

## Antibodies to *Helicobacter pylori* and Pepsinogen Levels in Children from Costa Rica: Comparison of Two Areas with Different Risks for Stomach Cancer

R. Sierra, N. Muñoz,<sup>1</sup> A. S. Peña, I. Biemond, W. van Duijn, C. B. H. W. Lamers, S. Teuchmann, S. Hernandez, and P. Correa

Instituto de Investigaciones en Salud, Universidad de Costa Rica, San José, Costa Rica [R. S., S. H.]; Unit of Field and Intervention Studies, International Agency for Research on Cancer, Lyon, France [N. M., S. T.]; Department of Gastroenterology, University Hospital, Leiden, the Netherlands [A. S. P., I. B., W. v. D., C. B. H. W. L.]; Louisiana State University Medical Center, New Orleans, Louisiana 70112-1393 [P. C.]

### Abstract

In children and adolescents from two areas of Costa Rica with contrasting gastric cancer risks, two factors suspected to be linked to the natural history of the disease were tested: serum antibodies to *Helicobacter pylori* and serum pepsinogen levels. One hundred fifty-five subjects from the high-risk area of Turubares were compared to 127 from the low-risk area of Hojanca. No significant differences were found in the prevalence of IgG or IgA antibodies to *Helicobacter pylori* between the two regions. The prevalence of IgG was 65.8% in the high-risk area and 72.4% in the low-risk area, and that of IgA was 43% in both areas. The levels of pepsinogen, especially pepsinogen C, were significantly elevated in subjects with *H. pylori* antibodies in their serum. The mean levels of pepsinogen C in those negative, positive, and strong positive for *H. pylori* antibodies were 8.7, 14.3, and 21.1 ng/ml. These findings suggest that *H. pylori*-associated gastritis, predominantly of antral localization, is very prevalent in Costa Rican children and adolescents. Such gastritis might be associated with a high prevalence of intestinal metaplasia and a high gastric cancer risk in the inland, but not the coastal rural populations. *H. pylori* may therefore be an insufficient cause whose role in gastric carcinogenesis is contingent upon the presence of other factors.

### Introduction

Stomach cancer is the most frequently occurring malignant tumor in Costa Rica (1), and the mortality rate from this tumor is the highest for any type of cancer in the world at the present time (2). Large intracountry differences in the incidence rates of gastric cancer are reported in this small country of 2.5 million inhabitants and 51,000

km<sup>2</sup>. The regions with the highest rates of gastric cancer (84.2/100,000 in males) and of precancerous lesions are situated in the highland area of the center of the country, and those with the lowest rates (25.4/100,000) are in the lowland and coastal areas (1, 3).

*Helicobacter pylori* infection has been linked to chronic gastritis, and recent studies have shown that it is also associated with an increased risk of gastric carcinoma (4, 5). Furthermore, it has been postulated that *H. pylori* infection during childhood might be one of the main determinants of stomach cancer risk later in life (6). Serum pepsinogen levels reflect the degree of gastric atrophy and therefore have been used as markers of precancerous lesions of the stomach (7, 8). Pepsinogen is present in the serum in two immunochemically distinct forms: PGI,<sup>2</sup> produced by the oxyntic cells of the body of the stomach decrease with increasing degrees of chronic atrophic gastritis, and PGII, which originates from the gland cells throughout the stomach and from the Brunner's glands in the duodenum, shows less variation with gastric atrophy (9).

In order to explore the reasons for contrasting gastric cancer risks in Costa Rica and to gather more information on the role of *H. pylori* infection in the early stages of gastric carcinogenesis, we have examined the prevalence of IgG and IgA serum antibodies for *H. pylori* and the serum levels of pepsinogens in a group of children and young adults from a rural area with a high incidence of gastric cancer (Turubares) and from a rural area at low risk for gastric cancer (Hojanca).

