**Helicobacter pylori** Infection and Gastric Atrophy: Risk of Adenocarcinoma and Squamous-Cell Carcinoma of the Esophagus and Adenocarcinoma of the Gastric Cardia

Weimin Ye, Maria Held, Jesper Lagergren, Lars Engstrand, William J. Blot, Joseph K. McLaughlin, Olof Nyrén

**Background:** An inverse association between *Helicobacter pylori* infection and esophageal adenocarcinoma has been reported that may be attributed to reduced acidity from inducing atrophic gastritis and from producing ammonia. We examined associations between *H. pylori* infection, gastric atrophy, and the risk of esophageal adenocarcinoma, esophageal squamous-cell carcinoma, and gastric cardia adenocarcinoma in a large population-based case–control study in Sweden. **Methods:** Self-reported data were obtained during interviews, and serum was collected from 97 patients with incident esophageal adenocarcinoma, 85 patients with incident esophageal squamous-cell carcinoma, 133 patients with incident gastric cardia adenocarcinoma, and 499 randomly selected control subjects. Serum antibodies against whole *H. pylori* cell-surface antigens (HP-CSAs) and cytotoxin-associated gene A (CagA) antigens were assessed by an IgG enzyme-linked immunosorbent assay and immunoblotting, respectively. Gastric atrophy was assessed by serum levels of pepsinogen I. Multivariable logistic regression with adjustment for potential confounding factors was used to evaluate associations. **Results:** *H. pylori* infection, assayed by HP-CSA or CagA antibodies, was statistically significantly associated with a reduced risk for esophageal adenocarcinoma (for HP-CSA antibodies, odds ratio [OR] = 0.3, 95% confidence interval [CI] = 0.2 to 0.6; for CagA antibodies, OR = 0.5, 95% CI = 0.3 to 0.8; for both, OR = 0.2, 95% CI = 0.1 to 0.5). Gastric atrophy was not associated with the risk for esophageal adenocarcinoma (OR = 1.1, 95% CI = 0.5 to 2.5). Serum CagA antibodies and gastric atrophy were associated with an increased risk for esophageal squamous-cell carcinoma (OR = 2.1, 95% CI = 1.1 to 4.0, and OR = 4.3, 95% CI = 1.9 to 9.6, respectively). The risk of gastric cardia adenocarcinoma was not associated with *H. pylori* infection. However, gastric atrophy was associated with an increased risk for gastric cardia adenocarcinoma (OR = 4.5, 95% CI = 2.5 to 7.8). **Conclusions:** Infection with *H. pylori* may reduce the risk of esophageal adenocarcinoma, but it is unlikely to do so by atrophy-reduced acidity. Gastric atrophy and infection with CagA-positive strains of *H. pylori* may increase the risk for esophageal squamous-cell carcinoma. **[J Natl Cancer Inst 2004;96:388–96]**

The incidence of esophageal adenocarcinoma has increased rapidly since the 1970s in several Western countries (1–3), including Sweden (4). It has been suggested that this increase is linked to declining rates of **Helicobacter pylori** infection in Western society (5). Indeed, epidemiologic evidence is accumulating that *H. pylori* infection, especially with strains carrying the cytotoxin-associated gene A (CagA), is associated with a reduced risk of adenocarcinoma of the esophagus or gastroesophageal junction (6–10), although a recent population-based case–control study found null results (11). It is postulated that *H. pylori* infection may induce atrophic gastritis, which results in a less acidic gastric reflexate, and *H. pylori* may also neutralize gastric acid by producing ammonia, independent of the presence or absence of gastric atrophy (12). This hypothesis has gained some support from the observed inverse relationship between indices of *H. pylori* infection and occurrence of gastroesophageal reflux symptoms (13). Our recent report (14) that patients with pernicious anemia who also had long-term achlorhydria did not have a decreased risk for esophageal adenocarcinoma, however, appears to contradict this hypothesis.

An *H. pylori* infection may also increase the risk of cancers such as esophageal squamous-cell carcinoma (15) by stimulating the production of nitrosamines. Mortality from esophageal cancer (predominantly squamous-cell carcinoma) was statistically significantly correlated with endogenous nitrosation ability in an ecologic study in China (16). Atrophic gastritis induced by *H. pylori* may form a milieu that favors bacterial overgrowth which, in turn, may increase intragastric nitrosation (17). Thus, infection with *H. pylori*, especially CagA-positive strains, may be a risk factor for esophageal squamous-cell carcinoma. However, only one study has investigated the relationship between a CagA-positive *H. pylori* infection and the risk of esophageal squamous-cell carcinoma; a nested case–control study (18) from China found a borderline statistically significantly increased risk for esophageal squamous-cell carcinoma associated with the presence of serum CagA antibodies.

