Multiplex *H. pylori* Serology and Risk of Gastric Cardia and Noncardia Adenocarcinomas

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Abstract

The reported associations with gastric adenocarcinoma and seropositivity to different *Helicobacter pylori* antigens using multiplex serology have not been consistent across studies. We aimed to investigate the association between 15 different multiplex serology antigens and the risk of gastric cardia (GCA) and gastric noncardia (GNCA) adenocarcinomas in northeastern Iran, a population with high rates of gastric adenocarcinoma. We included 272 cases of gastric adenocarcinoma (142 GCA, 103 GNCA, and 27 unspecified) and 524 controls who were individually matched to cases for age, sex, and place of residence in a population-based case–control study. Seropositivity to *H. pylori* was assessed using both multiplex serology and *H. pylori* IgG ELISA. Ninety-five percent of controls were seropositive to *H. pylori*. Of the 15 antibodies in the multiplex assay, 11 showed no significant association with gastric adenocarcinomas. CagA and VacA were associated with a significantly increased risk of all gastric adenocarcinoma and GNCA in multivariate models. Surprisingly, GroEL and NapA were significantly associated with a reduced risk of these tumors. Only CagA antigen was associated with significantly elevated risk of GCA. We found no associations between *H. pylori* seropositivity overall either by whole-cell ELISA test or multiplex serology, likely due to the high prevalence of seropositivity. Individual antigen testing showed that CagA positivity was associated with increased risk of both noncardia and cardia adenocarcinoma, which is similar to some other Asian populations, whereas two antigens were associated with lower risk of gastric cancer. This latter result was unexpected and should be retested in other populations. Cancer Res; 75(22): 4876–83.

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Introduction

*Helicobacter pylori* infection is the most important cause of gastric adenocarcinoma (1, 2), which is the third leading cause of cancer death worldwide (3). However, the progression from *H. pylori* infection to cancer depends on several factors, including virulence of *H. pylori* strain, anatomic subsite of infection in the stomach, other environmental factors, and host genetics (4).

With regard to anatomic subsite and geography, most Western studies have shown that *H. pylori* is a strong risk factor for noncardia gastric adenocarcinoma, whereas it is either not associated with, or is associated with a lower risk of cardia gastric adenocarcinoma (5, 6). By contrast, in some high-risk areas of the world for esophageal cancer, such as the Taihang Mountain region of China, *H. pylori* seropositivity is associated with modest increases in the risk of both cardia and noncardia gastric adenocarcinoma (7, 8). Because many of these populations have high infection rates, the risks associated with infection can appear smaller than in populations with lower infection rates. Genetic diversity of *H. pylori* strains may also play a role in these different patterns (9, 10). *H. pylori* strains carrying cytotoxin-associated gene A (cagA; refs. 11, 12) and vacuolating toxin (vacA) are more virulent than strains lacking these genes (13, 14). In addition to cagA and vacA, *H. pylori* has many other genetic variations that may confer higher or lower carcinogenic potential. Individual response to various antigenic protein can be assessed using specific serologic tests. The ability to simultaneously test for several of these antibodies was limited until recently. The recent advent of multiplex serology (15) has allowed investigators to efficiently study additional antigens that may be virulence or protective factors for outcomes following *H. pylori* infection. However, studies using this method have been limited; thus far, only five epidemiologic studies have used this method in relation to gastric adenocarcinoma, and they have found different proteins associated with this disease (16–19) and chronic atrophic gastritis (20).

The main aim of this study was to investigate the association between seropositivity to 15 different *H. pylori* antigens using the...
Multiplex serology method and gastric adenocarcinoma in a previously uninvestigated population with documented high rates of 

**Patients and Methods**

**Case and control selection**

This study had a case–control design. Methodologic details have been provided in a previous publication (21). Briefly, incident cases of gastric adenocarcinoma were enrolled from December 2004 to December 2011 in Atrak Clinic, a specialized clinic for upper gastroenterology cancers in Gonbad City, Golestan Province, Iran. All cases underwent upper gastrointestinal endoscopy by experienced gastroenterologists according to a standard protocol and all included case subjects were pathologically confirmed as adenocarcinomas by experienced pathologists at the Digestive Disease Research Institute (DDRI) laboratory at Tehran University of Medical Sciences. Endoscopy-captured images from the gastric adenocarcinomas were reviewed by an experienced DDRI gastroenterologist and the origin of each tumor was classified as cardia or noncardia. Esophageal adenocarcinoma cases were distinguished from cardia cancer if the endoscopist reported that the tumor originated from the lower one third of the esophagus, above the Z line and excluded from the current analysis. When localization of the anatomic origin of a tumor was not possible, the tumor origin was categorized as unspecified.