### Materials and Methods

Two areas were selected for the study: one county in Turubares with an age-adjusted incidence rate of stomach cancer of 49.2/100,000 and another in Hojanca with an incidence rate of 20.3/100,000 for the period of 1984 to 1988. These two counties were selected because, despite having different incidence rates of stomach cancer, they were similar in many respects: both were rural with a tropical climate (minimum temperature, 26°C; maximum temperature, 33°C) and have mestizo populations of low socioeconomic status, although the proportion of household heads who cultivated their own land was greater in the low-risk area (37.5%) than in the high-risk area (0%). A dietary survey conducted in the two study areas revealed similar dietary habits, although

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<sup>1</sup> To whom requests for reprints should be addressed, at International Agency for Research on Cancer, 150, cours Albert Thomas, F-69372 Lyon cedex 08, France.

<sup>2</sup> The abbreviations used are: PGI or PGA, pepsinogen I or pepsinogen A; PGII or PGC, pepsinogen II or pepsinogen C; AI, absorbance index.

Table 1 Prevalence of IgG and IgA-*H. pylori* antibodies by age and study area in Costa Rica

Study areas	Age group (years)	No. of subjects	Anti-IgG		Anti-IgA	
			% positive (AI > 0.32)	Mean	% positive (AI > 0.35)	Mean
High-risk	7-10	61	60.7	0.46	39.3	0.40
	11-13	48	66.7	0.51	47.9	0.37
	14-16	27	70.4	0.54	40.7	0.38
	17-20	19	73.7	0.53	47.4	0.46
	All ages	155	65.8	0.50	43.2	0.39
<i>P</i> (test for linear trend)			0.22		0.60	
Low-risk	7-10	36	57.1	0.41	30.6	0.29
	11-13	58	77.6	0.57	43.1	0.42
	14-16	22	80.9	0.65	54.5	0.46
	17-20	10	77.8	0.63	70.0	0.47
	Unknown	1				
All ages	127	72.4	0.54	43.3	0.40	
<i>P</i> (test for linear trend)			0.05		0.01	

the intake of certain nutrients was lower in the high-risk area. Details of this survey will be published elsewhere.

Two schools that were easily accessible by car and served the less isolated regions of the county were selected in each study area. All children attending the selected schools were invited to participate, and over 90% of them agreed to participate and were included in the study.

Blood samples were collected from children and young adults from the two study areas: 153 from the high-risk area and 123 from the low-risk area. Children 7-15 years of age were invited to participate at the schools, and those for whom consent was obtained from the parents (most of those invited) were included in the study. Subjects 15-20 years of age were invited to participate through local leaders and local health workers. A 5-10-ml blood sample was collected from each participant. On the day of collection serum samples were separated and stored at  $-20^{\circ}\text{C}$ . After a few weeks, the frozen samples were transferred to the Netherlands and analyzed for *H. pylori* antibodies. The analyses for pepsinogens were done 2 years after collection and storage at  $-20^{\circ}\text{C}$  in the same laboratory.

**Anti-*H. pylori* Antibodies.** IgG and IgA specific antibodies against *H. pylori* were measured by a modified enzyme-linked immunosorbent assay technique using conjugates labeled with immunoperoxidase specific for human IgA and IgG as described by Peña *et al.* (10). For standardization of the measurement of these antibodies, test conditions were chosen such that the absorbance of the standard reference serum was  $0.5 \pm 0.1$  for IgA and  $1.0 \pm 0.1$  for IgG. These values were used to correct the absorbance given by the sera under study. The results were expressed as the AI:

AI =

$$\frac{\text{Mean absorbance reading } (n = 2) \text{ of patient's serum} - \text{mean absorbance of blank reading}}{\text{Mean absorbance reading } (n = 2) \text{ of reference serum} - \text{mean absorbance of blank reading}}$$

Correlation analysis of the immunoblotting and enzyme-linked immunosorbent assay results showed that at a serum dilution of 1:200 and under the conditions defined above, a serum with an AI > 0.32 for IgG anti-*H. pylori* was positive above the 95% level. A correlation analysis between IgG and IgA showed that an AI > 0.353 for IgA-anti-*H. pylori* was positive above the 95% level.

