RELATIONSHIP BETWEEN *cag*A-POSITIVE *Helicobacter pylori* INFECTION AND RISK OF GASTRIC CANCER:
a case control study in Porto Alegre, RS, Brazil

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ABSTRACT – **Context** - Gastric cancer is the second most common cause of cancer related death worldwide. Although *Helicobacter pylori* has been classified as a class I carcinogen, the presence of infection is not a factor that alone is able to lead to gastric cancer, and one of the possible explanations for this is the existence of different strains of *H. pylori* with different degrees of virulence. **Objectives** - To investigate the association between *cag*A-positive *H. pylori* and gastric cancer, using polymerase chain reaction (PCR) for the detection of this bacterial strain. **Methods** – Twenty-nine patients with gastric cancer were matched by sex and age (± 5 years) with 58 patients without gastric cancer, submitted to upper gastrointestinal endoscopy. All patients were evaluated for the status of infection by *H. pylori* (through urease test, histological analysis and PCR for the genes *ureA* and 16SrRNA) and by *cag*A-positive strain (through PCR for *cag*A gene). **Results** - Evaluating the presence of infection by *cag*A-positive *H. pylori*, it was verified that the rate of infection was significantly higher in the group with gastric cancer when compared with the matched controls, occurring in 62.1% and 29.3%, respectively (OR = 3.95; CI 95% 1.543-10.096). **Conclusions** - There is an association between *cag*A-positive *H. pylori* strain and risk of gastric cancer.

HEADINGS - *Helicobacter pylori*. Helicobacter infections. *cag*A protein, *Helicobacter pylori*., Stomach neoplasms.

INTRODUCTION

Gastric cancer is the fourth more frequent neoplasm and the second most common cause of cancer related death worldwide[26], despite the decrease in the incidence in the last decades[12].

The process of gastric carcinogenesis is not yet completely understood, but environmental risk factors identified for the development of this neoplasm are: diet rich in salt and preserved foods (N-nitroso compounds)[10, 37] and poor in anti-oxidants, such as vitamin A and C[2, 10, 29], tobacco smoking[10, 17] and infection by *Helicobacter pylori*[6, 10, 15, 23, 35].

H. pylori was first isolated in 1982 by Warren and Marshall[39], and once its identification, this bacteria promptly became the target of several studies. In 1994, the World Health Organization and the International Agency for Research on Cancer determined that there was sufficient evidence in humans for the carcinogenicity of infection with *H. pylori*, classifying this bacteria as a class I carcinogen[15].

H. pylori infection is one of the most common in the human species. In different countries, the prevalence rate of infection may vary from 30% to 90%[4], depending mainly of the age and the social-economic level.

According to meta-analyzes about the relationship between soropositivity to *H. pylori* and gastric cancer, the odds ratio for infected patients is about 1.92[14].

Uemura et al.[38] and Hsu et al.[13], in prospective cohort studies of 7.8 and 6.3-year follow-up, found that gastric cancer developed, respectively, in 2.9% and 1,1% of those who were *H. pylori*-positive, compared with none of those who were not infected.

Perhaps, the presence of the infection is not a factor that alone is able to lead to gastric cancer, as not all individuals who are infected will develop the neoplasm and the rates of gastric cancer are low in some countries were the rates of *H. pylori* infection are high[33]. Possible explanations for these findings are: differences in host genetics, other associated environmental factors and the existence of different strains of *H. pylori* with different degrees of virulence[9].

H. pylori may cause damage to the gastric epithelial cell by mechanisms related to pathogenic factors of the bacteria, mainly by the proteins coded by the genes *vac*A, *cag*A, *ice*A and *bab*A[21].

*H. pylori cag*A-positive strains have been associated with gastric ulcer[7, 25], duodenal ulcer [7, 30] and gastric cancer[1, 7, 8, 28, 31].

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Approximately 60%-80% of *H. pylori* strains express a high molecular weight protein, denominated *Cag*A, coded by the *cag*A gene. *Cag*A protein is translocated into the intracellular region of gastric epithelial cell, allowing the bacteria to modulate pathways of the cellular metabolism of the host[5, 11], inducing cellular hyper proliferation[19], apoptosis[22] and leading to failure of gastric epithelial cell ability to maintain its normal cytoskeletal structure, an important prerequisite for neoplastic transformation[32]. Also, induce interleukin-8 (IL-8) production by the epithelial cells. The IL-8 leads to intense inflammatory response and production of oxygen free radicals that could cause DNA damage to adjacent cells. Accumulation of oxidative DNA damage could lead to genetic modifications of gastric epithelial cells that are carcinogenic[21].