We selected controls from healthy subjects enrolled in the Golestan Cohort Study (GCS), a cohort study enrolling 50,045 people in Golestan Province (22). Details of the methods of the GCS enrollment and follow-up have been published elsewhere (22). In summary, from January 2004 to June 2008, apparently healthy subjects, ages 40–75 years, were enrolled in the cohort study. We attempted to randomly select two controls from the cohort who were individually matched to each case for age (±5 years), sex, and place of residence (rural/urban). As the cohort study participants were limited to individuals 40 to 75 years of age at enrollment and to certain areas of the case catchment regions, we were not able to match two controls for all gastric adenocarcinoma cases enrolled in this study. Some cases had more than two controls, because some of the selected controls did not have adequate plasma samples.

Of the initial 331 potentially eligible cases, 59 were excluded because they did not have either serum samples (n = 37) or matched controls (n = 22). The remaining 272 cases were tumors originating in the cardia (n = 142), noncardia (n = 103), or an unspecified location (n = 27). From these cases, 245 had two, 24 had one, two had 3, and one had 4 controls (a total of 524 controls). This study was approved by the Institutional Review Board of the Digestive Diseases Research Institute, Tehran University of Medical Sciences, Iran.

**Questionnaires**

After obtaining written consent from the study participants, structured and validated questionnaires were administered to case subjects. The lifestyle questionnaire included information on age, sex, ethnicity, place of residence, education, lifelong history of opium and tobacco use, and indicators of socioeconomic status such as ownership of automobiles, motorcycles, televisions, refrigerators, freezers, vacuums, and washing machines, as well as house ownership, house size (m²), the presence of an indoor bath, and the occupation of the head of the family. The same questionnaires were administered at the cohort baseline to the cohort participants.

**Blood samples**

Twelve milliliters of blood was drawn from the case subjects after an overnight fast. This blood sample was taken on the day of endoscopy, before the procedure. Serum samples were separated and immediately stored at −80°C. Controls also provided 10 mL of blood at cohort baseline, from which plasma was separated and stored at −80°C. Serum samples of cases and plasma samples of controls were shipped on dry ice to the German Cancer Research Center (DKFZ), Heidelberg, Germany, for the multiplex serology and whole cell ELISA assays (23). A study have also shown that any differences between 

**H. pylori** multiplex serology test

Multiplex serology is a high-throughput method for detection of antibodies to up to 100 different antigens in large serologic studies (25). The method has been described in detail elsewhere (15, 26). Multiplex serology is based on a glutathione S-transferase (GST) capture immunosorbent assay combined with fluorescent-bead technology. 15 H. pylori proteins (GroEL, UreA, HP0231, NapA, HP0305, HpAa, CagY, CagM, CagA, HyuA, Cat-alase, VacA, HcpC, Cad, and Omp) were bacterially expressed and GST tagged, detailed information about these antibodies has been described elsewhere (15). Antigen specific cut-offs were calculated [mean median fluorescence intensity (MFI) + three standard deviations, excluding positive outliers] in 20 H. pylori negative sera, determined by commercial Ridascreen Helicobacter IgA, IgG (R-Biopharm) ELISA, run within the assay (15). H. pylori positive was defined as those seropositive to ≥4 antigens, based on previously evaluated results against ELISA (sensitivity 89%, specificity 82%) in a German population published studies (15). The assays have been validated for both serum and plasma samples (15, 17). Two of previous case control studies have used serum samples (16, 18) and one prospective study used plasma samples (17).

**H. pylori whole-cell antibody test**

In addition to the multiplex assay, serum samples were tested for 

**Statistical analysis**

Differences in baseline characteristics between cases and controls were compared using χ² tests (for categorial variables) and t tests (for continuous variables). Odds ratios (OR) and 95% confidence intervals (CI) for the associations between individual antibodies and risk of cardia, noncardia, and all gastric adenocarcinomas were estimated using unadjusted and adjusted conditional logistic regression. Using multiple correspondence
analyses we created a composite score with socioeconomic data, including ownership of automobiles, motorcycles, televisions, computer, refrigerators, freezers, vacuums, and washing machines, as well as house ownership, house size, the presence of an indoor bath, and the occupation of the head of the family in our previous papers (21, 28). The methods for creating this score and its association with cancer risk have been previously published (29).

