Gastric Cancer (1998) 1: 118–124

Original article

Severe atrophic gastritis with *Helicobacter pylori* infection and gastric cancer

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Abstract:
Background. **We conducted a case-control study to evaluate whether patients with severe gastric atrophy (indicated by serum pepsinogen concentration) have a high risk of gastric cancer.**
Methods. **At the time of diagnosis of gastric cancer, sera from 301 patients (cases) and 602 sex- and age-matched cancer-free individuals (controls) were tested for the presence of anti-*Helicobacter pylori* IgG antibody (HM-CAP enzyme-linked immunoassay [ELISA] kit; Kyowa Medix, Tokyo, Japan) and serum pepsinogen (PG) levels (PG I and II Riabead Kits; Dainabot, Tokyo, Japan). We defined positivity for pepsinogen a pepsinogen I concentration of less than 70 ng/mL and a PG I/II ratio of less than 3.0. We categorized the subjects according to serum pepsinogen levels and anti-*Helicobacter pylori* IgG antibody, creating four categories.**
Results. **Of the 301 cancer cases, 177 had positive serum pepsinogen levels, and 172 were positive for anti-*Helicobacter pylori* IgG antibody. The category in which subjects had positive serum pepsinogen levels and were negative for anti-*Helicobacter pylori* IgG antibody had the highest proportion (76.9%) of individuals with gastric cancer and the highest odds ratio (4.20) of the four categories. The odds ratios were 2.55 (95% confidence interval; 1.92–3.88) for positive serum pepsinogen levels and 0.93 (95% confidence interval; 0.63–1.27) for positive anti-*Helicobacter pylori* IgG antibody.**
Conclusion. **These results suggest that patients with positive serum pepsinogen levels who are negative for IgG antibody to *Helicobacter pylori*, constitute a high-risk group for gastric cancer. *Helicobacter pylori* infection is associated with the development of gastric cancer by providing a suitable environment i.e., severe gastric atrophy, for carcinogenesis of the gastric mucosa.**

Key words: *Helicobacter pylori*, pepsinogen, atrophic gastritis, gastric cancer, case-control study

Offprint requests to: F. Kitahara
Received for publication on Mar. 20, 1998; accepted on Aug. 20, 1998

Introduction

Gastric cancer is one of the leading causes of mortality in Japan and many risk factors have been implicated [1]. Many case-control studies have found an increased cumulative risk of gastric cancer and precancerous conditions in individuals with *Helicobacter pylori* antibodies [2–4]. Based on this epidemiologic evidence, the World Health Organization (WHO) International Agency for Research on Cancer concluded: There is sufficient evidence that *H. pylori* has a causal role in the chain of events leading to gastric cancer [5].

However, there are several problems with regard to the association between *H. pylori* infection and gastric cancer. First, there is no infected model of *H. pylori* with subsequent gastric cancer, and the mechanism of gastric cancer growth has not been made clear at the gene level. Secondly, atrophy and metaplasia are implicated in the pathogenesis of gastric cancer of the intestinal type, whereas gastric cancer of the diffuse type does not clearly coexist with atrophy and metaplasia.

Several reports have shown that the extent of atrophy in gastric mucosa without *H. pylori* infection does not increase with age [6–12]. Mucosa with severe atrophic gastritis is, however, associated with an increased risk of gastric cancer [6–9,13–18]. Therefore, it is now suggested that *H. pylori* infection, through the development of atrophic gastritis, is an indirect cause of gastric cancer.

We conducted a case-control study to evaluate the effect of *H. pylori* infection on the development of gastric cancer. We also investigated the risk of gastric cancer in patients with severe atrophic gastritis.