Under these conditions the sensitivities and specificities of the serological assay were 89% and 88% for IgG and 78% and 88% for IgA when compared with *H. pylori* detected by culture or Warthin-Starry stain (10).

**Pepsinogen Levels.** Pepsinogen A and C (PGI and PGII) were measured in duplicate by specific sensitive radioimmunoassays (11). Purified human Pepsinogens A and C were used as standard preparations. Serum samples were diluted 10-fold for Pepsinogen A (PGA) and 5-fold for Pepsinogen C (PGC) and were incubated with labeled pepsinogen and antibody in a final volume of 1 ml, for 4 days, at  $4^{\circ}\text{C}$ . Free pepsinogen was subsequently separated from antibody-bound pepsinogen by a double-antibody solid-phase technique, and the pellets were counted in a gamma scintillation counter. All samples were measured in the same assays. Intraassay variation varied from 3 to 10% for PGA and from 13 to 15% for PGC. The detection limit for the PGA radioimmunoassay was  $0.12 \mu\text{l/liter}$  and for the PGC radioimmunoassay  $1.8 \mu\text{l/liter}$ . Dilution curves of a serum sample containing high concentrations of the pepsinogens were parallel to the standard curve. Aliquots of a subsample of 20 sera were also tested at Dr. Samloff's laboratory using a radioimmunoassay as described elsewhere (7).

Differences in the mean values of *H. pylori* antibodies and pepsinogen levels were compared using the *t* test after logarithmic transformation and the Mann-Whitney *U* test. Correlation between pepsinogen levels and *H. pylori* antibodies was tested using the Spearman's rank test.

## Results

In the high-risk area a total of 65.8% of the subjects tested were positive for IgG *H. pylori* antibodies (AI >

Table 2 Mean values of serum PGA and PGC levels (ng/ml) by age and study area

Study areas	Age (years)	No. of subjects	PGA		PGC		PGA/PGC	
			Means	(±SD)	Means	(±SD)	Means	(±SD)
High-risk	7-10	61	35.5	(±11.3)	13.5	(±7.2)	3.3	(±2.5)
	11-13	48	39.8	(±13.8)	16.1	(±13.1)	3.2	(±1.5)
	14-16	27	39.7	(±13.6)	13.4	(±6.6)	3.3	(±1.3)
	17-20	19	38.4	(±11.8)	12.7	(±7.7)	4.1	(±3.0)
	All ages	155	37.9	(±12.6)	14.2	(±9.3)	3.4	(±2.1)
Low-risk	7-10	36	34.8	(±13.6)	11.0	(±7.2)	4.9	(±4.9)
	11-13	58	41.4	(±15.0)	15.2	(±8.6)	4.3	(±5.8)
	14-16	22	33.8	(±12.9)	14.4	(±11.2)	3.6	(±2.2)
	17-20	10	47.0	(±25.8)	18.1	(±12.7)	4.0	(±2.6)
	Unknown	1						
	All ages	127	38.6	(±15.5)	14.1	(±9.2)	4.3	(±4.8)

0.32), and 43.2% were positive for IgA antibodies ( $AI > 0.35$ ). The corresponding figures for the low-risk area were 72.4% and 43.3%. Table 1 shows the prevalence of IgG and IgA antibodies to *H. pylori* by age in the two study areas. An increase in the prevalence of *H. pylori* antibodies with increasing age was observed, but the test for linear trend for the percentage of positives in the four age groups was significant only in the low-risk area ( $P = 0.05$  for IgG and 0.01 for IgA). The distribution of IgG antibodies in both high- and low-risk areas was clearly bimodal. No significant differences in the prevalence of IgG and IgA antibodies to *H. pylori* was found between males and females or between the high- and the low-risk areas for gastric cancer.

Table 2 summarizes the results of the serum pepsinogen levels. No differences were observed in the median or log-transformed mean levels of PGA, PGC, and in the PGA:PGC ratio between the two study areas. In both areas the distributions of PGA and PGC were skewed to the right, and no difference between the high- and low-risk area was observed in any of the age groups with the exception of 7-10-year-old children, in whom PGC levels were significantly higher in the high-risk area than in the low-risk area ( $P = 0.022$ ).