The present study has the objective of investigating the association between *cag*A-positive *H. pylori* and gastric cancer, using polymerase chain reaction (PCR) for the detection of this bacterial strain.

METHODS

This study was approved by the Ethics Committee of the Hospital Nossa Senhora da Conceição, Porto Alegre, RS, Brazil.

Considering *cag*A-positive *H. pylori* infection prevalence of 64% in patients with gastric cancer and 30% in patients without this neoplasia, with confidence interval 95%, power 80% and case: control rate 1:2, the minimum sample size required to evaluate the relationship between *cag*A-positive *H. pylori* infection and risk of gastric cancer was 29 patients in the case group and 58 patients in the control group.

From August 2003 to December 2004 were studied prospectively 29 patients with gastric cancer, and they were matched by sex and age (± 5 years) with 58 patients without gastric cancer, all evaluated through upper gastrointestinal endoscopy at Hospital Nossa Senhora da Conceição.

The inclusion criteria for the case group were: histopathological diagnosis of gastric adenocarcinoma through biopsies obtained during endoscopic exam, age 18 years or older and consent to participate. The exclusion criteria were: upper gastrointestinal hemorrhage, therapy with antibiotics within the last 6 months, history of subtotal gastrectomy and diagnosis of gastric cancer established more than 6 months previously.

The inclusion criteria for the matched control group were: patients submitted to upper gastrointestinal endoscopy in which the diagnosis was not gastric cancer, age 18 years or older and consent to participate. The exclusion criteria were: upper gastrointestinal hemorrhage, therapy with antibiotics within the last 6 months and history of subtotal gastrectomy.

During endoscopy, were collected biopsies of gastric corpus, incisura angularis and antrum of all cases and controls patients. The gastric biopsies of all patients were evaluated for the detection of *H. pylori* (through 3 methods: urease test, histological analysis and PCR for the genes *ure*A and 16SrRNA) and the *cag*A gene (through PCR for the gene

*cag*A). Patients in whom the genes *ure*A and 16SrRNA were detected by PCR and/or two other methods for detection of *H. pylori* were positive were considered *H. pylori*-positive. Patients in whom the gene *cag*A was detected by PCR were considered *cag*A-positive.

Urease test
Carried out through one antrum and one corpus biopsies.

Histological analysis
Carried out through two antrum, one incisura angularis and two corpus biopsies. Sections were stained with H-E to grade the severity of gastritis and to evaluate the presence of atrophy and intestinal metaplasia, and with Giemsa to detect *H. pylori*.

Preparation of DNA for PCR amplification
The biopsy specimens for DNA analysis were placed in 0.9% NaCl, and the DNA was isolated directly from the biopsy specimens using the QIAamp tissue kit (QIAgen Inc., Santa Clarita, Calif, USA.).

H. pylori and *cag*A detection by PCR
One gastric biopsy specimen from the antrum was subjected to PCR, targing the *ure*A, 16SrRNA and *cag*A gene detection, using sets of synthetic oligonucleotides primers, as described elsewhere by Rota et al.[30]. The primers HPU18N (5'-CCCATTTGACTCAATGCGATG-3') and HPU54N (5'-TGGGATTAGCGAGTATGTCGG-3') were used to amplify a 132-bp product from the *16SrRNA* gene, and the primers UREA1 (5'-GCCAATGGTAAATTAGTT-3') and UREA2 (5'-CTCCTTAATTGTTTTTAC-3') were used to amplify a 394-bp product from the *ure*A gene. The forward and reverse primers *Cag*A/ConF (5'-GTGCCTGCTAGTTTGTCAGCG-3') and *Cag*A/Con-R (5'-TTGGAAACCACCTTTTGTATTAGC-3') were used to amplify a 402-bp fragment of the *cag*A gene (Figure 1).
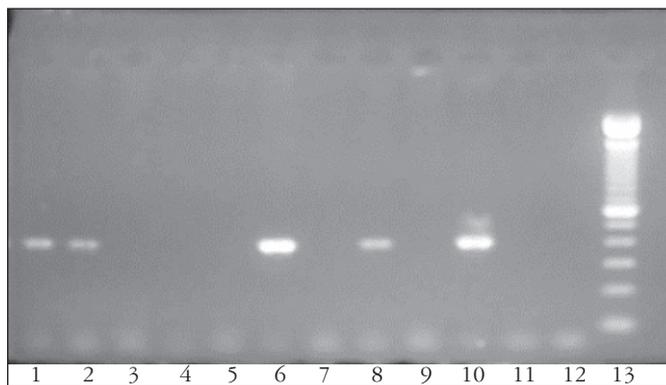