Subjects and methods

Study population

Between April 1989 and March 1997, 521 patients diagnosed with gastric cancer were admitted to the First

Department of Medicine, Yamanashi Medical University Hospital, in Yamanashi, Japan. Patients with multiple gastric cancers ($n = 65$) were excluded from our analysis, because the evaluation of tumor characteristics becomes complicated. Twenty-five patients who had previously undergone partial gastrectomy were also excluded, as atrophy of the gastric mucosa was marked in the gastric remnant, and its extent depended on the interval after surgery. Blood samples of 130 patients had not been stored at the time of diagnosis. Accordingly, 301 patients (213 men, 88 women, mean age at 64 years) were selected as case patients. Blood samples were drawn at the time of diagnosis in the 301 cases, and the separated sera were individually stored at less than −80 °C until tested. Control subjects ($n = 602$) were selected from among cancer-free, non-peptic ulcer, and non-gastric remnant patients determined by gastroscopy during health check-ups carried out at Yamanashi Koseiren Medical Center. The control subjects were matched to the cases according to sex and age (within 3 years). The blood samples for routine laboratory tests at the health check-up were drawn after the patients had fasted, and aliquots of the separated sera were individually stored at less than −80 °C until tested. We obtained 301 matched sets with one case and two controls. The study was accepted by the Ethics Committee of the Institution, and all participating patients gave their informed consent.

Serological test

H. pylori infection was determined by testing for the presence of anti-*H. pylori* IgG antibody, using an HM-CAP enzyme-linked immunoassay (ELISA) kit (Kyowa Medix, Tokyo, Japan), and subjects with a titer higher than 2.2 were considered positive for *H. pylori* infection. Serum pepsinogen (PG) concentrations were measured using pepsinogen I and II Riabead Kits (Dainabot, Tokyo, Japan), a modified radioimmunoassay method described previously [15]. We regarded as PG-positive those subjects with a PG I concentration of less than 70 ng/ml and a pepsinogen I : II ratio (I/II ratio) of less than 3.0 [16].

Methods

We categorized the subjects according to their serum PG concentration and anti-*H. pylori* IgG antibody titer (Table 1), creating four categories. Category A, positive for serum PG concentration and anti-*H. pylori* IgG antibody; category B, negative for serum PG concentration and anti-*H. pylori* IgG antibody; category C, positive for serum PG concentration and negative for anti-*H. pylori* IgG antibody; and category D, negative for serum PG concentration and positive for anti-*H. pylori* IgG antibody. We compared mean age, percentage of patients with gastric cancer to total subjects, and the odds ratios for each of the four categories, conducting a case-control study to evaluate the effects of *H. pylori* infection and atrophic gastritis (indicated by serum PG concentration), on the risk of gastric cancer.

Table 1. Four categories of subjects (cases and controls) based on serum pepsinogen concentration and anti-*Helicobacter pylori* antibody titer

	Positive PG	Negative PG
Positive *H. pylori* antibody	Category A	Category D
Negative *H. pylori* antibody	Category C	Category B

Positive PG, Subjects with serum pepsinogen (PG) I concentration less than 70 ng/ml and a PG I/II ratio of less than 3.0; negative PG, subjects with serum PG I concentration of more than 70 ng/ml or PG I/II ratio of more than 3.0; positive *H. pylori* antibody, subjects with a titer of more than 2.2; Negative *H. pylori* antibody, subjects with a titer of less than 2.1

The *statistical calculations* were carried out with a statistical software package (Stat view II; Abacus Concepts, Berkeley, CA, USA). Data values are summarized as means ± SD. Differences in mean age, serum PG I concentration, PG II concentration, and PG I/II ratio between the cases and controls were analyzed by the Mann-Whitney U-test. Pearson's χ^2 test was used to compare the prevalence of *H. pylori* infection and positive serum PG in cases and controls. The subjects mean ages in the categories were compared using the Mann-Whitney U-test. A matched case and control study design was used to identify cases and controls for the serum test. Odds ratios (OR), which approximate the relative risk, with 95% confidence intervals (CI) were determined using the Mantel-Haenzel method. Probability values less than 0.05 were considered significant.