The mean levels of both PGA and PGC were significantly higher for males than for females ( $t$  test for difference between means:  $P < 0.001$  for PGA and  $P = 0.002$  for PGC). No correlation between PGA and PGC levels and age was observed among *H. pylori*-positive or *H. pylori*-negative children.

The relationship between the levels of IgG *H. pylori* antibodies and pepsinogen levels is summarized in Table 3. Higher levels of pepsinogens are observed in those positive or strongly positive for *H. pylori* antibodies, and this tendency was more marked for PGC. A positive

correlation was found between the levels of both variables. The mean levels of PGC in those negative, positive, and strongly positive for *H. pylori* antibodies were 8.7, 14.3, and 21.1 ng/ml, respectively.

The values for PGA obtained in Dr. Samloff's laboratory were on average twice as high as those obtained in the Netherlands, but those for PGC were only 10% higher. The correlation coefficient between the measurements in the two laboratories was 0.76 for PGA ( $P < 0.001$ ), and for PGC it was 0.96 ( $P < 0.001$ ).

Based on the results of the 20 sera tested in the two laboratories a correction index was applied to the rest of the sera, and the following results were obtained: the proportion of children with low levels of PGA (below 20 ng/ml) decreased from 3.6% to 0.7% and those with a low PGA:PGC ratio ( $<1.0$ ) decreased from 2.5% to 0.4%.

Concerning the relationship between the levels of *H. pylori* antibodies and pepsinogen levels, the proportion (percentage) of those negative, positive, and strongly positive for *H. pylori* among those with high levels of PGA changed from 8.0, 18.3, and 26.5% to 64.8, 73.8, and 85%, and these proportions among those with high levels of PGC changed from 3.4, 18.3, and 42.7% to 5.7, 32.5, and 57.4%.

Our pepsinogen categories were chosen based on cutoff values used in previous studies to identify subjects likely to be affected by moderate or severe chronic atrophic gastritis (7). Since the use of these cutoff points in our study population might be questionable, we have plotted the serum values of PGA and PGC against the *H. pylori* IgG titers of those considered positive, as shown in Fig. 1. A positive correlation with *H. pylori* antibodies was observed for both PGA and PGC, but it was clearer for PGC (for PGA,  $r = 0.16$ ,  $P = 0.025$ ; for PGC,  $r = 0.36$ ,  $P < 0.001$ ).

Table 3 IgG *H. pylori* antibodies and pepsinogen levels

IgG <i>H. pylori</i> antibodies (AI)	No. of subjects	Pepsinogen A			Pepsinogen C		
		<20 ng/ml (%)	20-49 ng/ml (%)	≥50 ng/ml (%)	<10 ng/ml (%)	10-19 ng/ml (%)	≥20 ng/ml (%)
Negative (≤32)	88	5.7	86.4	8.0	63.6	33.0	3.4
Positive (33-74)	126	3.2	78.6	18.3	22.2	59.5	18.3
Strongly positive (≥75)	68	1.5	72.1	26.5	8.8	48.5	42.7
Total	282	3.6	79.4	17.0	31.9	48.6	19.5

