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Helicobacter pylori-CagA seropositivity and nitrite and ascorbic acid food intake as predictors for Gastric Cancer ☆

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Abstract

A hospital-based case-control study was carried out between 1994 and 1996 to evaluate the risk of gastric cancer (GC) according to *Helicobacter pylori*-CagA (+) seropositivity, nitrite and ascorbic acid intake. Three geographical areas of Mexico were selected on the basis of their contrasting dietary patterns and *H. pylori* seroprevalence. Nitrite and ascorbic acid consumption were estimated by interview among 211 cases and 454 matched controls. Serum antibodies against IgG *H. pylori* and CagA were detected by immunosorbent assays. The adjusted risk for GC was significantly higher among CagA+ subjects compared with those that were CagA negative (Odds Ratio (OR) = 2.04 95% Confidence Interval (CI) 1.37–3.02 P for trend $P < 0.001$), this effect remained significant among diffuse GC cases (OR 2.05 95% CI 1.25-3-36). No significant effects due to nitrite and ascorbic consumption or interactions of these nutrients with CagA seropositivity were detected. Seropositivity to *H. pylori* CagA+ strains may be an independent factor for diffuse GC in Mexico.

Keywords: *Helicobacter pylori CagA*; Gastric cancer; Ascorbic acid intake; Nitrite intake; Mexico

1. Introduction

Gastric cancer (GC) is the second leading cause of cancer deaths in the world accounting for more than half a million deaths, and ≈65% percent of them occur in less developed countries. Adjusted mortality GC rates vary from near $4.5 \times 100\,000$ habitants in Northern America, Northern and Western Africa to nearly $30 \times 100\,000$ in Eastern Asia [1]. In contrast to the markedly decreasing trend in incidence observed in developed countries [2], in some developing countries, such as Mexico, the incidence of this tumour has not decreased [3].

Helicobacter pylori infection is the strongest risk factor for GC [4]. Approximately a half of the population in the world is seropositive for *H. pylori*. However, less than 3% of infected individuals will develop GC [5]; thus others factors, such as the pathogenicity of *H. pylori* strains, host susceptibility and variation of dietary habits, may explain the variability in GC incidence in the world. Individuals infected with *H. pylori CagA+* strains, a high consumption of nitrites and low ascorbic acid intake might be at a higher risk of developing gastric cancer [6–8].

In line with expectations, some epidemiological studies [9–16] showed a 1.7-[10] to 10.4-[14] fold increase in the risk of GC adenocarcinoma in patients infected with *H. pylori cagA+*. Other studies showed an increased risk of GC due to nitrite consumption and the opposite effect for dietary ascorbic acid intake [17].

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Possible mechanisms of interaction have been proposed, such as a systemic reduction in the availability of dietary vitamin C due to *H. pylori* infection [18], a direct action of ascorbic acid derivatives that would reduce *H. pylori* growth [19], as well as the abolishment of the intragastric chemical reduction of swallowed nitrites in the fasting stomach of *H. pylori*-infected individuals [20]. Nevertheless, no study has evaluated a potential synergistic effect of *H. pylori* CagA+ seropositivity and dietary intake factors on the GC risk.

The aims of this study were to analyse the risk of GC with regard to the individual effects of *H. pylori* CagA (+) seropositivity, nitrite and ascorbic acid food intake, as well as to explore the potential effect of modifications among these factors.

2. Patients and methods

A hospital-based case-control study was carried out between 1994 and 1996, encompassing three geographical areas of Mexico, which were selected on the basis of their contrasting dietary patterns and *H. pylori* seroprevalence [21]. As a part of the study, a sera bank was created and used for this study to determine the presence or absence of IgG antibodies against *H. pylori* and its CagA protein.

3. Cases

Cases included all subjects with histologically-confirmed intestinal or diffuse adenocarcinoma of the stomach, without history of any other type of cancer, at least 20 years of age, and who lived for at least the previous six months in the study area. Recruitment was performed at 13 large public/social security hospitals, distributed as follows: 7 from Mexico City (Federal District), three from the city of Puebla (State of Puebla), and three from the city of Merida (State of Yucatan). One expert cancer pathologist determined the histology of the tumour (intestinal or diffuse) following the criteria established by Lauren [22]. Information about the anatomical subsite location of the tumour was not available for this study. In total, we identified 281 patients who met all of the eligibility criteria, and 261 agreed to enroll in the study, a participation rate of 92.9%. We were able to identify approximately 75% of all GC patients reported to the Mexico National Cancer Registry during the study period of recruitment in the study areas.