Results

Gastric cancer cases

The 301 gastric cancer cases consisted of 213 men and 88 women, with a mean age of 64.0 years. We categorized the cases into two each classes by age, sex, and depth of invasion the (the characteristics of these subgroup analyses are shown in Table 2). There was no significant difference in mean age between the men and the women. We classified depth of invasion as "early" or "advanced" stage. Early stage gastric carcinoma was defined as tumor localized at the deepest level within the submucosa, and advanced stage gastric carcinoma as tumor invading the muscularis propria or beyond. We

Table 2. Characteristics of cases (patients with gastric cancer)

Subgroup	Number (*n*)	Mean age (years)	Mean PG I	Mean PG I/II	PG-positive[a]	*H. pylori*-positive[a]
All cases	301	64.2	52.8	22.6	58.8%	57.1%
Sex						
Male	213	64.1	51.1	2.6	61.0%	63.4%*
Female	88	64.4	56.8	2.7	53.4%	42.0%
Depth of invasion						
Early stage	190	64.9	54.4	2.8	54.7%	60.5%
Advanced stage	111	62.9	50.3	2.5	65.8%	51.4%
Age (years)						
<59	85	48.4	54.4	2.8	52.9%	58.8%
≥60	216	70.4	52.3	2.6	61.1%	56.5%

* $P < 0.05$ compared with females by chi-square test
[a] See Table 1 footnote for definitions of positivity
Mean PG I, Mean PG I concentration (ng/ml); mean PG I/II, mean ratio of serum PG II concentration to serum PG I concentration; PG-positive, percentage of subjects positive for serum PG; *H. pylori*-positive, percentage of subjects with seropositivity for anti-*H. pylori* antibody; early stage, early gastric cancer, defined as that in which tumor is localized as the deepest within the submucosa; advanced stage, advanced gastric cancer, defined as that with the tumor invading the muscularis propria or beyond

did not find any difference in mean age between patients with early stage gastric carcinoma and those with advanced stage. There were no significant differences in mean serum PG I concentrations and PG I/II ratios between either men and women, patients with early or advanced stage gastric carcinoma or patients aged less than 59 years and those aged more than 60 years (Mann-Whitney U-test). We also found no differences in the percentages of patients who were PG-positive between men and women, between early stage and advanced stage gastric carcinoma, or between those aged less than 59 years and those aged more than 60 years (chi-square test). Men had a significantly higher positivity rate for *H. pylori* infection than women (chi-square test). There were no significant differences in *H. pylori* infection positivity rates between patients with early stage and advanced stage gastric carcinoma, or between those aged less than 59 years and those aged more than 60 years (chi-square test).

Comparison of cases and controls

The relevant characteristics of the 301 cases and 602 controls are shown in Table 3. We did not find any differences in mean serum PG I and PG II concentration between cases and controls. Cases had significantly lower PG I/II ratios than controls ($P < 0.001$, Mann-Whitney U-test) and significantly higher rates for positive serum PG concentration than controls ($P < 0.001$, chi-square test). There was no significant difference in the percentages positive for *H. pylori* infection between cases (57.1%) and controls (61.6%) (chi-square test).

Association between serum PG and H. pylori *antibody*

Table 4 shows the association between serum PG and *H. pylori* antibody in the cases.

Table 3. Characteristics of cases and controls

Characteristic	Cases	Controls
No of subjects	301	602
Sex ratio (m/f)	213/88	426/176
Mean age ± SD (years)	64.2 ± 12.2	63.8 ± 10.6
PG I (ng/ml)	52.8 ± 47.7	56.1 ± 31.3
PG II (ng/ml)	19.9 ± 13.5	17.5 ± 10.3
PG I/II ratio	2.6 ± 1.5*	3.9 ± 2.4
Positivity rate for PG (%)	177 (58.8%)**	216 (35.9%)
Positivity rate for *H. pylori* (%)	172 (57.1%)	371 (61.6%)

* $P < 0.001$ compared with controls by Mann-Whitney U-test;
** $P < 0.001$ compared with controls by chi-square test
Positive PG rate, percentage of subjects positive for serum PG; positive *H. pylori* rate, percentage of subjects with seropositivity for anti-*H. pylori* antibody

The association between serum PG and *H. pylori* antibody in controls is shown in Table 5. The mean ages of controls increased in the order category B to D to A to C. It is assumed that this order was strongly related to the development of atrophy in gastric mucosa.