FIGURE 1. PCR amplification of *cagA* gene: lanes 1, 2, 6 and 8: positive PCR products; lanes 3, 4, 5, 7 and 9: negative PCR products; lane 10: positive control; lanes 11 and 12: negative control (without DNA); lane 13: molecular weight marker

For statistical analysis, categorical variables were evaluated through x^2 test, Fischer exact or Yates correction. Significance was defined as *P* values <0.05. Continual variables were evaluated through Student *t* test. Odds ratio was used as association measure.

RESULTS

The characteristics of the 29 patients with gastric adenocarcinoma and the 58 matched controls are described in Table 1.

Case group – all patients presented with non-cardia gastric adenocarcinoma. While among men the most frequent histological classification was intestinal type adenocarcinoma (66.7%), among women, it was diffuse type adenocarcinoma (54.5%) (*P* = 0.039). Atrophy and/or gastric intestinal metaplasia were identified through histological analysis of biopsy samples from gastric segments without cancer in 65.5% of the patients.

Control group – the endoscopic findings were: normal exam (11 patients), enantematous gastritis (14 patients), erosive gastritis (13 patients), erosive duodenitis (5 patients), gastric ulcer (11 patients) and duodenal ulcer (4 patients). Atrophy and/or gastric intestinal metaplasia were identified through histological analysis of gastric biopsy samples in 32.7% of the patients, and were more frequent in *cag*A-positive *H. pylori* patients, however, the result did not achieve statistical significance (OR = 2.42, CI 95% 0.76-7.70).

Case vs control group – *cag*A-positive *H. pylori* infection prevalence was significantly higher in the case group, when compared with the control group, occurring in 62.1% and 29.3%, respectively (OR = 3.95; CI 95% 1.54-10.09) (Table 2).

Considering only *H. pylori*-positive patients, there was stronger relationship between *cag*A-positive strain and gastric cancer. The frequency of *cag*A-positive infection in the case group was 90% compared with 42.5% in the control group (OR = 12.18; CI 95% 2.71-52.9) (Table 2).

DISCUSSION

Despite *H. pylori* colonizes the stomach of about half of the world population, only 1%-2% of the infected individuals will evolve to gastric cancer. Among the possible explanations is the fact that specific *H. pylori* genotypes have been associated to more virulent presentations.

TABLE 2. Risk of distal gastric adenocarcinoma according to status of infection by *Helicobacter pylori cag*A+ strain

	Cancer patients		Matched controls		
	*cag*A+	*cag*A-	*cag*A+	*cag*A-	OR (CI 95%)
All patients	18	11	17	41	3,95 (1,54-10,09)
H. pylori + patients	18	2	17	23	12,18 (2,71-52,9)

Several case-control studies, in different countries, have investigated the association between *cag*A-positive *H. pylori* and gastric cancer, and most evidence in literature agrees this association does exist[3, 6, 16, 18, 20, 27, 28, 34].

Considering the known difference in the populational characteristics among the different countries and the fact that no study about this association has been previously carried out in south of Brazil, it becomes relevant to know the results obtained in our region.

The same rate of infection by *H. pylori* was identified in case and control groups. Possible reasons are: a) the sample size was not calculated to evaluate the relationship between *H. pylori* infection and gastric cancer, b) patients with gastric and duodenal ulcers (diseases also related to *H. pylori* infection) were included in the control group, and c) the fact that all patients in the case group presented with advanced cancer, and a significant parcel of these loose the colonization by *H. pylori* in the evolution of the disease[24], since the neoplastic tissue, as well as gastric epithelium severely atrophic and with intestinal metaplasia, are not the adequate habitat for development of this bacteria. Ekström et al.[6] describe that loss of infection may precede a cancer diagnosis by decades, and in these conditions the rate of *H. pylori* exposure may be underestimated.