In this article, we investigated whether *H. pylori* infection was associated with the risk for esophageal adenocarcinoma, esophageal squamous-cell carcinoma, and gastric cardia adenocarcinoma in a Swedish nationwide population-based case–control study in Sweden (e-mail: weimin.ye@meb.ki.se). **See “Notes” following “References.”**

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control study. We paid special attention to the role of serum antibodies against CagA. After _H. pylori_ has been eradicated, CagA antibodies appear to persist longer than antibodies against _H. pylori_ cell-surface antigens (HP-CSAs). Thus, use of CagA antibodies may lead to less misclassification of previous _H. pylori_ infections. We therefore used both serologic methods to detect relevant exposure to _H. pylori_ (19). We evaluated whether gastric atrophy, with its associated reduction of the gastric acid output, is in the causal pathway between _H. pylori_ infection and esophageal adenocarcinoma development. We also reasoned that if _H. pylori_ and gastric atrophy are both in the causal chain, then 1) their associations with esophageal adenocarcinoma should be in the same direction, 2) the inverse association between _H. pylori_ infection and the risk of esophageal adenocarcinoma should be confined to subjects with gastric atrophy, and 3) the risk for esophageal adenocarcinoma should be lower among subjects infected with _H. pylori_ who have gastroesophageal reflux symptoms than among those who do not have reflux. Results of similar tests for the first two points can also be used to assess whether gastric atrophy is in the causal pathway between CagA-positive _H. pylori_ infection and esophageal squamous-cell carcinoma risk.

**Patients and Methods**

**Study Design**

Study methods have been described in detail elsewhere (20). Briefly, eligible case patients were all of the newly diagnosed patients with adenocarcinoma of the esophagus or gastric cardia and half of the patients with squamous-cell carcinoma of the esophagus (i.e., those born on even-numbered days) from 1995 through 1997 in the entire native Swedish population who were younger than 80 years. Considerable efforts, including establishment of a comprehensive organization with contact persons at all 195 departments of general surgery, thoracic surgery, otorhinolaryngology, oncology, and pathology in Sweden and continuous collaboration with the six regional tumor registries, were made to ensure that every eligible case patient throughout the country was identified shortly after diagnosis. All tumors were uniformly classified histologically and topographically, and almost all (97%) biopsy and surgical specimens were reviewed by a single pathologist. Biopsy specimens were also obtained from nontumorous mucosa in and around the gastroesophageal junction. Endoscopists, surgeons, and pathologists gave standardized, detailed descriptions of the location of the tumors. Cancer of the gastric cardia was defined as a tumor with its center within 2 cm proximal or 3 cm distal of the gastroesophageal junction and without evidence of Barrett’s esophagus. To allay concerns about misclassification of gastric cardia adenocarcinoma, we also used a more stringent definition, i.e., a tumor within 1 cm proximal or distal to the gastroesophageal junction. Alternatively, we subdivided the gastric cardia adenocarcinoma tumors according to the location of tumor center, i.e., above or on or below the gastroesophageal junction. Squamous-cell carcinomas were classified as esophageal, even if the location was seemingly in the gastric cardia. If Barrett’s esophagus was detected adjacent to gastric cardia adenocarcinoma, the tumors were classified as esophageal. Control subjects were randomly selected from the continuously updated Swedish Population Register and frequency-matched to resemble the age (in 10-year strata) and sex distributions of case patients with esophageal adenocarcinoma.

**Interview**

The 618 case patients and 820 control subjects were interviewed face-to-face by specially trained professional interviewers from Statistics Sweden. The participation rates were 88%, 73%, 84%, and 73% among case patients with esophageal adenocarcinoma, case patients with esophageal squamous-cell carcinoma, case patients with gastric cardia adenocarcinoma, and population control subjects, respectively. The questions covered demographic characteristics, living conditions during childhood and adolescence (e.g., number of siblings and access to a refrigerator during childhood), gastroesophageal reflux symptoms, anthropometric measures, smoking status, alcohol consumption, dietary history, history of medication use, and occupational history. The interviewers could not be kept blinded to case-control status but were trained to treat both groups in a strictly equal manner.

**Serologic Data**

Interviewed subjects were asked to provide a venous blood sample, which was drawn from case patients during the initial hospital stay and from control subjects at their local health centers. After centrifugation, all serum samples were stored at −20 °C and transported to our laboratory for further storage at −70 °C until analyses were performed. In total, serum was collected from 97 patients with incident esophageal adenocarcinoma, 85 patients with incident esophageal squamous-cell carcinoma, 133 patients with incident gastric cardia adenocarcinoma, and 499 randomly selected control subjects. To increase the number of case patients with squamous-cell carcinoma, we also collected blood samples from patients born on odd-numbered days, 1 year after the initiation of the study. These patients were not interviewed (21).