Table 6 summarizes the association between serum PG and *H. pylori* antibody in cases and controls. Of the four categories, category C had the highest percentage of gastric cancer cases (64.3%). The proportion of gastric cancer cases increased in the order of B to D to A to C. This order was also related to increasing atrophy in the gastric mucosa. Thus, category C, with low serum PG concentration and negative for *H. pylori* IgG antibody, is a high risk group for gastric cancer. The odds ratios for the categories are also shown in Table 6. Category C had the highest odds ratio (4.20; 95% CI, 2.66–6.04) of the four categories.

Table 4. Association between serum PG concentration and *H. pylori* antibody in cases

	Positive PG	Negative PG
No positive for *H. pylori* antibody (%)	94 (31.2%)	78 (25.9%)
Mean age ± SD (years)	Category A 65.5 ± 10.3	Category D 61.5 ± 12.4
No negative for *H. pylori* antibody (%)	83 (27.6%)	46 (15.3%)
Mean age ± SD (years)	Category C 65.8 ± 13.0	Category B 63.2 ± 13.5

Positive PG, Subjects with serum PG I concentration less than 70 ng/ml and PG I/II ratio less than 3.0; negative PG, subjects with serum PG I concentration more than 70 ng/ml or PG I/II ratio more than 3.0; positive for *H. pylori* antibody, subjects with titer more than 2.2; negative for *H. pylori* antibody, subjects with a titer less than 2.1

Table 5. Association between serum PG concentration and *H. pylori* antibody in controls

	Positive PG	Negative PG
No positive for *H. pylori* antibody (%)	170 (28.2%)	201 (33.4%)
Mean age ± SD (years)	Category A 66.7 ± 6.9	Category D 62.8 ± 9.5
No negative for *H. pylori* antibody (%)	46 (7.6%)	185 (30.8%)
Mean age ± SD (years)	Category C 67.0 ± 8.3	Category B 58.3 ± 13.0

Table 6. Proportion of gastric cancer cases to total number of subjects, and odds ratios (ORs) for each category

Category	Gastric cancer cases (%)	OR	95% CI
A	35.6%	1.21	0.85–1.72
B	19.9%	0.36	0.25–0.53
C	64.3%	4.20	2.66–6.04
D	28.0%	0.77	0.54–1.10

See Table 1 for explanation of categories A–D
CI, Confidence interval

Association between H. pylori *infection and cancer*

The matched odds ratio of *H. pylori* infection and gastric cancer was 0.93 (95% CI, 0.63–1.21; Table 7). No significant association between *H. pylori* infection and gastric cancer was observed. The results of subgroup analyses by age, sex, and depth of tumor invasion are also shown in Table 7. The odds ratio in the group less than 59 years was high (1.53; 95% CI, 0.91–2.59).

Association between positive serum PG concentration and cancer

The matched odds ratios for positive serum PG concentration and gastric cancer was 2.55 (95% CI, 1.92–3.38; Table 7). Positive serum PG concentration was significantly associated with gastric cancer. The results of subgroup analyses are also shown in Table 7. Significant associations between positive serum PG concentration and cancer were observed in all subgroups.

Discussion

In recent years, there have been many studies of the relationship between *H. pylori* infection and gastric cancer [2–4,19–35]. The major findings of these 21 studies (including this study) are compared in Table 8.

Nine of the above studies reported a significant association between *H. pylori* infection and gastric cancer (OR, range, 2.6–13.3). In 19 of the studies, *H. pylori* infection was determined by serum IgG antibody to *H. pylori*. Ten of the studies were nested case-control studies, and the other 11 were cross-sectional case-control studies. Forman et al. [36] reported a significant trend towards an increased OR with increasing time between blood sample collection and cancer diagnosis, by multivariate analysis. The nested case-control studies seemed to show high ORs because the blood samples were collected long before the cancer diagnosis.

