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• *BRIEF REPORTS* •

Helicobacter pylori infection and low serum pepsinogen I level as risk factors for gastric carcinoma

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Abstract

AIM: To study whether examination of CagA antibodies could increase the odds ratio for gastric cancer in a case-control study, and how often other serum markers of gastric cancer risk could be found in *Helicobacter pylori*-negative patients.

METHODS: *H pylori* CagA and parietal cell antibodies (PCAs), and serum pepsinogen I (SPGI) levels were compared between patients with gastric cancer and controls who received endoscopic examination due to reasons other than gastrointestinal malignancy.

RESULTS: The odds ratio (OR) for gastric cancer was 2.9 (95% CI 1.4-5.8) in *H pylori* + patients, and 2.4 (95% CI 1.2-4.9) in CagA+ patients. When results of *H pylori* and CagA antibodies were combined, OR increased to 5.0 (95% CI 2.5-10.0). Furthermore, if cardia cancer patients were excluded, the OR increased to 6.8 (95% CI 3.1-14.8). Among patients with a low SPGI level, the OR was 12.0 (95% CI 4.1-35.3). However, the risk was significant only in the older age group. The number of patients with low SPGI was significantly higher in *H pylori* -/CagA+ patients as compared to other cancer patients.

CONCLUSION: Examination of both *H pylori* and CagA antibodies increases the OR for gastric cancer in our case-control study. CagA antibodies are important in detecting previous *H pylori* infection in advanced atrophic gastritis or cancer when spontaneous decline of *H pylori* antibodies occurs. SPGI may be helpful in screening elderly gastric cancer patients.

Key words: Gastric cancer; Risk factor; Atrophic gastritis

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INTRODUCTION

The incidence of gastric cancer has rapidly decreased in the Western countries during the last decade[1]. It remains, however, a major cause of cancer morbidity and mortality[1]. According to a recent meta-analysis, *Helicobacter pylori* (*H pylori*) infection increases the risk for non-cardia gastric cancer approximately six folds[2], whereas the development of cardia cancer has not been shown to be associated with *H pylori*[2]. Atrophic gastritis, a well documented risk factor for gastric cancer[3-5], has been estimated to develop in up to half the persons infected with *H pylori*[6-8]. However, in patients with severe atrophic gastritis, *H pylori* infection may be difficult to demonstrate because the bacteria disappear spontaneously and even *H pylori* antibody titers finally decline to normal[9-13]. It has previously been suggested that CagA antibodies last longer in serum after *H pylori* eradication than *H pylori* antibodies[14]. Patients with CagA-positive strains of *H pylori* have a higher risk for atrophic gastritis and gastric cancer than patients with CagA-negative *H pylori* gastritis[15,16].

Low serum pepsinogen I (SPGI) level has been shown to be an accurate indicator of atrophic corpus gastritis[17], and is used in screening patients with elevated risk for gastric cancer[18,19]. Although *H pylori* is the most important cause of atrophic gastritis, some patients may have atrophic changes without previous *H pylori* infection[20], so called autoimmune atrophic gastritis. However, autoimmune type of atrophic gastritis may be linked to a previous *H pylori* infection[21,22]. Parietal cell antibodies (PCAs) are found in autoimmune-type atrophic gastritis[23].

The present study was performed to measure *H pylori*, CagA and PCAs, to determine SPGI levels in patients with gastric cancer, and to compare the findings with those of patients who had undergone endoscopy due to reasons other than upper gastrointestinal malignancy.

MATERIALS AND METHODS

Patients and serum samples

Serum samples were randomly collected from 143 gastric cancer patients who were treated at Helsinki University

Central Hospital between September 1988 and July 2001. Eleven patients were treated in 1980s, 93 in 1990s and 39 in 2000-2001. Sera were stored at -20 ℃ until analyzed. There were 77 males and 66 females (age ranged from 32 to 89 years, mean age 64 years). There were 81 diffuse tumors and 61 intestinal-type tumors[24]. One tumor was unclassified. The tumor stages[25] were as follows: stage I in 33, stage II in 11, stage III in 38, and stage IV in 59 patients. In two patients the stage could not be defined. Fifteen of the tumors were located in the cardia and 128 were non-cardia tumors by location.

Control sera were collected from 108 patients who underwent endoscopy for some reasons other than gastrointestinal malignancy. The final diagnoses of the control patients are shown in Table 1. Fifty-nine of the controls were males and 49 were females (age ranged from 17 to 98 years, mean age 61 years). The control serum samples were collected in 2002 and stored at -80 ℃ until analyzed. All patients gave their informed consent before serum samples were collected.

