with non-specific gastritis (controls) were included, although in the literature it has been reported to be 5:1 [8,10].

Both gastric and duodenal biopsies were evaluated using light microscopy with standard hematoxylin and eosin (H&E) staining. Warthin-Starry staining was used to identify Hp where there was histopathological follicular gastritis but Hp was not visible with H&E staining.

The amount of gastric Hp and gastric inflammation was classified according to the Updated Sydney Classification System [12], in one of the three following groups: mild, moderate, and severe. Duodenitis is characterized by an increase in lamina propria inflammatory cells, regardless of atrophy (alteration of villus height/crypt depth ratio) (Figure 1A). If, in addition, neutrophils had infiltrated the lamina propria, the inflammation was classified as "active". Of each well oriented, not crushed duodenal biopsy, the alcian blue/periodic acid Schiff (alcian blue/PAS) staining was carried out for DGM identification (Figure 1B). For the purpose of the present study, DGM is defined by the presence of adjacent surface epithelial cells (in the apical portion or in the lateral side of the duodenal villous), without brush border and containing PAS-positive neutral mucin [13-15]. The DGM is classified as complete, incomplete, and intermediate, according to our criteria. In the metaplasic cells, abundant mucin characterizes the complete type; cubical cells contain little mucin in the incomplete type, whereas the intermediate type is between the first two. The slides were also observed and assessed for characteristics of the brush border, damage of the superficial enterocytes, intraepithelial lymphocytes count (with a normal average value of less than 1-5 in 20 enterocytes at the tip of five villi) and duodenal ulcer.

There was no follow up done to the patients. The Institutional Research Board approved this study.

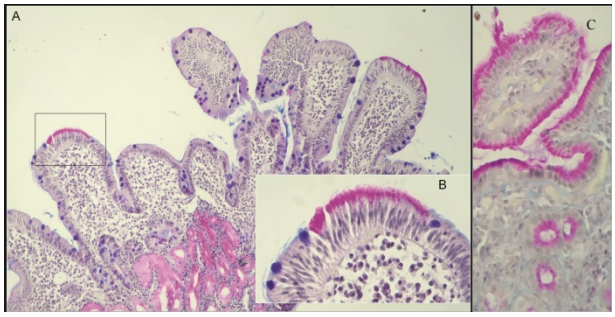

Figure 1: A. On alcian blue–periodic acid-Schiff staining, the duodenal epithelium shows gastric metaplasia. Note increase inflammatory infiltrate in lamina propria, also wide and short villi. B. Intermediate of A and C. C. Normal gastric epithelium

Statistical analysis

A descriptive statistic and bivariate analysis of the population conditions was performed. A stratified analysis was conducted for age and sex. The proportion of the pathological changes in the qualitative variables of both groups was compared using either a χ^2 test or a Fisher's exact test as appropriate. Ordinal variables were compared in tables of 2 x 3 looking for a linear association, with χ^2 of linear by linear trend. Comparison of risk factor between group data for continuous variables was assessed with the use of a test for independent variables or a Mann-Whitney test, as appropriate. Odds ratio (OR) and the 95% confidence intervals (CI 95%) of the main duodenal histopathological findings were calculated. A p value < 0.05 was considered as significant. The analysis was performed using the SPSS software, Version 11.0 (SPSS, Inc., Chicago, IL).

Results

A total of 306 slides were selected from a period of eight years, representing the total number of patients included in this study. A total of 506 biopsies were taken from duodenal bulbs (1 to 5 per patient, 1.6 on average). Ninety-five (31%) had been diagnosed with Hp gastritis, whereas 211 (69%) with non-specific gastritis. Hp density in the gastric biopsy and the frequency of the other histopathological duodenal changes are summarized in Table 1.

There was no difference related with patients' sex in any of the two forms of gastritis. There were 161 men [43 (27%) with Hp and 118 (73%) without Hp] and 145 women [47 (32%) with Hp and 98 (68%) without Hp].