4. Controls

At least two age (±5 years), gender and residence frequency matched controls were recruited for each case. Controls were identified from selected medical services of participating hospitals. Interviewers were instructed to identify potential controls by approaching patients and/or their companions in the waiting room when they were attending these services. Eligibility criteria included the absence of any, current or past, diagnosis of a malignant tumour; a diet related disease (gastritis, peptic ulcer, liver cirrhosis or diabetes mellitus); or immunosuppressive condition; and they also had to have lived for at least six months in the same area as the index case. The commonest diagnoses among the controls were circulatory system diseases other than hypertension (19.6%); diseases of the nervous system and the sensory organs with the exception of psychiatric disorders (15.4%); osteomuscular and connective tissue disorders (14.5%); injuries and poisonings (10.8%); and respiratory diseases (9.9%); other less common diagnoses were genitourinary diseases and skin problems. We also recruited some healthy companions of patients who were not willing to participate in the study as well as a group of subjects attending the hospitals for preventive purposes such as to have vaccinations. The participation rate for the control group was 94.6% (523 out of 553 eligible subjects).

5. Nitrite and ascorbic acid intake

Dietary habits, including nitrite and ascorbic acid intake, were estimated by means of personal structured interviews carried out by trained personal and using a semi-quantitative questionnaire. The questionnaire was adapted to capture specific food consumption that is typical of each geographical region in the study. More detailed information about the instrument is presented elsewhere in [21]. During the interview, all subjects were requested to refer their food consumption either one year before being diagnosed or one year before the date of the interview. Those individuals who reported caloric intakes below 700 kcal per day or above 4500 kcal per day were excluded from the analyses ($n = 73$), thus reducing the sample size in this phase to 234 cases and 468 controls.

6. Serological methods

A sample of 10 ml of blood was drawn from each case and control. Sera was obtained by centrifugation and kept frozen at −70 °C until tested. IgG antibodies against *H. pylori* infection were determined by means of an enzyme-linked immunosorbent assay (ELISA) commercial kit [23]. An individual was considered *H. pylori*-positive when the corresponding adjusted absorbance value was >0.99, otherwise the result was classified as negative. The sensitivity and the specificity for this

method are 98.5% and 98.1%, respectively. In addition, IgG antibodies against CagA were measured in seram by an ELISA test previously validated by our research group. The cut-off value for this test was $\geqslant 1.5$ absorbance units [24]. Bank seram samples for these analyses were available for 211 cases and 454 controls.

7. Statistical analyses

The general characteristics of the cases and controls (age, gender, etc.) were compared using the t-test and Chi^2 statistics. Prevalences of CagA+, as well as nitrite and ascorbic acid intakes, across the specific diagnostic groups and controls, were compared with the Chi^2 statistic. We further evaluated whether the following selected characteristics were significant determinants of CagA status among the controls: age, gender, years of education, change in socioeconomic status, place of residence, nitrite and ascorbic acid intake.

To evaluate the independent effects of *H. pylori* CagA status, dietary nitrite intake and ascorbic acid consumption, we used the following strategy: first, we created four categories as follows: *H. pylori* − CagA−, *H. pylori* + CagA−, *H. pylori* − CagA+, *H. pylori* + CagA+. Second, seropositiveness to CagA was first analysed as a dichotomous variable, using the values <1.5 and $\geqslant 1.5$ ELISA units as cut-off points and, in a further step, the quartile distribution based on the controls was obtained to establish cut-off points in four ascending categories of risk, that express the intensity of the serological response against CagA. Third, on the basis of the observed tertile distribution among controls, we created three categories of consumption (portions per day) of food sources of nitrites (ham, sausage, bacon, and hot sausage) and food sources of ascorbic acid (orange, melon, watermelon, papaya, mango, tangerine, strawberries, guava, lime, gooseberry, tomatoes, potatoes, lettuce, spinach, cauliflower, cabbage).

