Relation of atrophic gastritis with *Helicobacter pylori*-CagA⁺ and interleukin-1 gene polymorphisms

Rafaela Sierra, Clas Une, Vanessa Ramírez, Warner Alpízar-Alpízar, María I González, José A Ramírez, Antoine de Mascarel, Patricia Cuenca, Guillermo Pérez-Pérez, Francis Megraud

AIM: To determine the association of *H. pylori* CagA⁺ infection and pro-inflammatory polymorphisms of the genes interleukin (IL)-1RN and IL-1B with the risk of gastric atrophy and peptic ulcers in a dyspeptic population in Costa Rica, a country with high incidence and mortality of gastric cancer.

METHODS: Seven biopsy specimens, a fasting blood sample and a questionnaire concerning nutritional and sociodemographic factors were obtained from 501 consecutive patients who had undergone endoscopy for dyspeptic symptoms. A histopathological diagnosis was made. Pepsinogen concentrations were analyzed by enzyme linked immunosorbent assay (ELISA). Infection with *H. pylori* CagA⁺ was determined by serology and polymerase chain reaction (PCR). IL-1B and IL-1RN polymorphisms genotyping was performed by PCR-restriction fragment length polymorphism (PCR-RFLP) and PCR respectively.

RESULTS: In this dyspeptic population, 86% were *H. pylori* positive and of these, 67.8% were positive for CagA. Atrophic antral gastritis (AAG) was associated with CagA⁺ status [odd ratio (OR) = 4.1; *P* < 0.000] and fruit consumption (OR = 0.3; *P* < 0.00). Atrophic body gastritis (ABG) was associated with pepsinogen PGI/PGII < 3.4 (OR = 4.9; *P* < 0.04) and alcohol consumption (OR = 7.3; *P* < 0.02). Duodenal ulcer was associated with CagA⁺ (OR = 2.9; *P* < 0.04) and smoking (OR = 2.4; *P* < 0.04). PGI < 60 μg/L as well as PGI/PGII < 3.4 were associated with CagA⁺.

CONCLUSION: In a dyspeptic population in Costa Rica, *H. pylori* CagA⁺ is not associated with ABG, but it is a risk factor for AAG. The pro-inflammatory cytokine polymorphisms IL-1B + 3945 and IL-1RN are not associated with the atrophic lesions of this dyspeptic population.

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Key words: Atrophic gastritis; Pepsinogen; Peptic ulcers; *Helicobacter pylori*-CagA; Interleukins

Peer reviewer: Atsushi Nakajima, Professor, Yokohama City University Hospital, 3-9 Fukaura Kanazawaku, Yokohama 236, Japan


INTRODUCTION

Colonization with *H. pylori* is associated with atrophic gastritis, peptic ulcer and distal gastric cancer[1,2]. Nevertheless, many colonized individuals never develop these pathologies. Genetic characteristics of the host and the bacteria as well as environmental factors may be involved in the final clinical outcome[3]. The pathogenicity and virulence of *H. pylori* increases when the infecting strain expresses the cagA gene that codes for a highly immunogenic protein CagA, which is a marker for the presence
of the cag pathogenicity island (PAI). Moreover, the immune response of the host is considered a key event in the pathogenic process that leads to gastric cancer. A number of studies have reported that carriers of certain alleles in genes encoding proinflammatory and anti-inflammatory cytokines exhibit a stronger inflammatory response against H. pylori and a marked inhibition of acid secretion resulting in an increased risk of gastric cancer and its precursors, atrophic gastritis and intestinal metaplasia. However, this association has not been found in all studied populations.

The development of atrophic gastritis is central in the multi-step process which leads to gastric cancer and the risk increases with the severity and physical extension of the atrophic lesion. Serum levels of pepsinogen I (PGI) and the ratio of PGI/PGII serum levels decrease significantly with increased extension and severity of atrophic gastritis and gastric cancer. Therefore these parameters have been proposed as serological markers for those histopathological changes. Costa Rica is one of the countries with the highest incidence and mortality rates of gastric cancer worldwide. The prevalence of H. pylori associated gastritis is high from an early age.

Recently, we found an association between the PGI/PGII ratio and atrophic body gastritis in dyspeptic patients. In the present study, the same population was analyzed for associations of atrophic body and antral gastritis and presence of peptic ulcers with H. pylori CagA infection and pro-inflammatory interleukin-1 (IL-1) gene polymorphisms.