Serum immunoglobulin G (IgG) antibodies against HP-CSAs were measured with an enzyme-linked immunosorbent assay (ELISA) (Pyloriset EIA-G; Orion Diagnostica, Espoo, Finland) with a sensitivity of 98% and a specificity of 85% (22). We detected antibodies to CagA with an immunoblott assay (Helioblot 2.1; Genelabs Diagnostics, Singapore). Tests with ambiguous results were repeated to arrive at a definite classification. A low value of serum pepsinogen I is an indication of gastric atrophy. Serum pepsinogen I was measured by an immunoenzymometric assay (Gastroset PGI; Orion Diagnostica). The recommended normal range of pepsinogen I in serum is 28–158 μg/L. When pepsinogen I at 28 μg/L was used as the cut point, its sensitivity and specificity for detecting moderate and severe atrophic corpus gastritis were reported to be 81% and 99%, respectively (23). All persons involved in the analyses were blinded to case-control status of the subjects.

**Statistical Analyses**

We modeled the data by use of logistic regression to estimate relative risks in the form of odds ratios (ORs) with 95% confidence intervals (CIs). The confidence intervals were not adjusted for multiple comparisons. In multivariable modeling, our basic model included the frequency-matching variables of age (six categories) and sex. We further considered years of education (categorized into three classes), con-
The small number of subjects with gastric atrophy, we did not adjust for atrophy in the main analyses. A higher cutoff value (78 μg/L) when the recommended cutoff value of serum pepsinogen I (28 μg/L) was used to define gastric atrophy (23), we used a higher cutoff value (78 μg/L, the median value among control subjects), which gave a cleaner nonatrophic stratum and permitted more robust comparisons between the strata, when we performed stratified analyses by gastric atrophy status. All statistical analyses were performed with SAS, release 8.2 (SAS Institute, Cary, NC); PROC GENMOD was used for the logistic regression model. All statistical tests were two-sided.

**Ethical Considerations**

The study was approved by all regional ethics committees in Sweden. Individual written informed consent was obtained from study participants before the initial interview.

**RESULTS**

**Subjects**

The study included only those interviewed case patients and control subjects who provided serum samples. There were 97 case patients with esophageal adenocarcinoma, 85 with esophageal squamous-cell carcinoma, and 133 with gastric cardia adenocarcinoma. They constituted about 50% of all interviewed case patients. In addition, 499 control subjects were included, constituting approximately 60% of those interviewed. The main reason for nonparticipation among case patients was that the clinicians forgot to collect serum, whereas the main reason for nonparticipation among control subjects was failure to appear at the local health center. The median ages among participants included in the current study (both sexes) were 69 years for case patients with esophageal adenocarcinoma, 64 years for case patients with esophageal squamous-cell carcinoma, 65 years for case patients with gastric cardia adenocarcinoma, and 69 years for control subjects. The distributions of participants by sex, smoking status, alcohol consumption, and educational level are listed in Table 1. Consumption of fresh fruits and vegetables was lower among case patients with any of the three types of cancer under study than among control subjects (Table 1). Among case patients with esophageal adenocarcinoma, esophageal squamous-cell carcinoma, or gastric cardia adenocarcinoma, 54%, 9%, or 26%, respectively, reported reflux symptoms, and among control subjects, 17% reported reflux symptoms. No substantial differences in reflux symptoms, body mass index, and the number of siblings—a potential indicator of *H. pylori* infection (24)—were observed between those who provided serum and those who did not provide serum (Table 1).

Analyses among the 499 control subjects revealed that older age, current smoking status, fewer years of education, and lower consumption of fresh fruits and vegetables were associated with an increased prevalence of antibodies to *H. pylori*, whereas sex, alcohol consumption, symptomatic gastroesophageal reflux, and body mass index were unrelated to seroprevalence (Table 2). *H. pylori* infection, indicated by serum antibodies to HP-CSAs or CagA, was more common among those with serologic evi-

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**Table 1.** Characteristics of 315 case patients and 499 control subjects who provided serum and were interviewed and those who underwent a face-to-face interview

<table>
<thead>
<tr>
<th></th>
<th>Esophageal adenocarcinoma</th>
<th>Esophageal squamous-cell carcinoma</th>
<th>Gastric cardia adenocarcinoma</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present analysis</td>
<td>Interviewed part</td>
<td>Present analysis</td>
<td>Interviewed part</td>
</tr>
<tr>
<td>No. enrolled/No. eligible (%)</td>
<td>97/189 (51)</td>
<td>189/216 (88)</td>
<td>85/167 (51)</td>
<td>167/228 (73)</td>
</tr>
<tr>
<td>Male, No. (%)</td>
<td>88 (91)</td>
<td>165 (87)</td>
<td>59 (69)</td>
<td>120 (72)</td>
</tr>
<tr>
<td>Current smoker, No. (%)</td>
<td>23 (24)</td>
<td>43 (23)</td>
<td>54 (64)</td>
<td>101 (60)</td>
</tr>
<tr>
<td>High alcohol consumption, No. (%)</td>
<td>19 (20)</td>
<td>43 (23)</td>
<td>38 (45)</td>
<td>78 (47)</td>
</tr>
<tr>
<td>Low educational level, No. (%)</td>
<td>27 (28)</td>
<td>48 (25)</td>
<td>25 (29)</td>
<td>41 (25)</td>
</tr>
<tr>
<td>Low consumption of fresh fruits and vegetables, No. (%)</td>
<td>33 (34)</td>
<td>69 (37)</td>
<td>33 (39)</td>
<td>62 (37)</td>
</tr>
<tr>
<td>Positive reflux, No. (%)</td>
<td>52 (54)</td>
<td>113 (60)</td>
<td>8 (9)</td>
<td>25 (15)</td>
</tr>
<tr>
<td>High body mass index, No. (%)</td>
<td>50 (52)</td>
<td>98 (52)</td>
<td>20 (24)</td>
<td>47 (28)</td>
</tr>
<tr>
<td>Individuals with more than four siblings, No. (%)</td>
<td>28 (29)</td>
<td>60 (32)</td>
<td>23 (27)</td>
<td>52 (31)</td>
</tr>
</tbody>
</table>