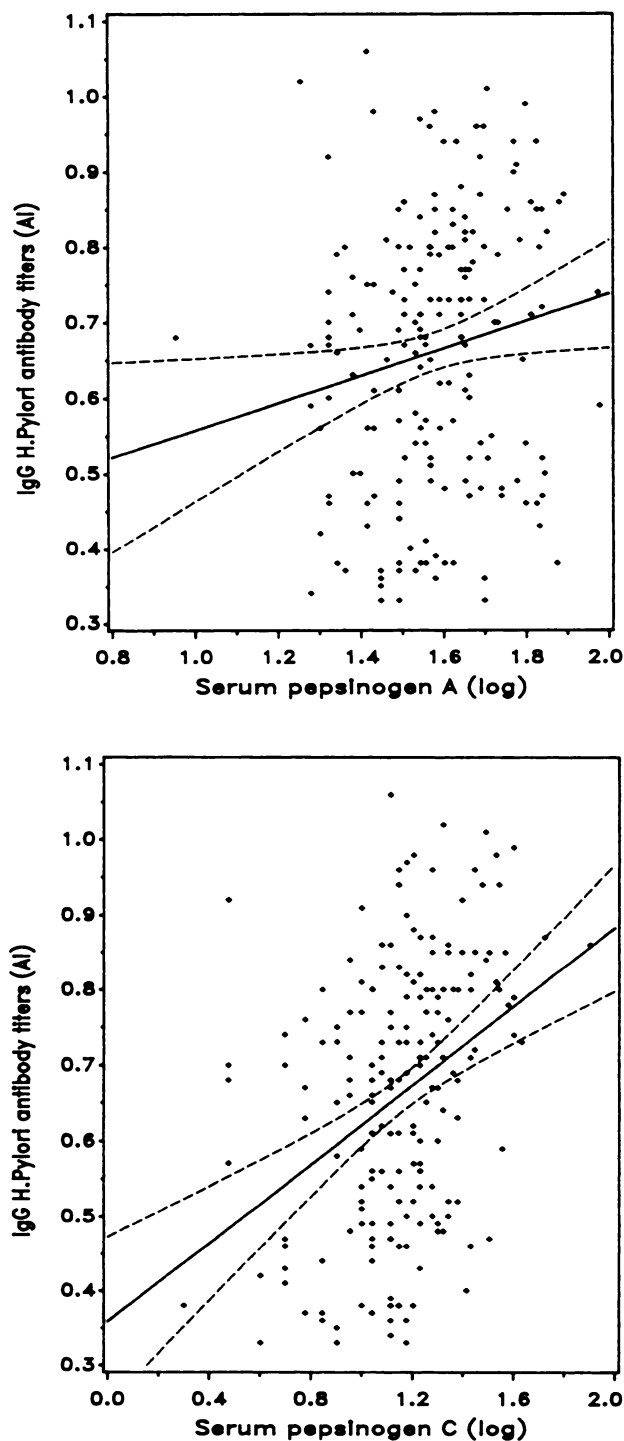


Fig. 1. Correlation between serum pepsinogen levels and IgG *H. pylori* antibody levels.

### Discussion

Our results show an extremely high prevalence (over 60.0%) of IgG and IgA *H. pylori* antibodies in children and young adults in Costa Rica. We measured both IgG and IgA antibodies because although IgG correlates well

with infection and is more sensitive than IgA, the latter has been found to be of particular value in the follow-up of patients (12). This finding is in sharp contrast with reports from developed countries at low risk for gastric cancer where *H. pylori* antibodies are found only exceptionally in people under 10 years of age and the prevalence in the age group between 10 and 20 years is usually less than 20% (13, 14). However, in Costa Rica the high prevalence of *H. pylori* antibodies was found both in high- and low-risk areas for precancerous lesions and for stomach cancer. Since *H. pylori* infection has shown intrafamilial clustering (15) we also compared the mean family size in both study areas and found no difference; it was 6.1 in the high-risk area and 6.3 in the low-risk area. Our results should be regarded with caution since they are based on a comparison of two areas only. In a recent ecological study conducted in serum samples from 46 counties in China, a significant but weak correlation was found ( $r = 0.40$ ;  $P = 0.02$ ) between the prevalence of *H. pylori* antibodies and mortality rates for gastric cancer (16).

Recent reports on the prevalence of *H. pylori* infection in different populations have pointed out remarkable differences between developing and developed countries. The former display a higher prevalence (over 60%) and an earlier age of onset of infection than the developed or affluent countries (13, 14, 17–21). This pattern has led to the hypothesis that an early age of *H. pylori* infection is an etiological factor in gastric carcinogenesis and that a lower rate of such infection in children may account for the decline in gastric cancer rates reported in most developed countries. However, this hypothesis does not account for some of the international differences in gastric cancer risk. Thus, some developing countries in which the *H. pylori* prevalence in children and adults is high display high rates of gastric cancer, *i.e.*, Peru (22), Colombia, China, and Costa Rica (23), but others, such as Algeria, Ivory Coast, and Vietnam (22), appear to have low rates of cancer.