MATERIALS AND METHODS

Patients
As previously described, between January and July 2000, 800 consecutive patients referred to the endoscopy service at the Calderón Guardia Hospital in San José for dyspeptic symptoms were interviewed. This hospital is a tertiary hospital that, however, also provides gastroenterology service at primary and secondary levels. Patients were excluded if they were under 18 years of age, had not resided in Costa Rica for the previous two years, had received H. pylori eradication therapy, had taken antibiotics during the 3 mo preceding endoscopy, had taken bismuth compounds at the time of endoscopy, had a history of gastric surgery, suffered from diseases associated with coagulation, or presented digestive bleeding. A total of 501 patients fulfilled the inclusion criteria and signed an informed consent form before 25 mL of fasting blood was obtained. The project had been previously approved by the ethic committees of the University of Costa Rica and the Hospital. A questionnaire with information concerning sociodemographic factors, family history, general health status, consumption of salt, coffee, alcohol, fruit, vegetables, and smoking habits was filled out. Blood pressure and height and weight were recorded to calculate the body mass index.

Endoscopy procedure
Seven biopsies were obtained: two biopsies from the middle part of the antrum were taken from the major and minor curvatures and two from the gastric body at the anterior and posterior walls of the central body for histopathological diagnosis. One biopsy from the antrum and one from the body were taken for culture and one from the antrum for rapid urease test.

Histological diagnosis
Sections of formalin-fixed and paraffin-embedded biopsies, stained with haematoxylin and cosin, were used for the histopathological diagnosis according to the Sydney Classification. All slides were independently read by two specialists in gastric pathology (IA Ramirez in San José, Costa Rica, and A De Mascarel in Bordeaux, France). Cases with discrepant diagnoses were reviewed by both pathologists until a consensus was reached. A patient was considered to have: (1) Atrophic body gastritis (ABG), if atrophy or metaplasia was present in any of the biopsies from the body; (2) Atrophic antral gastritis (AAG), when atrophy was found in any of the biopsies from the antrum and not in the body; (3) Non-atrophic gastritis (NAG), in the case of gastritis without atrophy or normal mucosa, when no pathology was found in the antrum or the body and no granulocytic activity or lymphoid follicles were observed. In 22% of the patients, there was not histopathological diagnosis because there was not sufficient tissue in the biopsies.

H. pylori status
Infection with H. pylori was determined by histology, culture, and serology. For histological detection, the slides were stained with toluidine blue. One biopsy from the antrum and one from the body were cultured for H. pylori. Biopsies from the antrum and corpus were ground in brain-celula brood with an electric homogenizer. The suspensions were plated on two in-house media: a Wilkins Chalgren agar containing 10% human blood and antibiotics and a Columbia blood agar without antibiotics incubated for 10 d in a microaerohic atmosphere at 37°C. Standard identification was performed. Serum antibodies to H. pylori were measured by an in-house enzyme linked immunosorbent assay (ELISA) developed in our laboratory and based on a modification of a previously described ELISA. The antigen preparation and determination of cutoff points was previously reported. A patient was considered positive for H. pylori when positive by any of the three methods: culture or histology or serology and negative when none of the methods were positive.

CagA status
The cagA status was determined by polymerase chain reaction (PCR) on isolated strains using primers cagA1-cagA2 and, if negative, a second set of primers, cagA3-cagA4, were used. Serum antibodies to CagA were measured by ELISA as described by Blaser et al. A patient was considered positive for H. pylori CagA infection when positive by PCR and/or serology.

Serum pepsinogen concentrations
The concentrations of PGI and PGII in sera were
measured by an enzyme immunoassay (EAI) (Eiken Chemical Company, Tokyo, Japan) according to the manufacturer's recommendations. The validation of pepsinogen levels for this population was described previously.[8] In order to detect as many patients as possible with ABG, a cut-off point that favored sensitivity was selected. The optimal cut-off point for this population had been set at a PGI/PGII ratio of 3.4, which gave a sensitivity of 91.2% and a specificity of 38.5%.[8] The predictive value for a positive sample was 11.2% and for a negative 98.1%. Pepsinogen levels could not be measured in 5% patients because insufficient amounts of blood were obtained or because the patients did not accept being bled.