*This category included smoking of cigarettes, cigars, and pipes and was assessed as of 2 years before the interview.
†High alcohol consumption was defined as consumption of more than 70 g of alcohol per week.
‡Low educational level was defined as less than 7 years of formal education.
§Low consumption of fresh fruits and vegetables was defined as the lowest tertile.
¶Refers to subjects who had symptoms of heartburn and/or regurgitation at least 50 times a year.
The prevalence of *H. pylori* antibodies was lower among case patients with esophageal adenocarcinoma than among control subjects; 19% versus 40%, respectively, were positive for HP-CSAs, and 43% versus 59% were positive for CagA (Table 3). In multivariable analyses adjusted for age, sex, and other possible confounders including years of education, body mass index, tobacco smoking status, and consumption of fruit and vegetables, *H. pylori* infection determined by HP-CSA antibodies was associated with a statistically significantly reduced risk of esophageal adenocarcinoma (OR = 0.3, 95% CI = 0.2 to 0.6) (Table 3). Among subjects without gastric atrophy (pepsinogen I level ≥78 μg/L), the association was similar (OR = 0.3, 95% CI = 0.2 to 0.7). Likewise, no dramatic differences in the risk for esophageal adenocarcinoma emerged when we stratified subjects by the presence or absence of symptomatic reflux (OR = 0.4 [95% CI = 0.2 to 1.1] versus OR = 0.2 [95% CI = 0.1 to 0.6] for those with and without symptomatic reflux, respectively; P for interaction = .20). When *H. pylori* infection status was determined only by serum CagA antibody status, the overall relative risk for esophageal adenocarcinoma associated with *H. pylori* infection was also reduced (OR = 0.5, 95% CI = 0.3 to 0.8) (Table 3). Among subjects without gastric atrophy (pepsinogen I level ≥78 μg/L), the association was similar (OR = 0.4, 95% CI = 0.2 to 0.8). Likewise, no dramatic differences in the risk for esophageal adenocarcinoma were detected in analyses stratified by symptomatic reflux (P for interaction = .55).

When we analyzed *H. pylori* infection data by combining HP-CSA and CagA status and used subjects seronegative in both assays as the unexposed reference group, a different pattern emerged. Only subjects seropositive for both HP-CSA and CagA had a reduced risk for esophageal adenocarcinoma (OR = 0.2, 95% CI = 0.1 to 0.5); a reduced risk for esophageal adenocarcinoma was not observed among subjects who were seropositive in one test but not in the other (Table 3). Neither excess nor reduced risk for esophageal adenocarcinoma was associated with gastric atrophy, as indicated by low serum pepsinogen I concentration (<28 μg/L) (OR = 1.1, 95% CI = 0.5 to 2.5). However, a high level of pepsinogen I (>158 μg/L) was statistically significantly associated with increased risk for this tumor (Table 4).

### Esophageal Squamous-Cell Carcinoma

Patients with esophageal squamous-cell carcinoma (born on even-numbered days) compared with control subjects had a similar prevalence of HP-CSA antibodies (38% versus 40%) and had a higher prevalence of CagA antibodies (74% versus 59%) (Table 3). After controlling for age, sex, years of education, smoking status, alcohol consumption, and consumption of fruits and vegetables, *H. pylori* infection status assessed by measuring antibodies to CagA was associated with a statistically significantly increased risk for esophageal squamous-cell carcinoma (OR = 1.1, 95% CI = 1.1 to 4.0). This effect was stronger in those with gastric atrophy (pepsinogen I level <78 μg/L) (OR = 3.3, 95% CI = 1.3 to 8.7) than in those without gastric atrophy (OR = 1.1, 95% CI = 0.4 to 3.1) (P for interaction = .07). When we combined the results from the HP-CSA and CagA assays as a measure of *H. pylori* infection status, the relative risk for esophageal squamous-cell carcinoma was higher for the group that was seropositive for CagA but seronegative for HP-CSAs (OR = 3.0, 95% CI = 1.4 to 6.2) than for other groups, using those seronegative for both tests as the referent (Table 3). When we used the overall model to assess associations with gastric atrophy status, we found that gastric atrophy status was strongly associated with the risk of esophageal squamous-cell carcinoma (OR = 4.3, 95% CI = 1.9 to 9.6) (Table 4).
Table 3. *Helicobacter pylori* infection and the risk of esophageal adenocarcinoma, esophageal squamous-cell carcinoma, and gastric cardia adenocarcinoma*