We estimated the adjusted risk (by age, gender, residence, energy, change in socioeconomic level and years of education) of GC according to the individual *H. pylori*/CagA status and dietary variables, using unconditional logistic regression models. In addition, the GC risks due to *H. pylori*/CagA status were also adjusted in a second model by adding ascorbic acid and nitrite intake. The GC risk estimate due to nitrite intake was, in turn, further adjusted by the *H. pylori*/CagA status and ascorbic acid and, lastly, the effect of ascorbic acid intake was adjusted by nitrite intake and *H. pylori*/CagA status. All of these models were fitted to the total number of cases with adenocarcinoma of the stomach and, in separate steps, stratifying by GC histological types.

To test for trend, we entered as ordinal variables in the corresponding logistic models, the four categories of the CagA serological response, the four groups of the *H. pylori*-CagA status, the tertile distribution of nitrite intake and the tertile distribution of ascorbic acid consumption.

An exploratory analysis for a potential interaction between CagA+ seropositivity and nitrites and ascorbic acid dietary intake was performed using a multiplicative approach. In this step, we incorporated the product of the CagA status and nitrite intake categorical values adjusting by ascorbic acid intake, and the product of the CagA status and ascorbic acid intake adjusting by nitrite consumption, to separate models, and looked at the change in the likelihood ratio statistic (ΔG^2).

8. Results

The general characteristics of the study population are presented in Table 1. As expected from the study design, the distribution of age and gender was similar in the cases and controls. The percentage of residents in Mexico City was higher, but non-significantly, than those in the other two areas of the study. No important differences regarding the mean years of education and the change in socioeconomic status (e.g., current level minus socioeconomic level in childhood) were found. The prevalence of individuals with CagA+ serum was significantly higher in the cases than controls (78.7 vs. 65.9). A higher, but non-significant, prevalence of high nitrite consumers (tertile 3) was observed for the cases compared with the controls (39.3 vs. 32.6). The tertile distribution of ascorbic acid was similar between the cases and controls.

The percentage of cases with the diffuse type of GC found in this study population was higher than the percentage of the intestinal type (64.4% vs. 35.6%). On average, cases of the diffuse type of GC were younger (mean age 55.4 vs. 59.6 years, $P < 0.01$). A similar gender distribution was found among cases of diffuse GC, but, contrastingly, there was a predominance of males (64%) over females (36%) among cases of the intestinal type of GC (data not shown).

As shown in Table 2, no significant patterns of CagA+ individuals or high nitrite/ascorbic acid consumption were observed among the hospital controls, according to their original diagnosis. The analysis of variance (ANOVA) comparisons yielded P values of 0.109, 0.921 and 0.413 for the distribution of *H. pylori* CagA+ subjects, nitrite and ascorbic acid intake, respectively.

In Table 3, we evaluated the association of potential factors related to GC incidence and CagA status among controls. The distribution of CagA− and CagA+ individuals was similar according to the selected variables, thus none were significantly associated with CagA status.

Table 1
General characteristics of the study population

Characteristics	Cases (211)	Controls (454)
Age (years)		
X (min–max)	58.27 (28–86)	57.55 (28–81)
Gender (%)		
Females	42.7	43.6
Males	57.4	56.4
Residence (%)		
Mexico City	43.1	39.4
Puebla	27.0	31.9
Yucatan	29.9	28.6
Years of education		
X (min–max)	5 (0–20)	4.47 (0–18)
Change in socioeconomic level[a] (%)		
Same or less	52.2	48.8
Improvement	47.8	51.2
CagA[b] (%)		
Negative	21.3	34.1
Positive	78.7	65.9
Nitrite intake[c] *(portions/day)* (%)		
0–0.11	32.2	35.2
0.12–0.26	28.4	32.2
0.27-2.25	39.3	32.6
Ascorbic acid intake[d] *(portions/day)* (%)		
0.23–2.69	35.1	33.5
2.70–3.91	27.5	33.5
3.92–14.18	37.4	33.0

X = mean.
[a] Current level – socioeconomic level in childhood.
[b] Negative $\leqslant 1.5$ absorbance units, otherwise positive, $P < 0.05$, Chi^2.
[c] Consumption of one portion of any of the next food items: ham sausage, bacon and/or hot sausage.
[d] Consumption of one portion of any of the next food items: orange, melon, watermelon, papaya, mango, tangerine, strawberries, guava, lime, gooseberry, tomatoes, potatoes, lettuce, spinach, cauliflower, cabbage.