**IL-1B and IL-1RN genotyping**

IL-1B polymorphism analysis was performed by PCR-restriction fragment length polymorphism (PCR-RFLP). 100 ng of genomic DNA were amplified with the primers and PCR conditions previously reported.[8] The PCR products for IL-1B + 3954 were digested with the restriction enzyme TagI and the allele designation for this polymorphism was the same as previously reported.[8]

Genomic DNA (100 ng) was amplified using the same primers and PCR conditions as previously reported for IL-1RN.[8] For statistical analysis purposes and because of the low frequency of alleles 3, 4 and 5, this polymorphism was treated as biallelic by dividing alleles into short and long categories, in which the short allele has two repeats (allele 2) and long allele has more than two repeats (allele L). For 110 (22%) patients genotyping was not done because of insufficient leukocyte samples or problems in the isolation or analysis of the DNA.

**Statistical analysis**

χ² statistics were used for comparing genotype frequencies among the groups studied and to assess Hardy-Weinberg equilibrium for each of the loci studied. Allelic frequencies were assessed using Estimating Haplotype Frequencies (EH) (available at ftp://linkage.rockefeller.edu/software/eh/). Polytomic logistic regression was used to compare genotypic frequencies among the groups studied here using STATA/SE 8.0 (STATA Corporation, College Station, TX).

A logistic regression model was used to calculate the odd ratios (ORs) for (dependent variable): (1) ABG compared to dyspepsia without atrophic gastritis; (2) AAG compared to dyspepsia without atrophic gastritis; (3) Gastric ulcer compared to all of the others; (4) Duodenal ulcer compared to the rest; (5) Levels of PGI < 60 µg/L compared to levels of PGI > 60 µg/L; and (6) Values of PGI/PGII < 3.4 compared to values of PGI/PGII > 3.4. The systematic independent variables were H pylori CagA infection, IL-1B + 3954 allele T carriers, IL-1RN allele 2 carriers, and those that had been associated with the dependent variables in a previous study of the same population: age, sex, overweight, frequency of alcohol and fruit consumption and the PGI/PGII ratio for atrophic gastritis and age; gender and cigarette smoking for peptic ulcers.[8] The logistic regression analyses were performed including either, only H pylori infected individuals or, all of the participants. The association was determined as an OR at a confidence interval of 95%. Statistical significance was set at P < 0.05. The software STATA/SE 8.0 (STATA Corporation, College Station, TX) was used for the statistical analysis.

**RESULTS**

In this study, 501 patients were included, 338 women with an average age of 46.3 years and 163 men with an average age of 46 years. The mean age was higher in groups with the most severe pathologies (Table 1).

**Pepsinogen**

PGI y PGII concentrations could be measured in 479 patients. Serum concentrations of PGI as well as the PGI/PGII ratio were lower in patients with ABG as compared to other dyspeptic patients (Table 1). Dyspeptic patients with a ratio below 3.4 were at increased risk of having ABG compared to those with a ratio higher than 3.4 (Table 2).

**H pylori CagA**

The frequency of H pylori infection was 86%, out of which 67.8% were CagA⁺, however the prevalence varied according to the pathology. Patients with a normal histopathological diagnosis, although few, showed a lower incidence of infection with H pylori and H pylori CagA⁺ (15.0% and 5.3% respectively) as compared to the rest, NAG (89.9% and 55.5%), AAG (93.3% and 81.6%) or ABG (82.9% and 58.8%). The prevalence of infection with H pylori and H pylori CagA⁺ for gastric ulcer was 100% and 52.2% respectively, and for duodenal ulcer 98% and 85% respectively. Among patients with PGI/PGII < 3.4, the prevalence of infection with H pylori was 87.7% and with H pylori CagA⁺ 61.3% while that of patients with ratios > 3.4 were at 82.1% and 50.0%. H pylori CagA⁺ was associated with a PGI/PGII ratio < 3.4 and PGI < 60 µg/L (Table 3).

**Polymorphisms: IL-1RN and IL-1B + 3954**

Genotyping of the cytokine polymorphisms was successfully performed in 371 patients (74%). Genotypic distributions of IL-1RN and IL-1B + 3954 polymorphisms were not significantly different among the studied groups (Table 4). There was Hardy-Weinberg
Atrophic gastritis

In AAG

OR 95% CI  P

Age > 50 yr 16 0.8-3.0 0.2
Men 0.6 0.3-1.2 0.2
Overweight (BMI > 25.9) 0.7 0.3-1.2 0.2
Fruit consumption
0-1 times/wk 1.0
2-6 times/wk 0.4 0.2-0.8 0.01
More than 6 times/wk 0.3 0.2-0.7 0.00
Alcohol consumption
No consumption 1.0
Weekends or more 3.0 0.8-12 0.2
H pylori
CagA status* 4.1 1.9-9.0 0.000
IL-1B + 3954
T carriers vs CC 1.2 0.5-3.2 0.7
IL-1RN
2 carriers vs LL 1.4 0.8-2.6 0.3
PGI/PGII < 3.4 1.4 0.7-2.8 0.3

Logistic regression, AAG and ABC vs non-atrophic gastritis.