<table>
<thead>
<tr>
<th>H. pylori infection</th>
<th>Control subjects, No. (%)</th>
<th>Case patients with esophageal adenocarcinoma</th>
<th>Case patients with esophageal squamous-cell carcinoma</th>
<th>Case patients with gastric cardia adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>OR (95% CI)†</td>
<td>OR (95% CI)‡</td>
<td>No. (%)</td>
</tr>
<tr>
<td>HP-CSA Negative</td>
<td>301 (60)</td>
<td>79 (81)</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>HP-CSA Positive</td>
<td>198 (40)</td>
<td>18 (19)</td>
<td>0.3 (0.2 to 0.6)</td>
<td>0.3 (0.2 to 0.6)</td>
</tr>
<tr>
<td>CagA Negative</td>
<td>206 (41)</td>
<td>55 (57)</td>
<td>Referent</td>
<td>0.5 (0.3 to 0.8)</td>
</tr>
<tr>
<td>CagA Positive</td>
<td>293 (59)</td>
<td>42 (43)</td>
<td>0.5 (0.3 to 0.8)</td>
<td>0.5 (0.3 to 0.8)</td>
</tr>
<tr>
<td>HP-CSA and CagA</td>
<td>195 (39)</td>
<td>50 (52)</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>HP-CSA−, CagA−</td>
<td>11 (2)</td>
<td>5 (5)</td>
<td>1.9 (0.6 to 5.9)</td>
<td>1.5 (0.4 to 4.9)</td>
</tr>
<tr>
<td>HP-CSA+, CagA−</td>
<td>106 (21)</td>
<td>29 (30)</td>
<td>1.1 (0.6 to 1.8)</td>
<td>1.0 (0.6 to 1.7)</td>
</tr>
<tr>
<td>HP-CSA−, CagA+</td>
<td>187 (37)</td>
<td>13 (13)</td>
<td>0.3 (0.1 to 0.5)</td>
<td>0.2 (0.1 to 0.5)</td>
</tr>
<tr>
<td>HP-CSA+, CagA+</td>
<td>49 (10)</td>
<td>8 (8)</td>
<td>1.1 (0.5 to 2.5)</td>
<td>0.8 (0.4 to 2.1)</td>
</tr>
</tbody>
</table>

*OR = odds ratio; CI = confidence interval.
†Other variables included in the model were age, sex, years of education, smoking status, alcohol consumption, and level of consumption of fruits and vegetables.
‡Other variables included in the model were age, sex, years of education, body mass index, smoking status, and level of consumption of fruits and vegetables.

adjusted for age and sex, the risks for esophageal squamous-cell carcinoma associated with serum antibodies against both HP-CSAs and CagA and gastric atrophy were consistent with the results for those born on even-numbered days (OR = 1.0, 95% CI = 0.6 to 1.8; OR = 2.0, 95% CI = 1.1 to 3.6; and OR = 3.9, 95% CI = 1.9 to 7.8, respectively).

**Gastric Cardia Adenocarcinoma**

Patients with gastric cardia adenocarcinoma had a somewhat lower overall prevalence of HP-CSA antibodies than control subjects (34% versus 40%), but they had a similar prevalence of CagA antibodies (60% versus 59%) (Table 3). *H. pylori infection, measured with either antibody, was not associated with risk for gastric cardia adenocarcinoma (for HP-CSA antibodies, OR = 0.8, 95% CI = 0.5 to 1.2; for CagA antibodies, OR = 1.0, 95% CI = 0.7 to 1.6). Essentially no association emerged in any stratum when the results of the two assays were combined. However, gastric atrophy, as indicated by low serum pepsinogen I concentration (<28 μg/L), was associated with a statistically significantly increased risk for gastric cardia adenocarcinoma (OR = 4.5, 95% CI = 2.5 to 7.8) (Table 4). Interestsingly, the risk for gastric cardia adenocarcinoma was higher among those with a high level (>158 μg/L) of serum pepsinogen I (OR = 2.9, 95% CI = 1.6 to 5.2) than among those with a normal level (28–158 μg/L) of pepsinogen I.