Table 1 Final diagnoses of 108 control patients

Diagnosis	Number of patients	*H pylori*-positive (*H pylori* + and/or CagA+) patients (%)
Non-ulcer gastrointestinal bleeding	26	15 (58)
Esophagitis	23	12 (52)
Dyspepsia	21	11 (52)
Gastric ulcer	14	12 (86)
Barrett's esophagus	7	2 (29)
Duodenal ulcer	4	3 (75)
Benign gastrointestinal tumor	4	2 (50)
Others	9	5 (56)
All	108	62 (57)

All subjects who had received *H pylori* eradication therapy before serum samples were collected (seven cancer patients and 23 controls), were excluded from the analysis.

Serum antibodies to *H pylori* were measured by an in-house enzyme immunoassay (EIA). The antigen used was an acid glycine extract from *H pylori* NCTC 11637[26]. The lower limits for the raised titers were 700 for IgG antibodies and 70 for IgA antibodies[27]. In an outpatient series, the assay showed a specificity of 93% and a sensitivity of 100% as compared to histology[27].

CagA antibodies were measured with a commercial immunoblot method (I.D. Blot *H pylori* IgG, DPC, Los Angeles, California, USA) according to instructions of the manufacturer. Briefly, serum samples at a dilution of 1:50 were incubated with nitrocellulose strips for 30 min at room temperature. The strips were washed with buffer and incubated with an enzyme-labeled anti-IgG antibody. After washing, the strips were treated with the substrate solution. Positive bands were estimated visually. A band at 120-kDa indicated the presence of CagA antibodies.

SPGI concentrations were measured using an immunoenzymometric assay (Gastroset PG1, Orion Diagnostica, Espoo, Finland). SPGI levels <28 μg/L indicated the presence of atrophic corpus gastritis.

PCAs in serum were determined with a commercial enzyme immunoassay (Varelisa Parietal Cell Antibodies, Pharmacia Diagnostics, Freiburg, Germany) according to instructions of the manufacturer. Serum samples at a dilution of 1:101 were incubated for 30 min in microtiter wells coated with the H^+/K^+ATPase antigen. The microtiter wells were washed with buffer, and an enzyme-labeled anti-human IgG conjugate was added and incubated for 30 min. After washing, enzyme substrate was pipetted into the wells, incubated for 10 min, and the reaction was stopped. Absorbances (optical densities) at 450 nm were recorded with a microplate reader and converted to PCA concentrations. According to instructions of the manufacturer, concentrations <10 U/mL were considered normal.

Statistical analysis

Categorical data were analyzed using χ^2 or Fisher's exact tests, and continuous data with Mann-Whitney *U* test. *P* values less than 0.05 were considered statistically significant. Logistic regression analysis was used to evaluate and compare relative risks (odds ratio, OR) for cancer attributed to the serum markers of risk factors for gastric cancer. The analysis was performed in a backward and forward stepwise manner and the best fit of the model was assessed with likelihood ratio test. The final multivariate model included only the covariates with statistical significance in the model.

RESULTS

H pylori and CagA antibodies

The prevalence of *H pylori*, CagA and either *H pylori* and/or CagA antibodies in 143 gastric cancer patients, or 108 controls are shown in Table 2. The prevalence of CagA antibodies was higher in *H pylori* antibody positive cancer patients than in *H pylori* + control patients (95/101, 94.1% *vs* 35/42, 83.3%, $P = 0.056$, χ^2). There was no significant difference between the histological type of cancer and the prevalence of *H pylori* or CagA antibodies. In patients with tumors located in the cardia, antibodies (*H pylori* + and/or CagA+) were found less often than in patients with tumors located elsewhere in the stomach (10/15 [66.7%] *vs* 117/128 [91.4%], $P = 0.014$, Fisher's exact test).

Table 2 Number (%) of *H pylori* (*H pylori* +), CagA (CagA+) and *H pylori*+ and/or CagA+ persons in 143 gastric cancer patients and 108 controls

	Cancer patients *n* (%)	Controls *n* (%)	*P* (χ^2-test)
H pylori +	101 (70.6)	42 (38.9)	<0.001
CagA+	120 (83.9)	55 (50.9)	<0.001
H pylori + and/or CagA +	127 (88.8)	62 (57.4)	<0.001

Among the non-cardia cancer patients, 11 (8.6%) of the patients had no evidence of *Hp* infection (*H pylori* - and CagA-); most of them had diffuse-type and stage IV tumors (Table 3). The effects of tumor stage on the prevalences of *H pylori* and CagA antibodies are shown in Table 4.