When *H. pylori* IgG status was considered with the CagA status, we obtained a set of non-significant estimates for the adjusted risk to develop GC which were 1.76 (95% CI 0.81–3.81) for those subjects classified as *H. pylori*−CagA+ and 1.63 (95% CI 0.89–2.97) for *H. pylori* + CagA+, the comparison category being those who were *H. pylori*−CagA− and the corresponding test for trend was statistically significant ($P = 0.002$). The adjusted risk of GC was significantly higher among CagA+ subjects compared with those that were CagA-negative with a value of 2.04 (95% CI 1.37–3.02) being reported. In addition, when the magnitude of the response to CagA was considered, individuals in the upper quartile had a risk of 2.53 (95% CI 1.49–4.30) compared with the reference group of those in the CagA lower quartile. The increase in the adjusted risk for GC in relation to seropositivity for CagA was highly significant ($P < 0.0001$). A non-significant increase in GC risk due to high nitrite consumption was observed in this population, as well as, a non-significant protective effect due to ascorbic acid intake. Otherwise the interactions between CagA status and nitrite intake ($P = 0.919$) and between CagA status and ascorbic acid consumption ($P = 0.874$) were all non-significant (Table 4).

The Odds Ratios for *H. pylori* seropositivity and CagA status categories were borderline significant for diffuse GC (intestinal cases could not be evaluated due to insufficient numbers), with a significant linear trend ($P = 0.018$). The dichotomous effect of CagA status remained significant only among the diffuse GC cases (OR 2.05 95% CI 1.25–3.36). The Odds Ratio for diffuse GC for those in the upper quartile of CagA seropositivity compared with those in the lowest quartile was 2.63 (95% CI 1.37–5.07, *P* for trend 0.003). The effect of CagA status was borderline significant for intestinal GC cases. The Odds Ratio for those in the upper quartile of CagA seropositivity compared with those in the lowest

Table 2
Prevalence of CagA+ serum antibodies, nitrite and ascorbic acid consumption for controls by diagnosis

Diagnoses (454)	CagA+[a] (%)	Nitrite intake[b] (%)	Ascorbic acid intake[c] (%)
Circulatory system (89)	57.3	61.8	61.8
Nervous system and sensory organs (70)	67.1	67.1	74.3
Osteomuscular and connective tissue (66)	66.7	66.7	71.2
Injuries and poisoning (49)	59.2	57.1	69.4
Respiratory diseases (45)	68.9	62.2	64.4
Genitourinary system (37)	75.7	73.0	70.3
Skin (26)	53.9	65.4	50.0
Healthy (42)	83.3	66.7	59.5
Others (30)	66.7	66.7	70.0
All (454)	65.9	64.8	66.5

[a] CagA-positive $\geqslant 1.5$, $Chi^2 = 0.109$.
[b] Percentage of individuals who consume between 0.12 and 2.25 portions/day (tertiles 2, 3) of food sources of nitrite, $Chi^2 = 0.921$.
[c] Percentage of individuals who consume between 2.70 and 14.18 portions/day (tertiles 2, 3) of food sources of ascorbic acid, $Chi^2 = 0.413$ analysis of variance (ANOVA). *P* values were 0.109, 0.921 and 0.413 for the distribution of *H. pylori* CagA+ subjects, nitrite and ascorbic acid intake, respectively.