In total, 44 patients had the center of their cardia adenocarcinomas within 1 cm of the gastroesophageal junction. In a subanalysis of this group, the point estimates for *H. pylori* seroprevalence remained essentially unchanged (for HP-CSA antibodies, OR = 0.8, 95% CI = 0.4 to 1.7; for CagA antibodies, OR = 1.1, 95% CI = 0.5 to 2.1). When we compared subjects by level of pepsinogen I, as a measure of gastric atrophy, higher risks for gastric cardia adenocarcinoma were found for those with low (<28 μg/L) or high levels (>158 μg/L) of pepsinogen I than for those with normal levels (28–158 μg/L), although point estimates were lower than those in the main study (for low levels, OR = 1.9, 95% CI = 0.7 to 5.0; for high levels, OR = 2.6, 95% CI = 1.1 to 6.2). We also subdivided patients with gastric cardia adenocarcinoma into two groups: the 69 case patients with the tumor above or on the gastroesophageal junction and the 64 case patients with the tumor below the gastroesophageal junction. *H. pylori* seroprevalence was not associated with risk for gastric cardia adenocarcinoma in any of these groups (for the tumor above or on the gastroesophageal junction:

Table 4. Levels of pepsinogen I and the risk of esophageal adenocarcinoma, esophageal squamous-cell carcinoma, and gastric cardia adenocarcinoma*

<table>
<thead>
<tr>
<th>Level of pepsinogen I, μg/L</th>
<th>Control subjects, No. (%)</th>
<th>Esophageal adenocarcinoma</th>
<th>Esophageal squamous-cell carcinoma</th>
<th>Gastric cardia adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>OR (95% CI)†</td>
<td>OR (95% CI)‡</td>
<td>No. (%)</td>
</tr>
<tr>
<td>Normal (28–158 μg/L)</td>
<td>400 (80)</td>
<td>59 (61)</td>
<td>Referent</td>
<td>56 (66)</td>
</tr>
<tr>
<td>Low (&lt;28 μg/L)</td>
<td>49 (10)</td>
<td>8 (8)</td>
<td>1.1 (0.5 to 2.5)</td>
<td>0.8 (0.4 to 2.1)</td>
</tr>
<tr>
<td>High (&gt;158 μg/L)</td>
<td>50 (10)</td>
<td>30 (31)</td>
<td>4.5 (2.5 to 8.2)</td>
<td>10 (12)</td>
</tr>
</tbody>
</table>

*Serum pepsinogen I level of 28–158 μg/L was defined as normal; low level (<28 μg/L) of pepsinogen I indicates presence of gastric atrophy. OR = odds ratio; CI = confidence interval.
†Adjusted for age, sex, years of education, body mass index, smoking status, and level of consumption of fruits and vegetables.
‡Adjusted for age, sex, years of education, smoking status, alcohol consumption, and level of consumption of fruits and vegetables.
for HP-CSA antibodies, OR = 0.9, 95% CI = 0.5 to 1.6; for CagA antibodies, OR = 0.8, 95% CI = 0.5 to 1.4; for the tumor below the gastroesophageal junction: for HP-CSA antibodies, OR = 0.6, 95% CI = 0.3 to 1.2; for CagA antibodies, OR = 1.4, 95% CI = 0.8 to 2.6). However, risk for gastric cardia adenocarcinoma above the gastroesophageal junction was higher for individuals with a high level of pepsinogen I (>158 μg/L) (OR = 3.8, 95% CI = 1.9 to 7.6) than for those with a normal level of pepsinogen I, although the odds ratio for such tumors among subjects with a low pepsinogen I level (<28 μg/L) was close to unity. In contrast, a low pepsinogen I level was associated with a more than 10-fold increased risk for gastric cardia adenocarcinoma below the gastroesophageal junction (OR = 10.9, 95% CI = 5.4 to 22.2), whereas the increased risk associated with high pepsinogen I levels was modest.

**Sensitivity Analysis**

To demonstrate consistency, we reanalyzed our data by using HP-CSA antibody results determined by immunoblotting. The associations between H. pylori infection and the risks of the three types of cancer were remarkably similar to those observed with HP-CSA antibody results determined by ELISA (data not shown). For instance, for esophageal adenocarcinoma, the presence of H. pylori determined by using HP-CSA antibodies was statistically significantly associated with a reduced risk (OR = 0.4, 95% CI = 0.2 to 0.6), and the reduced risk was still confined to the HP-CSA-positive and CagA-positive stratum (OR = 0.3, 95% CI = 0.2 to 0.6).

The interpretation of weak CagA bands in the immunoblot assays is controversial. Because we postulated that previous infections might result in only a faint response, in our main analyses, we classified even a faint CagA band as positive. In a sensitivity analysis, we reanalyzed our data by using a higher cutoff value for CagA, as recommended by the manufacturer, and found it had little impact on our results. As in the original analysis, the risk for esophageal adenocarcinoma among the CagA-positive subjects compared with the CagA-negative subjects was statistically significantly reduced (OR = 0.4, 95% CI = 0.2 to 0.6), and the risk reduction was essentially confined to subjects who were HP-CSA–positive and CagA-positive (OR = 0.2, 95% CI = 0.1 to 0.5). Also consistent with the original analysis was the increased risk of esophageal squamous-cell carcinoma among CagA-positive subjects, particularly among those without a concomitant HP-CSA seroresponse (OR = 3.0, 95% CI = 1.4 to 6.5).