Table 3 Characteristics of 11 non-cardia cancer patients who did not show any evidence of previous *H pylori* infection (*H pylori* - and CagA-)

Case	Age (yr)	Sex	SPGI	PCA	Histological type of cancer	Tumor stage
1	54	Male	Normal	Normal	Intestinal	4
2	65	Male	Normal	Normal	Intestinal	4
3	76	Male	Normal	Normal	Intestinal	4
4	62	Female	Normal	Normal	Diffuse	1
5	75	Female	Low	Normal	Diffuse	2
6	44	Female	Normal	Normal	Diffuse	3
7	32	Female	Normal	Elevated	Diffuse	4
8	54	Male	Normal	Normal	Diffuse	4
9	69	Female	Normal	Normal	Diffuse	4
10	77	Male	Low	Elevated	Diffuse	4
11	78	Female	Normal	Normal	Diffuse	4

Table 4 Effect of tumor stage on the prevalence of *H pylori* (*H pylori* +) and CagA (CagA+) antibodies in 126 non-cardia gastric cancer patients

	Stage 1 (%) (*n*=31)	Stage 2 (%) (*n*=10)	Stage 3 (%) (*n*=30)	Stage 4 (%) (*n*=55)
H pylori +	80.6	70.0	73.3	67.3
CagA+	90.3	80.0	93.0	81.8
H pylori + and/or CagA +	96.8	90.0	96.7	85.5

Serum pepsinogen I

Cancer patients had significantly lower SPGI levels than the controls (mean SPGI 75.1 μg/L *vs* 116.9 μg/L, $P<0.001$, Mann-Whitney U test). Accordingly, the number of patients with a low SPGI level (<28 μg/L) was higher in the group of cancer patients (50 out of 143 *vs* 4 out of 108, $P<0.001$, Fisher's exact test). Patients with intestinal-type cancer had a lower mean SPGI level than those with diffuse-type tumors (mean SPGI 57.8 μg/L *vs* 88.9 μg/L, $P = 0.004$, Mann-Whitney U test). In those cancer patients who had CagA antibodies but not elevated *H pylori* antibodies (*H pylori* -/CagA+), the SPGI level was significantly lower than in other cancer patients (mean SPGI 39.2 μg/L *vs* 82.7 μg/L, $P = 0.001$, Mann-Whitney *U* test). Also the number of patients with a low SPGI level was more in *H pylori* -/CagA+ patients than in the other cancer patients (16/25 [64.0%] *vs* 34/118 [28.8%], $P = 0.002$, Fisher's exact test).

Parietal cell antibodies

PCAs were found more commonly in cancer patients than in controls (24/143 cancer patients [16.8%] *vs* 8/108 controls [7.4%], $P = 0.020$, Fisher's exact test). PCAs were detected in 9 out of 61 (14.8%) patients with intestinal-type cancer and in 15 out of 81 (18.5%) with diffuse-type tumors. PCAs were found almost as often in *H pylori* - and CagA- patients (11.3%) as in *H pylori* positive (*H pylori* + and/or CagA+) patients (13.2%). The number of patients with PCAs was more in low SPGI level patient group than in normal SPGI level group (14 out of 54 [27.8%] *vs* 18 out of 197 [9.1%], $P = 0.002$, χ^2).

Logistic regression analysis

The OR of *H pylori* infection, as determined by elevated *H pylori* antibody titers, was 2.9 (95%CI 1.4-5.8) in all gastric cancers, and 3.1 (95%CI 1.5-6.3) in non-cardia cancer. When *H pylori* and CagA antibodies were combined, the OR increased to 5.0 (95%CI 2.5-10.0) in all gastric cancers and to 6.8 (95%CI 3.1-14.8) in non-cardia cancer (Table 5). The effect of the histological type of cancer on ORs is shown in Table 6.

Table 6 Odds ratios (with 95% confidence intervals) of risk factors for intestinal and diffuse types of gastric cancer

	Intestinal type (*n* = 61)	Diffuse type (*n* = 81)
Low SPGI	27.7 (8.5-89.5)	8.2 (2.5-27.0)
PCA+	NS	NS
H pylori + and/or CagA +	4.1 (1.5-10.7)	6.0 (2.6-13.9)

NS = not significant.