Table 3
Association between selected potential risk factors of gastric cancer and CagA status among controls

	CagA−(155) (%)	CagA + (299) (%)	*P*-value[a]
Age (years)			
20–39	11.0	10.0	0.943
40–59	38.7	39.8	
60+	50.3	50.2	
Gender (%)			
Females	40.7	45.2	0.359
Males	59.4	54.9	
Years of education (%)			
<6	59.5	57.2	0.649
⩾ 6	40.5	42.8	
Change in socioeconomic level (%)			
Same or less	52.9	46.6	0.204
Improved	47.1	53.4	
Residence (%)			
Mexico City	33.6	42.5	0.166
Puebla	36.1	29.8	
Yucatan	30.3	27.8	
Nitrite intake[b] *(portions/day)* (%)			
0–0.11	31.0	37.5	0.289
0.12–0.26	32.3	32.1	
0.27–2.25	36.8	30.4	
Ascorbic acid intake[c] *(portions/day)*			
0.23–2.69	31.0	34.8	0.689
2.70–3.91	35.5	32.4	
3.92–14.18	33.6	32.8	

[a] *P*-value from Chi^2.
[b] Consumption based on a portion of any of the next food items: ham sausage, bacon and/or hot sausage.
[c] Consumption of one portion of any of the next food items: orange, melon, watermelon, papaya, mango, tangerine, strawberries, guava, lime, gooseberry, tomatoes, potatoes, lettuce, spinach, cauliflower, cabbage.

quartile was 2.07 (95% CI 0.89–4.80, *P* for trend 0.108). There were no significant increases in GC risk due to high nitrite consumption and the non-significant protective effect due to ascorbic acid intake remained after stratifying by histological type of GC (Table 5).

9. Discussion

This study confirms that the evidence of infection with more virulent strains of *H. pylori* CagA+ is a cofactor for GC risk and suggests that this condition has an impact that is independent of that produced by nitrite and ascorbic acid consumption.

The positive association between CagA+ seropositivity and GC risk has been reported by others [9–16]. Interestingly, the risk for gastric cancer increased with the magnitude of the CagA antibody response and the dose–response relationship was highly significant. To our knowledge, this observation has not been previously reported. Patients with the intestinal type of earlier gastric tumours have higher antibody titres to *H. pylori* antigens than patients without cancer [25]. In addition, in Mongolian gerbils infected with *H. pylori* the progression to gastric cancer was mainly observed among animals with higher *H. pylori* antibody titres. These results led to the suggestion that high antibody titres are indicative of severe gastric mucosal inflammation and its long-term persistence might cause metaplasia of the gastric mucosa [25]. The dose–response of the antibody titres to CagA and risk of gastric cancer observed in our study is in accordance with the above observation, and suggests that antibody titers to CagA might be associated with the developmental stage of gastric cancer. However, this observation should be interpreted with caution since the dose–response test was no longer significant when CagA-negative subjects were excluded from the analysis.

We found no interaction between nitrate and ascorbic acid consumption with CagA status on the GC risk. Simultaneous assessments of dietary and infectious risk factors with regard to GC risk are rarely done. You and co-workers [26], reported that *H. pylori* antibodies, cigarette smoking and low levels of dietary vitamin C contribute to the progression of precancerous lesions to gastric cancer, these authors estimated the independent effect of GC cofactors, but did not report on the evaluation of interactions. Thus, further research is needed to confirm the negative results reported in this manuscript. This may be partially explained by an insufficient statistical power and/or the possibility that the inflammatory response induced by *H. pylori* CagA+ strains produces a more powerful effect on the GC risk than those due to nitrite and ascorbic acid intake, thus obscuring the simultaneous evaluation of these factors.

It has been suggested that *H. pylori* causes intestinal and diffuse types of GC by different mechanisms, and also that inflammation may be critical in the carcinogenesis of intestinal-type tumours, but not in the diffuse-type of the disease. The more inflammatory phenotype of *H. pylori* CagA+ has been linked to gastric atrophy that is a precursor lesion for the intestinal type of GC [27,28]. However, epidemiological evidence on the relationship between CagA status and histological type of GC is inconsistent. Some studies showed an association with both tumour types [10,12,14,29], whilst others only found an association with the intestinal type [9,11]. One study found an association with the diffuse type of GC alone [10]. In this study, we found that cases of diffuse GC were mainly linked to CagA+ status; however, we cannot rule out that intestinal GC cases could also be linked, since a marginal statistical result was obtained. If