Five of the ten nested case-control studies showed a positive association between *H. pylori* infection and gastric cancer. Of these five studies, three were conducted in countries with a low prevalence of *H. pylori*

Table 7. Odds ratios for gastric cancer according to anti-*H. pylori* antibody and serum PG concentration

	Positive for *H. pylori* antibody Odds ratio (95% CI)	Positive PG Odds ratio (95% CI)
All cases	0.93 (0.63–1.27)	2.55 (1.92–3.38)
Sex		
Male	0.87 (0.61–1.62)	2.77 (1.98–3.86)
Female	0.74 (0.44–1.24)	2.11 (1.26–3.53)
Depth of invasion		
Early stage	0.95 (0.68–1.33)	2.16 (1.56–3.80)
Advanced stage	0.66 (0.44–0.98)	3.43 (2.28–5.17)
Age (years)		
<59	1.53 (0.91–2.59)	5.71 (3.25–10.01)
≥60	0.64 (0.46–0.90)	2.04 (1.46–2.84)

Table 8. Comparison of odds ratios for association between *H. pylori* seropositivity and gastric cancer in 21 case-control studies

Author	Country	Design	Time (years)	*H. pylori* (+) Cases	*H. pylori* (+) Controls	OR	CI	Age	Assess
Parsonnet [4]	USA	NCCS	14.2	84.4%	60.6%	3.6	1.8–7.3	54	IgG
Nomura [3]	J-Ame	NCCS	13	94%	76%	6.0	2.1–17.3	59	IgG
Forman [2]	UK	NCCS	6	69%	47%	2.8	1.0–8.0	54	IgG
Talley [33]	USA	NCCS	3.7	52%	38%	2.7	1.4–5.0	63	IgG
Hansson [25]	Sweden	NCCS	2.6	80%	61%	2.6	1.4–5.0	67	IgG
Lin [30]	Taiwan	NCCS	3.1	69%	59%	1.6	0.7–2.6	58	IgG
Blaser [23]	J-Ame	NCCS	21	87.4%	77.7%	1.9	0.9–4.0	59	cagA
Aromaa [20]	Finland	NCCS	13	86.9%	82.9%	1.5	0.7–3.2	62	IgG
Webb [34]	China	NCCS	2.4	54.1%	56.1%	0.9	0.6–1.5	61	IgG
Watanabe [32]	Japan	NCCS	3.2	91.1%	75.6%	1.8	0.6–5.7	63	IgG
Archimen-dinitis [19]	Greece	CSCCS		72.3%	68%	1.2	0.5–3.0	62	IgG
Fukuda [24]	Japan	CSCCS		76.2%	73.9%	1.0	0.7–1.5	57	IgG
Kikuchi [27]	Japan	CSCCS		88.6%	39%	13.3	5.3–35.6	34	IgG
Asaka [21]	Japan	CSCCS		87.2%	58.5%	2.4	1.2–4.8	60	IgG
Kato [26]	Japan	CSCCS		70.8%	68.5%	1.1	0.7–1.6	62	IgG
Kokkola [29]	Finland	CSCCS		72%	44%	3.3	1.4–7.5	37	hist
Barreto [22]	Japan	CSCCS		82%	60%	3.0	1.7–5.3	56	IgG
Kim [28]	Korea	CSCCS		60%	51.9%	1.4	0.9–2.2	57	hist
Kuipers [35]	Italy	CSCCS		77%	79%	0.9	0.3–2.2	67	IgG
Lopez [31]	Mexico	CSCCS		82.5%	87.5%	1.4	0.7–2.8	56	IgG
Our study	Japan	CSCCS		57.1%	61.6%	0.9	0.6–1.2	64	IgG

Time, Interval (years) between blood sample collection and gastric cancer diagnosis; *H. pylori* (+) cases, percentage of patients positive for *H. pylori* infection among-gastric cancer cases; *H. pylori* (+) controls, percentage of subjects positive for *H. pylori* infection among matched controls; OR, odds ratio; CI, confidence interval; age, mean age of gastric cancer cases; assess, assessment of *H. pylori* infection; J-Ame, Japanese American; NCCS, nested case-control study; CSCCS, cross-sectional case-control study; IgG, anti-*H. pylori* IgG antibody; hist, histopathology