Characteristics	n	%

Helicobacter pylori in the gastric biopsy		
Absent	211	69
Present		
+ (mild) ++ (moderate) +++ (intense)	64 20 11	20.9 6.5 3.6
Duodenal gastric metaplasia		
Absent Incomplete Intermediate Complete	275 20 10 1	89.9 6.5 3.3 0.3
Chronic duodenitis		
Absent Mild Moderate Severe	6 142 139 19	2.0 46.4 45.4 6.2
Activity of the duodenitis		
Absent Present	257 49	84 16
Helicobacter pylori in duodeno		
Absent Present	300 6	98 2
Alteration of Villus height: crypt depth ratio		
Absent Present: Partial atrophy	121 185	39.5 60.5
Crypt hyperplasia		
Absent Present	124 182	40.5 59.5
Damage to the superficial enterocytes		
Absent Present	229 77	74.8 25.2
Brush border loss		
Absent Present	272 34	88.9 11.1
Abnormal count of intraepithelial lymphocytes		
Absent Present	297 9	97.1 2.9
Duodenal ulcer Absent	295	96.4
Present	11	3.6

Table 1: Histopathological findings of 306 gastric and duodenal biopsies

The age of the patients ranges between 1 and 16 years, with an average of 8.06 (± 4.7 years). For the cases with gastric Hp the average age is 9.96 (± 4.4) years, whereas for the comparative group it is 7.2 (± 4.6), p<0.001. When analyzing the association between duodenitis intensity (mild, moderate or severe) and Hp vs. non-Hp gastritis, we found an increase in the intensity of the lamina propria inflammation, although it was not significant (p=0.086).

The bivariate analysis of the duodenal findings related with gastritis according to each one of our dependent variables in the cases with and without Hp, are presented in Table 2. DGM appears in greater proportion in the cases where gastritis shows a lower density of Hp (p<0.025). The alterations of villus height/crypt depth ratios and the damage to the superficial enterocytes have greater frequency with smaller density of Hp.

Duodenal biopsy: Histopathological findings	**Without Hp n=211 (%)**	**Relation between chronic duodenitis and gastric Hp density n=95 (%)**				
			64 + (67.36)	**20 ++ (21.05)**	**11 +++ (11.57)**	**p***
Chronic duodenitis	205 (97.2)	95 (31.0)				0.086
Mild Moderate Severe	101 (47.86) 93 (44.07) 11 (5.21)	41 (43.15) 46 (48.42) 8 (8.42)				
Duodenal gastric metaplasia (DGM)	16 (7.5)	15 (15.78)	10 (10.52)	2 (2.1)	3 (3.15)	0.025
Incomplete Intermediate Complete	11 (5.21) 5 (2.36) 0	9 (9.47) 5 (5.26) 1 (1.05)				
Active duodenitis	30 (14.2)		14 (14.7)	4 (4.21)	1 (1.05)	0.536
Duodenal HP	0 (0)		1 (1.05)	4 (4.21)	1 (1.05)	0.000
Alteration of villus height:crypt depth ratios	132 (62.6)		41 (43.15)	9 (9.47)	3 (3.15)	0.025
Crypt hyperplasia	81 (38.4)		23 (24.21)	3 (3.15)	0 (0)	0.003
Damage to the superficial enterocytes	38 (18)		28 (29.47)	7 (7.36)	4 (4.21)	0.001
Loss of the brush border	15 (7.1)		12 (12.63)	4 (4.21)	3 (3.15)	0.001
Abnormal count of intraepithelial lymphocytes	6 (2.8)		2 (2.1)	1 (1.05)	0 (0)	0.99
Duodenal ulcer	4 (1.9)		3 (3.15)	3 (3.15)	1 (1.05)	0.005
*p. $\chi 2$ of linear by linear trend. Intensity. Hp density: + mild, ++ moderate, +++ severe						

Table 2: Bivariate analysis of the histopathological duodenal findings in cases with and without gastric *Helicobacter pylori* (Hp)

In chronic duodenitis, there seems to be no relation between activity and Hp density. The difference in the abnormal count of intraepithelial lymphocytes between the two groups is not statistically significant; none of these patients were diagnosed with coeliac disease.

Other duodenal findings were duodenal giardiasis (one case), and a pathological eosinophilic infiltrate (more than 20 per high-powered field) that was found in thirteen cases, where allergy diagnosis was suggested, both of them have etiologic relevance.

Figure 2 shows the relation between age and Hp density (p<0.001). The comparison between duodenal histopathological findings in both groups is presented in Table 3. It shows the association between DGM, duodenal Hp, crypt hypertrophy, damage to the superficial enterocytes, brush border loss, and duodenal ulcer. In comparison with non-Hp gastritis, Hp gastritis presents a greater association with DGM (p<0.045), duodenal Hp (p<0.001), duodenal ulcer (p<0.018), brush border loss (p<0.001) and damage of the superficial enterocyte (p<0.001).