Table 4
Adjusted effect of *H. pylori*/CagA status, nitrite and ascorbic acid intake on gastric cancer risk

	Cases	Controls	OR[a] (95% CI)	OR (95% CI)
Hp/CagA				
Hp−/CagA−	18	48	1.0 –	1.0[b] –
Hp+/CagA−	27	107	0.70 (0.35–1.43)	0.72 (0.35–1.46)
Hp−/CagA+	22	40	1.74 (0.80–3.76)	1.76 (0.81–3.81)
Hp+/CagA+	144	259	1.57 (0.87–2.86)	1.63 (0.89–2.97)
P trend			0.003	0.002
CagA[c]				
Negative	45	155	1.0 –	1.0[b],[d] –
Positive	166	299	2.00 (1.35–2.96)	2.04 (1.37–3.02)
CagA				
0.506–1.20	28	113	1.0 –	1.0[b] –
1.21–2.34	49	114	1.84 (1.07–3.19)	1.87 (1.08–3.23)
2.35–6.26	65	114	2.45 (1.44–4.15)	2.50 (1.47–4.25)
6.26–37.76	69	113	2.49 (1.47–4.22)	2.53 (1.49–4.30)
P trend			0.001	0.0001
Nitrites consumption (portions/day)				
0–0.11	68	160	1.00 –	1.0[e] –
0.12–0.26	60	146	0.93 (0.60–1.42)	0.95 (0.62–1.46)
0.27–2.25	83	148	1.17 (0.77–1.79)	1.24 (0.81–1.90)
P trend			0.447	0.320
Ascorbic acid consumption (portions/day)				
0.23–2.69	74	152	1.0 –	1.0[f] –
2.70–3.91	58	152	0.69 (0.45–1.06)	0.66 (0.43–1.02)
3.92–14.18	79	150	0.82 (0.51–1.31)	0.78 (0.48–1.26)
P trend			0.362	0.267

OR – Odds Ratio; CI – Confidence Interval; Hp – *H. pylori*.
[a] Adjusted by age, gender, energy, residence, change in socioeconomic level and years of education.
[b] Adjusted by age, gender, residence, energy change in socioeconomic level, years of education, nitrite and ascorbic acid consumption.
[c] Negative $\leqslant 1.5$ ascorbic acid, otherwise positive, $P < 0.05$, Chi^2.
[d] *P* for interaction between CagA status and nitrite intake = 0.919; *P* for interaction between Hp/CagA status and Ascorbic Ac intake = 0.874.
[e] Adjusted by age, gender, residence, energy change in socioeconomic level, years of education, Hp/CagA status and ascorbic acid consumption.
[f] Adjusted by age, gender, residence, energy change in socioeconomic level, years of education, Hp/CagA status and nitrite consumption.

CagA+ status increases the risk of both intestinal and diffuse GC, differences in the prevalence of intestinal and diffuse cases across the published studies might explain the heterogeneity of their results. Further research is needed to understand the potential relationship between CagA status and diffuse GC risk, which might well have an alternative mechanism for GC carcinogenesis, as recently suggested by Shibatta and co-workers [30], who showed that CagA+ *H. pylori* infection is associated with a higher prevalence of *p53* mutation or the inhibition of T cell proliferation [31].

The Odds Ratios for GC and *H. pylori*/CagA were lower than those estimated for CagA status alone, suggesting that non-differential measurement affected more the former category. It is possible that antibodies against CagA have a greater specificity than those against *H. pylori* and/or there is an enhanced production of this protein in GC cases; thus, the evaluation of CagA would be a more valid marker of *H. pylori* infection as reported by others in [29,34], and the corresponding Odds Ratios would be less attenuated than those estimated for *H. pylori* and CagA together. In addition, it is important to remark that the risk for GC was slightly higher for subjects who were *H. pylori*−CagA+ (1.76), than those who were *H. pylori* + CagA+ (1.63). Previous studies documented a decrease in antibody response to *H. pylori* antigens in patients with GC, which is probably due to a decrease in *H. pylori* colonisation as the gastric mucosa becomes malignant and is no longer suitable for *H. pylori* growth [32,33] and our results are in line with this hypothesis.