Table 4: Genotype groups n (%) of the samples.

Normal NOAG AAG ABG

IL-1RN
1/T 11/17 (65) 124/253 (49) 33/76 (43) 13/25 (52)
1/L 6/17 (35) 107/253 (42) 31/76 (41) 8/25 (32)
2/T 0/17 (0) 22/253 (9) 12/76 (16) 4/25 (16)
2/L 0.16 0.27 0.29 0.28
Allelic freq. T 0.22 0.26 0.29 0.28

H pylori CagA status* 4.1 1.9-9.0 0.000
IL-1B + 3954
T carriers vs CC 1.2 0.5-3.2 0.7
IL-1RN
2 carriers vs LL 1.4 0.8-2.6 0.3
PGI/PGII < 3.4 1.4 0.7-2.8 0.3

1Logistic regression, AAG and ABC vs non-atrophic gastritis.

Table 5: Age gender and smoking.

Age > 50 yr 3.3 1.2-9.1 0.002
Men 0.3 0.9-1.3 0.1
Present smoker 1.2 0.3-4.7 0.8
H pylori
CagA status* 2.5 1.4-4.3 0.002
IL-1B + 3954
T carriers vs CC 1.2 0.5-3.2 0.7
IL-1RN
2 carriers vs LL 1.4 0.8-2.6 0.3
PGI/PGII < 3.4 1.4 0.7-2.8 0.3

1Logistic regression, Peptic ulcer vs no peptic ulcer.

older than 50 years, those consuming alcohol and those with a PGI/PGII ratio < 3.4. Patients infected with CagA + strains of H pylori and those with lower consumption of fruit were at higher risk of developing AAG, (Table 2). The presence of pro-inflammatory alleles, IL-1B + 3954T and IL-1RN*2, did not confer an enhanced risk of any type of atrophy (Table 2).

Peptic ulcers

Duodenal ulcer was more frequent than gastric ulcer (52/24), in both genders. However, it was twice as common in males and the average age of patients with duodenal ulcer (average 46; 95% IC, 42.5-50.2) was lower than that of those with gastric ulcer (average 58; 95% CI, 52.2-63.6). Males, smokers and patients infected with H pylori CagA + were at increased risk of duodenal ulcer (Table 5).

DISCUSSION

While NAG associated with H pylori is more prevalent in industrialized countries, atrophic gastritis is more common in the developing world. The reason for this is probably a combination of effects caused by H pylori and other infections in early childhood, as well as other environmental factors and genetic composition. Atrophic gastritis initiates in the antrum and may extend upwards towards the gastric body, often resulting in more severe atrophic gastritis with increased age. It has been reported in sev-
eral studies that infection with *H. pylori* CagA+ increases the risk of atrophic gastritis and gastric cancer. In the present study, infection with *H. pylori* CagA- was associated with atrophic gastritis of the antrum but not of the body, results which are in accordance with those reported by Oksanen et al, 2000. Indeed, in a stomach affected by ABG, the microenvironment is more hostile for the survival and growth of *H. pylori* promoting spontaneous eradication of the bacteria and a progressive decline in concentrations of serum antibodies to *H. pylori*. Atrophic gastritis has been considered as a consequence of prolonged gastritis caused by *H. pylori* and the association between *H. pylori* infection and gastric cancer appears stronger when the infection is recorded several years before the onset of cancer. Therefore, in our study, the association of *H. pylori* CagA+ with ABG may be underestimated taking into account that patients belong to a population in which *H. pylori*-associated gastritis is prevalent from childhood. *H. pylori* and its virulence factor CagA may be involved in processes during the early stages of inflammation of the antrum that lead to extension of atrophic gastritis towards the gastric body.

In concordance with other studies, infection with *H. pylori*-CagA+ is associated with low PGI/PGII ratios. Several studies have reported an association of low PGI concentrations and low PGI/PGII ratios with precancerous lesions as well as with gastric cancer. In the study population, PGI/PGII values below 3.4 were previously shown to be associated with ABG but not with AAG. The studies performed in Costa Rica to date indicate low specificities of the pepsinogen test for the detection of gastric cancer and ABG (64% and 38.5% respectively). Nevertheless, its high negative predictive values (99.5% and 98%) could make the PG test useful to eliminate, from subsequent steps of screening programs, persons that are unlikely to develop gastric cancer.