**Risk Factors for Symptomatic Gastroesophageal Reflux**

H. pylori infection (assessed by HP-CSA antibodies, CagA antibodies, or the two combined) was not associated with occurrence of symptomatic reflux. Gastric atrophy, as indicated by low levels of pepsinogen I (<28 μg/L), was not associated with a reduced risk of symptomatic reflux, but high levels of pepsinogen I (158 μg/L) increased the risk for symptomatic reflux (OR = 1.9, 95% CI = 1.2 to 3.0). These results were similar regardless of whether the entire group of case patients and control subjects was analyzed or whether the analysis was confined to control subjects only (data not shown).

**DISCUSSION**

In this study, a CagA-positive H. pylori infection was strongly associated with a reduced risk of esophageal adenocarcinoma. Gastric atrophy, as indicated by a low level of pepsinogen I, was unrelated to this risk, and the inverse relationship between H. pylori infection and esophageal adenocarcinoma persisted in the atrophy-free stratum. Furthermore, no departure from the heterogeneity of the odds ratio was detected in analyses stratified by symptomatic reflux. Therefore, the apparent protection afforded by H. pylori may be mediated by mechanisms other than gastric atrophy. In contrast to the decreased risk of esophageal adenocarcinoma associated with H. pylori infection, patients with CagA serum antibodies had a statistically significantly increased risk of esophageal squamous-cell carcinoma. A statistically significant increased risk for esophageal squamous-cell carcinoma associated with gastric atrophy and an almost complete absence of an association between CagA antibodies and the risk of esophageal squamous-cell carcinoma in the nonatrophy stratum suggest that gastric atrophy may be an intermediate step in the pathway from a CagA-positive H. pylori infection to squamous-cell carcinoma.

Strengths of our study include the population-based design, the almost complete case patient ascertainment, the efforts made to minimize misclassification of case patients, and the high participation rate in the interview phase (20). Detailed interview data enabled us to control for a range of conceivable confounding factors. However, selection bias resulting from nonparticipation might have distorted our study findings in an unknown direction. Patients with the most aggressive or end-stage disease were possibly less likely to be recruited to the interview. The relatively low proportion of interviewed case patients and control subjects who provided serum may further raise concerns about the internal validity of the study. Our results could have been biased if characteristics of the subjects who did and did not provide serum differed. Although the similar distribution of demographic and exposure variables obtained at interview among participating and nonparticipating case patients and control subjects suggests that selection bias is unlikely to have substantially influenced our results, further studies, preferably with prospective design and complete follow-up, will be needed to confirm our findings. Another limitation of our study is the relatively small size of the groups; this problem resulted in limited statistical power for some of the subgroup analyses and prevented stratified analyses by sex and some other possible risk modifiers. Moreover, we had to rely on serologic methods to determine H. pylori infection status, including CagA status, and the presence of gastric atrophy. Misclassification of these variables could have distorted our study findings. The misclassification is likely to be non-differential, which might bias our results toward no association. Although cancer status may theoretically influence the antibody levels, the differential associations with the three types of cancers suggest that our findings are not explained by this effect.

Our study confirms and expands on a study by Chow et al. (7), who also found an inverse, albeit weaker, association between CagA-positive H. pylori infection and the risk of esophageal adenocarcinoma. Our data might also indicate areas in which to search for underlying mechanisms. A prevailing hypothesis postulates that the apparent protection associated with
H. pylori infection is mediated via gastric atrophy and a reduced load of esophageal acid (12). This hypothesis finds some support in a number of clinical cross-sectional studies (13) that have reported an inverse association between H. pylori infection and risk of gastroesophageal reflux, including esophagitis, hiatal hernia, and Barrett’s esophagus. However, in these studies, possible confounding was generally not taken into account, and selection bias may explain the observed results because control subjects for endoscopy are more likely to be H. pylori–positive. Moreover, although an earlier study (25) found that eradication of H. pylori provokes reflux, recent studies (26–33) have found conflicting results. The lack of association between gastric atrophy and a decreased risk of esophageal adenocarcinoma in our study and the absence of risk modification for the relationship between H. pylori and adenocarcinoma by gastric atrophy status or reflux status further challenge the hypothesized mechanism. Doubt is also cast on this mechanism by the slightly increased—instead of decreased—risk of esophageal adenocarcinoma among patients with pernicious anemia and achlorhydria, noted in a recent cohort study (14), and by the lack of association of esophageal and gastric cardia adenocarcinoma with proinflammatory genotypes (which enhance gastric acid inhibition and promote the development of atrophic gastritis) (34). The relative unimportance of hydrochloric acid in esophageal carcinogenesis is also corroborated in studies that use animal models (35). Hence, the mechanism by which CagA-positive H. pylori is associated with a reduced risk for esophageal adenocarcinoma clearly warrants further investigation and might provide important clues to the unexplained increase in esophageal adenocarcinoma in Western populations.