DISCUSSION

In the present study, 89% of gastric cancer patients and 91% of non-cardia cancer patients were *H pylori* seropositive (*H pylori* + and/or CagA+). When CagA-positive patients were considered, the prevalence of *H pylori* positivity increased by 17%, and the OR in *H pylori* gastritis for non-cardia gastric cancer rose from 3.1 to 6.8. The present result is in line with a recent Swedish study, in which CagA immunoblot detected previous *H pylori* infection in an additional 21% of gastric cancer patients[28]. It has been shown that OR for gastric cancer in *H pylori* infection increases when time interval between serum collection and cancer diagnosis becomes longer[29]. This may be due to spontaneous decline of *H pylori* antibodies in some patients as the gastric carcinogenesis proceeds. However, although seronegative in ordinary *H pylori* antibody tests, some patients may still have CagA antibodies as a marker of *H pylori* infection. Although CagA positivity was very common both in the *H pylori* + gastric cancer patients and in *H pylori* + controls in our study, some of the cancer patients may have been infected with CagA negative *H pylori* strains.

A low SPGI value is the best indicator of gastric cancer

Table 5 Odds ratios (with 95% confidence intervals) of risk factors for the development of gastric cancer

	All gastric cancers	Non-cardia cancers only	Patients aged <55 yr[1]	Patients aged >55 yr[2]
Low SPGI	12.0 (4.1-35.3)	12.5 (4.2-37.4)	NS	16.4 (2.1-128.8)
PCA+	NS	NS	NS	NS
H pylori+ and/or CagA+	5.0 (2.5-10.0)	6.8 (3.1-14.8)	16.7(3.1-88.7)	3.1 (1.2-8.0)

NS= not significant. [1]38 cancer patients <55 yr were compared to 40 age-matched controls. [2]105 cancer patients >55 yr were compared to 68 age-matched controls.

with the highest OR especially in the intestinal type of cancer and in patients older than 55 years of age. Patients with atrophic gastritis are often asymptomatic, and screening for SPGI may increase the detection rate of early neoplastic lesions in the stomach[18].

In our study, the presence of PCAs was not a significant risk factor for gastric cancer although it was observed more frequently in cancer patients. Recently, a large study from Sweden showed only 2.2 fold gastric cancer risk in pernicious anemia patients[30]. A larger case-control study might show an increased risk for gastric cancer in PCA-positive patients. It seems to give only little additional value in preoperative diagnosis of gastric cancer. In our study, only one gastric cancer patient without any evidence of previous *H pylori* infection and a normal SPGI level showed PCAs.

In the present study, most gastric cancer patients had evidence of *H pylori* infection (*H pylori* + and/or CagA+) but in some of the seronegative patients with advanced atrophic gastritis and in those with stage IV tumors, spontaneous eradication occurred. When patients with stage IV tumors were excluded, only two non-cardia cancer patients were *H pylori* seronegative and had normal SPGI and PCA levels.

Our control material collected from patients at our department could hardly represent a "normal" population. The majority of the control patients had gastrointestinal bleeding or esophagitis, and many patients with peptic ulcer disease were also included. Thus, both patient groups known to have a high prevalence of *H pylori*, such as peptic ulcer patients[31,32] and those with a low prevalence of *H pylori*, such as gastroesophageal reflux disease patients[33,34] were included as controls. Although the control samples were collected later than the samples from cancer patients, and *H pylori* prevalence is rapidly decreasing in developed countries such as Finland[35,36], the overall *H pylori* seroprevalence in the controls was still 57%. However, though the problems existed in selecting the control patients, the OR for gastric cancer in *H pylori*-positive subjects in our study was approximately the same as in a recent meta-analysis[2]. *H pylori* infection was observed less frequently in patients with cardia cancer than in patients with non-cardia gastric cancers, which is also in line with previous studies[2]. In our study, the odds ratio was even higher in cancer patients aged less than 55 years as compared to the older ones. Corresponding results have also been published earlier[37].

In summary, *H pylori* infection increases the risk for non-cardia gastric cancer by 6.8-fold, but is not associated with cardia cancer. Detection of CagA antibodies can significantly increase the number of *H pylori*-positive patients. Low SPGI level is a significant risk factor for gastric cancer only in elderly patients. The value of PCAs in preoperative diagnosis of gastric cancer seems to be limited.

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