Our results fail to demonstrate an association between AAG, ABG, and pro-inflammatory polymorphisms IL-1B + 3945 or IL-1RN. The control group consisted of dyspeptic patients of which a large majority suffered from some inflammatory condition (NAG). In a previous study, performed by our group, among participants in a program at the Center for Early Detection of Gastric Cancer in Costa Rica, it was found that IL-1B + 3945T and IL-1RN*2 were associated with an increased risk of gastric cancer: OR = 3, P = 0.007 and OR = 2.9; P = 0.03 respectively. In this study, the control groups were normal individuals as judged by X-ray (double contrast, gastric study).

The combination of IL-1B + 3954 and IL-1RN pro-inflammatory genotypes, or that of proinflammatory alleles and CagA, did not reveal any association with any particular pathology when included in the logistic regression analysis (data not shown).

Recent reports concerning the association of polymorphisms enhancing the expression of the gene IL-1B with atrophic gastritis, gastric cancer and peptic ulcer, diverge in their results. Studies link these polymorphisms to a reduction in acid secretion, gastric inflammation, atrophy, and gastric cancer, whereas others do not. These contradictory results may be related to the characteristics of the studied population, the methodology used, regional differences with regard to the frequencies of pro- or anti-inflammatory polymorphisms, the prevalence and time of infection with *H. pylori* and the characteristics of the infecting strain as well as diet and other environmental factors that interact with and influence the final result of the pathological process initiated by the infection.

The results of the present study show that consumption of fruit, even in modest quantities, diminishes the risk of AAG but has no effect on more advanced atrophy, ABG. It has been suggested that the protective properties of fruit are due to its content of antioxidants that would counteract the oxidative stress induced by *H. pylori*. The data presented here are in accordance with reports from other laboratories stating that CagA+ individuals are at higher risk of developing duodenal ulcers. Although patients with duodenal ulcer have reduced risk of gastric cancer, *H. pylori* predisposes to both conditions. These different consequences may be a result of the host response to the infection. It has been reported that an increase in acidity predispose for duodenal ulceration whereas hypochlorhydia is associated with a higher risk of developing gastric cancer.

In summary, this study does not permit the conclusion that there exists no association between infection with CagA+ *H. pylori* and ABG because *H. pylori* is spontaneously eradicated with the severity and extension of atrophic lesions. Future epidemiologic investigations should eliminate the inherent biases of sub-detection of *H. pylori* and specially *CagA*. The true relationships between *H. pylori* and diseases of the upper gastrointestinal tract are highly complex. It is of crucial importance to identify the factors that direct the initial inflammatory reactions to different gastric pathologies and decipher the mechanisms involved those processes, not only for the understanding of carcinogenesis but also for the prevention and detection of diseases related to *H. pylori*.

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Costa Rica has a high incidence and mortality from gastric cancer. Infection with *H. pylori* and proinflammatory interleukin (IL) gene polymorphisms have been associated with precancerous gastric lesions and gastric adenocarcinoma. Atrophic gastritis may lead to the development of cancer. This study addresses the association of atrophic antral and body gastritis with infection
H. pylori-CagA+ strains and IL-1 gene polymorphisms in a dyspeptic population in an area at high risk of gastric cancer in Costa Rica.

**Research frontiers**

In a dyspeptic population in Costa Rica, H. pylori-CagA+ is not associated with atrophic body gastritis, but it is a risk factor for atrophic antral gastritis (AAG). The pro-inflammatory cytokine polymorphisms IL-1β +3945 and IL-1RN are not associated with the atrophic lesions.

**Innovations and breakthroughs**

Studies of various populations have indicated an association between gastric cancer and H. pylori-CagA as well as proinflammatory gene variants. The etiology of gastric cancer varies among populations. The present study shows that H. pylori-CagA is related to active AAG but not to more advanced atrophy of the gastric body in a high-risk population. It is speculated that the bacteria may have disappeared at this stage. Furthermore, the study demonstrates no relation between two proinflammatory IL-1 gene polymorphisms and atrophy of the stomach in dyspeptic patients.

**Applications**

In developing countries with high incidence of H pylori infection and dyspepsia it is not feasible to screen large populations with endoscopy. The information generated here may be used to create a battery of markers to detect risk factors for gastric cancer from a blood sample. Screening and intervention may then be concentrated on people with a high-risk profile.

**Peer review**

The report is interesting. It could be useful in clinical settings. It indicated that in a dyspeptic population in Costa Rica, H pylori CagA+ is a risk factor for AAG.

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