Discussion

The limits of duodenal normality in duodenal bulb biopsies are difficult to define because chronic inflammatory cells in duodenal bulb lamina propria vary greatly in number from villus to villus; therefore the assessment of duodenal bulb inflammation is highly subjective. In our study lamina propria chronic inflammatory cells is the main criterion for duodenitis and might be the reason because of duodenitis percentage bing so high (98%), in contrast to what other authors report (Table 4) [1,6,7], particularly with the 6.08% reported by Gormally et al. [7] which can be explained by the difference in criteria used for the diagnosis, theirs being more specific. They only diagnose duodenitis when there are neutrophils in the lamina propria and an increase in the density of mononuclear cells; whereas we relate the presence of neutrophils only with active duodenitis. Then the subtle morphologic criteria for the diagnosis of chronic duodenitis, when mild, as in most of our cases (46.4%), chronic duodenitis might be overdiagnosed. From a practical point of view, since the diagnosis of

duodenitis is subjective and difficult (specially on the milder changes), a correct histological diagnosis and classification of duodenitis could be reached in nearly all our patients if the simultaneous histological examination of bulb mucosa and endoscopically healthy mucosa of the lower duodenum is established as a routine method, as has been already suggested [16]. The histopathological criteria for mild duodenitis must be well established in order to eliminate its overdiagnosis.

Nº (%)	**Histopathological findings in the duodenal biopsy N=306**									
	CD 300 (98)	**DGM 31 (10.1)**	**Act D 49 (16.1)**	**Hp D 6 (1.9)**	**V:C AR 185 (60.4)**	**HC 182 (59.4)**	**SED 77 (25.1)**	**BBL 34 (11.1)**	**IEL AC 9 (2.9)**	**DU 11 (3.5)**
With Hp n=95	95 (31.6)	15 (48.4)	19 (38.8)	6 (100)	53 (28.6)	26 (24.2)	39 (50.6)	19 (55.8)	3 (33.3)	7 (63.6)
Without Hp n=211	205 (68.4)	16 (51.6)	30 (61.2)	0 (0)	132 (71.4)	81 (75.8)	38 (49.4)	15 (44.2)	6 (66.7)	4 (36.4)
OR CI	2.76 0.33-23.2	2.2 1.02-4.8	1.5 0.8-2.84	14.3 1.7-120	.755 0.46-1.23	0.6 0.33-0.95	3.17 1.85-5.43	3.26 1.57-6.75	1.114 0.27-4.55	4.116 1.17-14.4
p*	0.352	0.045	0.2	0.001**	0.26	0.032	0.001	0.001	0.881**	0.018**

Helicobacter pylori; OR, odds ratio; CI, confidence interval; CD, chronic duodenitis; DGM, duodenal gastric metaplasia; Act D, active duodenitis; HpD, duodenal Helicobacter pylori; V:C AR, villous:crypta alteration ratio; HC, crypt's hyperplasia; SED, superficial enterocytes damage; BBL, brush border loss; IEL AC, intraepithelial lymphocytes, abnormal count; DU, duodenal ulcer.*p $\chi 2$. ** p. Fisher's exact test (wait frequency < 5).

Table 3: Analysis of the duodenal histopathological changes in children with gastritis, with and without Hp

Author	**N° cases**	**Sex M:F**	**Age (main)**	**Hp**	**Duodenitis (%)**	**DGM (%)**		**Ulcera (%)**
						Hp	**Without Hp**	
Shabib et al. [9]	64	2.1:1	13.3 (± 3.4)	31	26 (40.6)	13 (42)	1 (3)	19 (29)
Gormally et al. [6]	148	1.2:1	8.8 (0.3-17.7)	25 (17 %)	9 (6.08)	11 (44)	23 (19)	7 (4.7)
Elitsur et al. [8]	173	0.88:1	11.1 (± 4.5)	60 (34.6)	19 (11)	7 (12)	16 (14.1)	0
This series	306	1.1:1	8.08 (± 4.7)	95 (31)	300 (98)	15(15.7)	16 (7.5)	11 (3.6)

Table 4: Comparison of different series for the main histopathological findings in the duodenal biopsy from children with Hp-associated gastritis

However, duodenitis is not related with Hp gastritis ($p<0.35$), contrary to what we expected; nevertheless, the finding of duodenal ulcer is significantly more frequent in the cases with Hp gastritis ($p<0.018$).