The presence of confounding variables might explain the association between CagA+ seropositive and GC risk. However, we performed additional analyses adding variables previously linked to GC in Mexico such as cigarette smoking status and capsaicin consumption to the multivariate models, and no differences were found (data not shown).

Selection of controls is a key issue in hospital-based studies, since CagA+ prevalence might be associated with the diseases affecting the controls, thus biasing the results in any direction. In the present study, we evaluated not only the distribution of CagA+ seropositivity, but also nitrite and ascorbic acid intake, and no

Table 5
Adjusted effect of *H. pylori*/CagA status, nitrite and ascorbic acid intake on gastric cancer risk by histological type

	Cases[a]		Intestinal OR (95% CI)	Diffuse OR (95% CI)
	Intestinal	Diffuse		
Hp/CagA				
Hp−/CagA−	8	10	ND	1.0[b]
Hp+/CagA−	8	16	ND	0.85 (0.34–2.11)
Hp−/CagA+	5	15	ND	2.33 (0.91–5.99)
Hp+/CagA+	50	77	ND	1.77 (0.82–3.82)
P trend				0.018
CagA[c]				
Negative	16	26	1.0[b]	1.0[b]
Positive	55	92	1.70 (0.93–3.09)	2.05 (1.25–3.36)
CagA				
0.506–1.20	9	17	1.0[b]	1.0[b]
1.21–2.34	19	26	1.96 (0.84–4.57)	1.74 (0.87–3.49)
2.35–6.26	21	35	2.28 (0.99–5.24)	2.37 (1.22–4.61)
6.26–37.76	22	40	2.07 (0.89–4.80)	2.63 (1.37–5.07)
P trend			0.108	0.003
Nitrites consumption (portions/day)				
0–0.11	28	33	1.0[d]	1.0[d]
0.12–0.26	14	42	0.47 (0.23–0.98)	1.39 (0.82–2.36)
0.27–2.25	29	43	1.10 (0.59–2.04)	1.31 (0.75–2.27)
P trend			0.806	0.362
Ascorbic acid consumption (portions/day)				
0.23–2.69	24	40	1.0[e]	1.0[e]
2.70–3.91	19	35	0.69 (0.35–1.36)	0.77 (0.45–1.31)
3.92–14.18	28	43	0.77 (0.37–1.59)	0.89 (0.49–1.63)
P trend			0.464	0.678

ND – not determined due to insufficient numbers.
[a] Numbers that add up less than the total number of cases presented in Table 4 are due to missing values in the variable of interest.
[b] Adjusted by age, gender, residence, energy change in socioeconomic level, years of education, nitrite and ascorbic acid consumption.
[c] Negative $\leqslant 1.5$ absorbance units, otherwise positive, $P < 0.05$, X^2
[d] Adjusted by age, gender, residence, energy change in socioeconomic level, years of education, Hp/CagA status and ascorbic acid consumption.
[e] Adjusted by age, gender, residence, energy change in socioeconomic level, years of education, Hp/CagA status and nitrite consumption.

significant patterns emerged from the diagnoses of controls, thus the possibility of a selection bias in this group should be low. However, since controls with peptic ulcer disease were not included in the study, the possibility of an overestimation between *H. pylori* CagA status and gastric cancer risk cannot be ruled out.

Our results support those from an ecological study, which found a relationship between the seroprevalence of CagA+ and GC mortality rates in Mexico. The seroprevalence of antibodies against *H. pylori cagA+* strains varied from ≈64% in the states with a high mortality rate of GC to 48% in those with low rates of GC [35]. However, no information about the histological type of gastric cancer according to Lauren's classification is available at the national level to evaluate whether diffuse GC varies according to CagA seroprevalence and also whether this link is a determinant of the constant GC trend in Mexico, in contrast to the declining trends observed in most countries worldwide.

In summary, this study demonstrated that in our population seropositivity to *H. pylori* CagA+ strains is related to the development of GC. Our results also suggest that *H. pylori* CagA+ seropositivity may be related to the predominance of diffuse GC in Mexico.

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