The present study demonstrates that *cag*A-positive *H. pylori* is associated significantly with non-cardia gastric cancer (OR = 3.95; CI 95% 1.543-10.096). However, a stronger association between *cag*A-positive *H. pylori* and gastric cancer was identified when we compared only *H. pylori*-positive patients of both groups (OR = 12.18; CI 95% 2.71-52.9). Demonstrating that, in our region, once we identify the infection by *H. pylori*, 90% of gastric cancer patients present infection by *cag*A-positive strain.

In the future, studies evaluating the relationship between *cag*A-positive *H. pylori* strains and gastric cancer should associate the use of PCR for *cag*A gene and serology for *Cag*A

TABLE 1. Characteristics of case and matched-control groups

	n	Male n (%)	Mean age	*H. pylori* + n (%)	*cag*A + n (%)
Non-cardia gastric cancer	29	18 (62,1%)	65,8	20 (68,9%)	18 (62,1%)
Intestinal	16	12 (75%)	67,1	10 (62,5%)	9 (56,2%)
Diffuse	8	2 (25%)	56,5	6 (75%)	5 (62,5%)
Indeterminated	5	4 (80%)	76,6	4 (80%)	4 (80%)
Matched-control	58	36(62,1%)	64,1	40 (68,9%)	17 (29,3%)
Normal exam or enantematous gastritis	25	16 (64%)	66,8	17(68%)	9 (36%)
Erosive gastritis and/or duodenitis	18	10 (55,5%)	59,8	11 (61,1%)	2 (11,1%)
Peptic ulcer	15	10 (66,7%)	64,7	12 (80%)	6 (40%)

antigen in an attempt to identify not only patients with actual infection by *cag*A-positive *H. pylori* (situation in which PCR presents elevated sensibility), but also patients that presented infection by this strain in a recent past of up to 32 months (situation which can be detected only by serology)[36].

CONCLUSIONS

We conclude that exists an association between *cag*A-positive *H. pylori* infection and distal gastric cancer in Porto Alegre, Brazil (OR = 3.947; CI 1.543-10.096). The behavior of the infection by this bacterial strain strengths the hypothesis that it plays an important rule in the pathogenesis of gastric cancer. Thus, we might consider the possibility that the *cag*A-positive *H. pylori* infection can be a marker for individuals at an increased risk for the development of non-cardia gastric cancer.

Nevertheless, to suggest a change in the management of *H. pylori* infected patients, it is necessary the accomplishment of additional long term prospective studies, evaluating the impact of the eradication of *cag*A-positive strain infection on the risk of development of gastric cancer.

Meine GC, Rota C, Dietz J, Sekine S, Prolla JC. Relação entre infecção por *Helicobacter pylori* linhagem *cag*A-positiva e risco de câncer gástrico: um estudo caso-controle em Porto Alegre, Brasil. Arq Gastroenterol. 2011;48(1):41-5.

RESUMO – ***Contexto*** - O câncer gástrico é a segunda causa mais comum de mortes relacionadas à neoplasia em todo o mundo. Embora o *Helicobacter pylori* seja classificado como um carcinógeno classe I, a presença da infecção não é um fator que isoladamente possa conduzir ao câncer gástrico e, uma das possíveis justificativas, é a existência de diferentes linhagens de *H. pylori* com diferentes graus de virulência. ***Objetivo*** - Investigar a associação entre *H. pylori cag*A-positivo e câncer gástrico, utilizando a reação em cadeia de polimerase (PCR) para a detecção desta linhagem bacteriana. ***Métodos*** – Vinte e nove pacientes com câncer gástrico foram pareados por sexo e por idade (± 5 anos) com 58 pacientes sem câncer gástrico, submetidos a endoscopia digestiva alta. Todos os pacientes foram avaliados quanto à presença de infecção pelo *H. pylori* (com teste da urease, análise histológica e PCR para os genes *ure*A e 16SrRNA) e pela linhagem *cag*A desta bactéria (com PCR para o gene *cag*A). ***Resultados*** - Avaliando a presença de infecção por *H. pylori cag*A-positivo, verificou-se que a taxa da infecção era significativamente mais alta no grupo de pacientes com câncer gástrico, quando comparado com o grupo controle, ocorrendo em 62,1% e em 29,3%, respectivamente (OR = 3,95; CI 95% 1,543-10,096). ***Conclusões*** - Há associação entre *H. pylori cag*A-positivo e risco de câncer gástrico.

DESCRITORES - *Helicobacter pylori*. Infecções por helicobacter. *cag*A proteína, *Helicobacter pylori.* Neoplasias gástricas.

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