When comparing our series with others of similar characteristics [5,6,8], we find a smaller percent (3.6%) of patients with duodenal ulcer. Only six cases with Hp gastritis had duodenal Hp. A subgroup of patients with Hp gastritis younger than a year of age (4.2%) had mild (+) Hp density. As Calva et al. report [17], we found that in the studied population Hp gastritis is more frequent in the group of older patients ($p<0.001$), regardless of it being high or low density, in addition, we also found that cases with higher gastric Hp density are less frequent (Figure 2).

In order to identify DGM in the pediatric age, a duodenal biopsy is necessary for children presenting chronic abdominal pain. To date, neither our service nor any pediatric hospital in Mexico City uses a routine method to identify DGM, thus, it cannot be diagnosed. The same happens with staining to identify duodenal Hp. We suggest the use of alcian blue/PAS to identify DGM and modified Giemsa's for Hp. In our study, it has been demonstrated that DGM is frequently present in patients with Hp gastritis ($p<0.025$) but DGM is not a consequence exclusively of Hp presence, it can also be found in non-Hp gastritis cases, although in a small proportion (7.5%). Chronic duodenitis with DGM is associated with damage to the superficial enterocytes ($p<0.001$) and loss of brush border ($p<0.001$). This means that DGM, damage to the superficial enterocyte, and loss of brush border always go together.

Our results don't show relation between the gastritis type and presence of duodenitis activity, alterations of the villous/crypt ratio, or abnormal count of intraepithelial lymphocytes (Table 3).

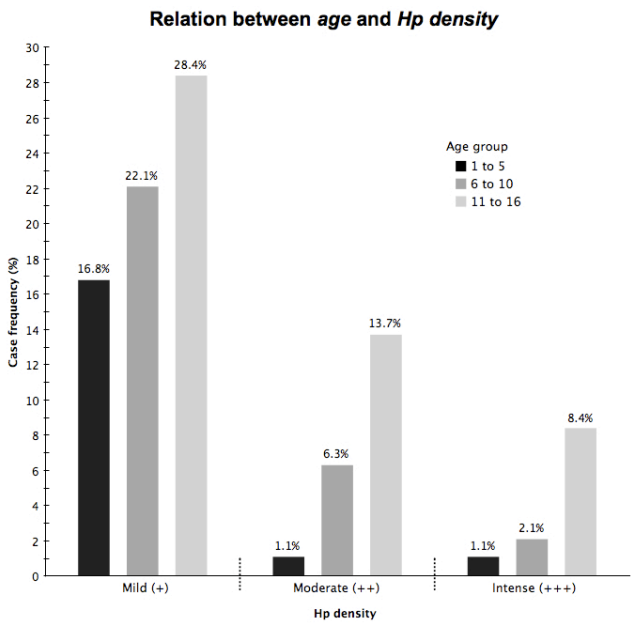

Figure 2: The graphic shows that Hp gastritis is more frequent in older patients ($p<0.001$). The distribution of children for age is: from 1 to 5 111 (36.27%); 6 to 10, 91 (29.7%) and 11 to 16, 104 (33.9 %). In addition, we also found that with increasing gastric Hp density the frequency of cases decreases

It has been suggested that duodenitis development follows the colonization of metaplasic duodenal epithelium by Hp [5,6], nevertheless, our observations do not support this opinion, because all our cases with DGM are associated with duodenitis, and because 51.6% of total DGM cases are not associated with gastric Hp. On the other hand, it has been reported that the gastric epithelium in the duodenum is not necessarily a protective change; it can be either a congenital or an ectopic condition [8]. Although it is definitely clear that DGM has an intimate relation with duodenal ulcer, a considerable evaluation between both conditions is required. One would presume that DGM would predispose to DU, but this is not clear here and is also no clear from the literature.