To our surprise, we found that a high level of serum pepsinogen I was associated with an increased risk for esophageal and gastric cardia adenocarcinoma. The underlying mechanism for this finding is not clear. It was reported that duodenal ulcer and treatment with omeprazole may increase the level of serum pepsinogen I (36). Because gastroesophageal reflux is a strong risk factor for esophageal and cardia adenocarcinoma and because the use of inhibitors of gastric acid secretion, such as omeprazole, is obviously overrepresented in patients with adenocarcinoma in the gastroesophageal junction, our findings may be influenced by confounding or reversed causation.

Previous studies (18,37) using HP-CSA antibodies as the only marker of H. pylori infection found no association between H. pylori and esophageal squamous-cell carcinoma. Our results are consistent with these observations. However, similar to a recent nested case–control study in a high-risk area of China (18), we found an increased risk for esophageal squamous-cell carcinoma among CagA-seropositive subjects. This increased risk was not observed in the stratum of subjects who were presumably free of gastric atrophy. Furthermore, a closer look revealed that this positive association was most pronounced among CagA-positive subjects who were H. pylori–negative as assessed by HP-CSA antibodies. In our validation study, this category consisted of individuals who frequently lacked an H. pylori infection, as determined by culture and histologic examination, but who also frequently had gastric atrophy, paralleling that seen among CagA-positive and HP-CSA–positive patients (Ye W, Held M, Enroth H, Kraaz W, Engstrand L, Nyrén O: unpublished results). In this study, hypopepsinogenemia was associated with a statistically significantly elevated risk for esophageal squamous-cell carcinoma. Confounding by other factors is an unlikely explanation because the results remained unchanged after control for possible confounders, including smoking status (38,39) and alcohol consumption (40). These observations suggest that CagA-positive H. pylori infection may increase the risk of esophageal squamous-cell carcinoma by a pathway involving gastric atrophy. N-Nitrosoamines appear to be an important environmental risk factor for this tumor type (15) because O2–methyleneoxyguanosine adducts, a product of DNA alkylation by nitrosoamines and other alkylation substances, were found in subjects living in a high-risk area for esophageal squamous-cell carcinoma (41). Severe atrophic gastritis induced by H. pylori infection forms a milieu that favors overgrowth of anaerobic bacteria capable of increasing intragastric nitrosation (17). Gastric nitrosoamines can conceivably reach the esophageal mucosa through reflux or the superficial gastroesophageal venous plexus. Cytochrome P450 isoforms, abundant in the esophagus, can activate nitrosoamines into carcinogenic substances (42). Thus, endogenous nitrosoamines may be involved in the etiology of esophageal squamous-cell carcinoma, as shown by the results of an ecologic study (16) in China in which mortality from esophageal cancer in 69 counties was statistically significantly correlated with endogenous nitrosation ability, measured by an N-nitrosopropyl test. Moreover, in a cohort of pernicious anemia patients with long-term achlorhydria, we observed a statistically significantly increased risk of esophageal squamous-cell carcinoma (14). Further studies are needed to clarify the role of atrophic gastritis and endogenous nitrosoamines in the carcinogenesis of squamous-cell carcinoma.

In patients with an H. pylori infection, colonization in the cardia is common (43). Although evidence is accumulating that H. pylori colonization may be linked to carditis and intestinal metaplasia in the cardia (44), results in previous studies (37,45–47) and in this article do not support an important role of H. pylori infection in the development of gastric cardia adenocarcinoma.

In this population-based study from Sweden, infection with CagA-positive strains of H. pylori was strongly and inversely related to the risk of esophageal adenocarcinoma through a mechanism that does not appear to involve gastric atrophy and hypoacidity. However, CagA seropositivity, particularly that accompanied by HP-CSA seronegativity, was associated with an increased risk of esophageal squamous-cell carcinoma. The presence of gastric atrophy was also a statistically significant risk factor for esophageal squamous-cell carcinoma and, in the absence of atrophy, CagA-positive H. pylori infection was unrelated to the risk of esophageal squamous-cell carcinoma. Hence, atrophy may be an important mediator of the observed association between CagA seropositivity and esophageal squamous-cell carcinoma. Further studies are warranted to explore the mechanism by which CagA-positive H. pylori is inversely associated with the risk of esophageal adenocarcinoma, to confirm the positive association between CagA-positive H. pylori and esophageal squamous-cell carcinoma, and to elucidate the possible role of gastric atrophy and intragastric nitrosation in this putative carcinogenic pathway.

Note added in proof. A preliminary report of some of the results in this article has been published (48).


NOTES

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