infection (United States and United Kingdom) [37]. The study of Nomura et al. [3] reported a high seropositivity rate of *H. pylori* infection in cases, and the study of Hansson et al. [25] reported a low seropositivity rate of *H. pylori* infection in their matched controls. The remaining five nested case-control studies were conducted in countries with a high prevalence of *H. pylori* infection (Japan, Taiwan, China, and Finland) [37]. Four of the 11 cross-sectional case-control studies reported a positive association between *H. pylori* infection and gastric cancer. Two of these four studies were conducted in a younger population (aged less than 50 years). Case-control studies in young populations have high ORs as the seropositivity rate of *H. pylori* infection in the matched controls is relatively low. The study of Barreto and colleagues [22] had a low seropositivity rate of *H. pylori* infection in controls, and that of Asaka and colleagues [21] had a high seropositivity rate of *H. pylori* infection in cases. Two factors that influence the OR are the prevalence rate of the risk factor in the population, and the interval between serum collection and diagnosis.

Our results do not support the hypothesis that *H. pylori* infection plays a role in gastric cancer carcinogenesis, as our study showed a low prevalence of *H. pylori* infection in the gastric cancer cases. Most cancer cases with seronegativity for *H. pylori* infection belonged to category C (positive for serum PG concentration and negative for *H. pylori* infection). Category C had the highest percentage of gastric cancer cases of all categories. Most of the subjects in category C had extensive mucosal atrophy (indicated by low PG I and low PG I/II ratio), and this may cause spontaneous loss of *H. pylori* from the stomach. Our results suggested the following theory. First, *H. pylori* infection caused anti-*H. pylori* IgG antibody seropositivity, in result subjects in category B changed to those in category D. Next, chronic gastritis due to *H. pylori* infection, caused extensive mucosal atrophy, in result subjects in category D changed to those in category A. Finally, the extensive mucosal atrophy led to the spontaneous loss of *H. pylori* from the stomach, in result most of subjects in category A changed to those in category C. Indeed, the gastric mucosa in category C subjects showed the greatest extent of mucosal atrophy, due to *H. pylori* infection, and subjects in this category had the highest risk of gastric cancer among our four categories. Previous studies have also suggested that loss of *H. pylori* colonization and subsequent loss of seropositivity may occur frequently in conditions such as severe atrophic gastritis and intestinal metaplasia [24,32,38]. Thus, false-negative results for *H. pylori* infection may have been frequent in gastric cancer cases in blood samples taken at the time of cancer diagnosis, because loss of seropositivity may have occurred in the gastric cancer patients with severe gastric atrophy.

Serum PG concentrations (low PG I and low PG I/II ratio) are considered to be a reliable marker of chronic atrophic gastritis [18,39–41], and are useful for the screening of gastric cancer [6–9,13–18]. The severity of atrophic gastritis is well correlated with serum PG I concentration and with PG I/II ratio [39,42]. Therefore, atrophic gastritis was associated with a significantly increased risk of gastric cancer (OR, 6.59; 95% CI, 3.45–12.57) in our study, as we used the serum PG concentration as a marker of extensive mucosal atrophy.

In conclusion, our results suggest that *H. pylori* infection leads to severe atrophic gastritis and intestinal metaplasia in the gastric mucosa, with gastric cancer eventually occurring in this environment. Therefore, it appears that *H. pylori* infection is one of the indirect causes of gastric cancer, via the development of atrophic gastritis. Individuals who have severe atrophy and intestinal metaplasia in the gastric mucosa have the highest risk of gastric cancer. False-negative assessments of *H. pylori* infection may be frequent in this high-risk group.

H. pylori seems not to act as an initiator in the development of gastric cancer, but as a promoter in the progression from normal mucosa to severe atrophy. However, it seems that *H. pylori* infection alone is neither necessary nor sufficient for the development of gastric cancer. Dietary factors, such as excessive salt intake and diets low in fresh fruits and vegetables; smoking; and genetic factors may still play important roles in gastric cancer carcinogenesis [30,43–47]. The role of *H. pylori* infection in the complex multifactorical carcinogenesis of gastric cancer needs to be explored further in the light of the multistep theory of gastric carcinogenesis.

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