In Table 4, we compare the duodenal histopathological findings of our cases to three similar series [6,8,9]. We account for the greatest number of cases. Many additions to the study would have added interest, but are feasible in a retrospective study. Some of those additional bits of information are: urease test on gastric biopsies, IL1 beta polymorphism analysis, gastric pH and serum gastrin levels. The following step in this study would be a follow up of all patients with DGM, correlate the anatomopathological findings with clinical data, including the pharmacological treatment received before de GI biopsies; thus improving the quality of attention to each one of these children by monitoring one of the risk factors related with the development of duodenal ulcer: DGM. It is necessary that gastroenterologists accurately report endoscopic findings and precise biopsy site in order to be able to establish a suitable clinical-pathological correlation.

The strongest feature of this study is that it is the first qualitative comparative study in Mexico about duodenal architectural features between children with and without Hp gastritis; nevertheless, it is limited by the absence of more detailed clinical information. Prospective and longitudinal studies of pediatric duodenal biopsies in patients with Hp gastritis would allow better knowledge in the development of duodenal ulcer and duodenal gastric metaplasia.

Acknowledgment

We thank Mr. Jose Alberto Castillo Naranjo, histotechnologist of the Service of Pathology of the UMAE Hospital of Ped CMN S. XXI, IMSS, for his valuable collaboration in the accomplishment of special staining.

References

1. Torres J (2000) [Epidemiologic and clinical aspects of Helicobacter pylori infection in children]. Rev Gastroenterol Mex 65: 13-19.
2. Brown LM (2000) Helicobacter pylori: epidemiology and routes of transmission. Epidemiol Rev 22: 283-297.
3. Torres J, Leal-Herrera Y, Perez-Perez G, Gomez A, Camorlinga-Ponce M, et al. (1998) A community-based seroepidemiologic study of Helicobacter pylori infection in Mexico. J Infect Dis 178: 1089-1094.
4. Torres J, Pérez-Pérez G, Goodman KJ, Atherton JC, Gold BD, et al. (2000) A comprehensive review of the natural history of Helicobacter pylori infection in children. Arch Med Res 31: 431-469.
5. Mittal SK, Mathew JL (2003) Helicobacter pylori infection in children: a review. Trop Gastroenterol 24: 106-115.
6. Gormally SM, Kierce BM, Daly LE, Bourke B, Carroll R, et al. (1996) Gastric metaplasia and duodenal ulcer disease in children infected by Helicobacter pylori. Gut 38: 513-517.
7. Elitsur Y, Triest WE (1997) Is duodenal gastric metaplasia a consequence of Helicobacter pylori infection in children? Am J Gastroenterol 92: 2216-2219.
8. Shabib SM, Cutz E, Drumm B, Sherman PM (1994) Association of gastric metaplasia and duodenitis with Helicobacter pylori infection in children. Am J Clin Pathol 102: 188-191.
9. Wyatt JI, Rathbone BJ, Dixon MF, Heatley RV (1987) Campylobacter pyloridis and acid induced gastric metaplasia in the pathogenesis of duodenitis. J Clin Pathol 40: 841-848.
10. Chen XY, Liu WZ, Shi Y, Zhang DZ, Xiao SD, et al. (2002) Helicobacter pylori associated gastric diseases and lymphoid tissue hyperplasia in gastric antral mucosa. J Clin Pathol 55: 133-137.
11. Rotimi O, Cairns A, Gray S, Moayyedi P, Dixon MF (2000) Histological identification of Helicobacter pylori: comparison of staining methods. J Clin Pathol 53: 756-759.
12. Dixon MF, Genta RM, Yardley JH, Correa P (1996) Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 20: 1161-1181.
13. Harris AW, Walker MM, Smolka A, Waller JM, Baron JH, et al. (1996) Parietal cells in the duodenal bulb and their relation to Helicobacter pylori infection. J Clin Pathol 49: 309-312.
14. Serra S, Jani PA (2006) An approach to duodenal biopsies. J Clin Pathol 59: 1133-1150.
15. Rubio CA (2002) A simple method to demonstrate duodenal gastric metaplasia. J Clin Pathol 55: 520-523.
16. Schmitz-Moormann P, Pittner PM, Reichmann L, Massarat S (1984) Quantitative histological study of duodenitis in biopsies. Pathol Res Pract 178: 499-507.
17. Calva-Rodríguez R, Luna-Alcántara JJ, Lagunes-Yannelli B, Rivera-Domínguez ME, Calva-Cerqueira D, et al. (2006) [Prevalence and risk faactors of Helicobacter pylori infection in three populations of children in Puebla, Mexico]. Rev Gastroenterol Mex 71: 440-445.