In addition, the association between *H. pylori* infection, gastritis, and gastric cancer is not straightforward: diffuse antral gastritis related to duodenal ulcer is probably caused by *H. pylori*, but it is not associated with an increased risk of gastric cancer. In the case of multifocal atrophic gastritis, a precursor lesion of gastric cancer, other factors in addition to *H. pylori* are suspected to play an important etiological role, *i.e.*, excessively salted foods and low intake of certain micronutrients such as  $\beta$ -carotene and vitamin C (17). In this type of gastritis, *H. pylori* infection could be another source of chronic irritation of the gastric mucosa, inducing excessive cellular proliferation (17, 18) and in this way enhancing carcinogenesis. The role of *H. pylori* would then be similar to that of salt (NaCl), which is not a carcinogen in itself but markedly enhances the carcinogenesis of genotoxic agents (24). In Costa Rica, the critical genotoxic damage to the gastric cells might be induced by *N*-nitrosocompounds. This possibility is supported by a parallel study carried out in the children who are the subject of the present report. After proline ingestion, the excretion of nitrosoproline in the urine was higher in the children from the high-risk area than in children from the low-risk area, indicating a higher potential of *in vivo* formation of *N*-nitrosocompounds in the high-risk area (25).

Our results in Costa Rican children confirm the findings of high levels of PGA in *H. pylori*-positive children with histologically confirmed gastritis as reported by Oderda *et al.* (26–27). Although we did not interview or examine the children for gastrointestinal symptoms, it is presumed that most of them were asymptomatic or had minor symptoms that did not prevent them from school attendance. At the moment the present manuscript was submitted, no publication existed showing a strong correlation of PGC and *H. pylori* antibody levels. However, subsequently similar findings have been reported before and after treatment of *H. pylori* infection (28) and in Dutch healthy blood transfusion donors (29).

Errors in the measurement of pepsinogen levels are unlikely. PGC levels showed an excellent correlation between the U.S. and Dutch laboratories, but the correlation of PGA was less satisfactory.

It has been shown that *H. pylori* stimulates pepsinogen secretion *in vitro* independently of toxic damage to cells and probably through mediation of a peptide smaller than *M*, 12,000 (30). Thus, although endoscopy and gastric biopsies were not performed in the present study, the serum pepsinogen levels and their correlation with the *H. pylori* antibody titers suggest that a high proportion of these children actually had gastritis. Similar findings have been reported in children with nonspecific abdominal pain (26, 27). The much higher levels of PGC observed in the present study among those found to be positive or strongly positive for *H. pylori* antibodies than among the negative subjects suggest that there is increased liberation of PGC as a result of antral gastritis.

In general, gastric cancer risk is higher in populations with low levels of blood pepsinogens, especially PGA (31). On the other hand, our and other studies (27) suggest that *H. pylori* elevates blood levels of PG, especially PGC. This apparent incongruence may be explained by the fact that low PG levels reflect mostly atrophic and intestinalized mucosa, which is not colonized by *H. pylori*. Anti-*H. pylori* antibodies are very prevalent in populations at high gastric cancer risk, even though morphological detection of *H. pylori* in them is low (32). In patients with extensive metaplasia, *H. pylori* infection in the nonatrophic mucosa is insufficient to raise PG blood levels.

Our results indicate that Costa Rican children and adolescents have a high prevalence of *H. pylori*-associated antral gastritis. Such gastritis might be associated with a high prevalence of intestinal metaplasia and a high gastric cancer risk in the highlands but not in the coastal rural populations. *H. pylori* infection could therefore be regarded as an insufficient cause of intestinal metaplasia and of gastric cancer, whose effect is dependent on the simultaneous presence of other factors, such as certain vitamin deficiencies and exposure to genotoxic compounds.

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