Multivariable models were adjusted for education (no formal education vs. any), ethnicity (Turkmen vs. non-Turkmen), tobacco use, opium consumption (ever vs. never), and composite socioeconomic status (SES) wealth score as continues variable. Controls were individually matched to cases for age, sex, and place of residence, so we did not include these variables in our risk models.

Phi correlation coefficients and their statistical significance were calculated for dichotomized (positive vs. negative) results of multiplex antibodies that were associated with risk of gastric cancer. Antibody levels were reported as continuous variables, but because they were not normally distributed, we presented correlations using dichotomous nominal variables and phi correlation coefficients. Using the multiplex method, each person was considered *H. pylori* positive if he/she was seropositive for antibodies to at least four out of the 15 tested antigens (15). We also conducted an exploratory, *ad hoc* analysis based on the previous findings. In this exploratory analysis, we used combination of CagA and VacA seropositivity as the two established virulence factors for gastric cancer to predict the risk of cancer. Finally, we checked if there is any differences in proportion of antibody positivity or cancer risk between subjects of Turkmen and non-Turkmen ethnicities. All statistical analyses were done using Stata statistical software, version 11 (Stata Corp.).

**Results**

Table 1 shows the baseline characteristics of the cases and controls. Overall, the proportion of *H. pylori* positive subjects using multiplex serology assay (94.6% of the controls) was not significantly different from the seropositivity rate using the whole-cell ELISA test (91.8% of the controls; Table 1).

*H. pylori* positivity was not significantly associated with cancer (i.e., all gastric, cardia, and noncardia adenocarcinomas) by either multiplex serology or whole-cell ELISA (Table 2). Based on the whole-cell ELISA test, the adjusted ORs (95% CI) were 1.3 (0.7–2.5) for all gastric adenocarcinoma, 1.1 (0.5–2.5) for cardia, and 1.2 (0.4–3.4) for noncardia adenocarcinomas. Using multiplex serology results, the respective point estimates were 1.2 (0.5–2.6), 1.3 (0.4–3.5), and 0.7 (0.2–2.6).

The proportions of cases and controls seropositive for each of the 15 *H. pylori* antigens, and related risk estimates for gastric adenocarcinoma and its subtypes are shown in Table 2. Following multivariable adjustment, four of the 15 antibodies were statistically significantly associated with gastric cancer: GroEL, NapA, CagA, and VacA. GroEL (OR, 0.4; 95% CI, 0.2–0.9) and NapA (OR, 0.3; 95% CI, 0.2–0.7) were significantly associated with a reduced risk, and CagA (OR, 3.4; 95% CI, 1.4–8.1) and VacA (OR, 2.8; 95% CI, 1.4–5.5) antibodies were associated with a significant increased risk of noncardia gastric adenocarcinoma. For gastric cardia adenocarcinoma, a single antigen, CagA, was associated with an increased risk of this cancer (ORs, 1.9; 95% CI, 1.1–3.7).

The phi coefficient correlations between CagA, VacA, NapA, and GroEL, the four antibodies that were significantly associated (positively or inversely) with risk of all gastric adenocarcinoma are shown in Table 3. There was a weak positive correlation between

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**Table 1. Baseline characteristics of the cases and matched controls**

<table>
<thead>
<tr>
<th></th>
<th>All gastric adenocarcinoma</th>
<th>Matched controls</th>
<th>Cardia adenocarcinoma</th>
<th>Matched controls</th>
<th>Noncardia adenocarcinoma</th>
<th>Matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>272</td>
<td>524</td>
<td>142</td>
<td>276</td>
<td>103</td>
<td>195</td>
</tr>
<tr>
<td>Age (years), mean (SD)</td>
<td>65.2 (10.8)</td>
<td>63.7 (9.2)</td>
<td>66.3 (11.1)</td>
<td>64.4 (9.1)</td>
<td>64.0 (10.6)</td>
<td>62.8 (9.4)</td>
</tr>
<tr>
<td>Female gender, N (%)</td>
<td>69 (25.4)</td>
<td>133 (25.4)</td>
<td>31 (21.8)</td>
<td>63 (22.8)</td>
<td>30 (29.1)</td>
<td>53 (27.2)</td>
</tr>
<tr>
<td>Urban residence, N (%)</td>
<td>88 (32.5)</td>
<td>166 (31.7)</td>
<td>47 (33.3)</td>
<td>86 (31.2)</td>
<td>33 (32.0)</td>
<td>61 (31.3)</td>
</tr>
<tr>
<td>Ethnicity, N (%)</td>
<td>129 (47.4)</td>
<td>320 (61.1)</td>
<td>82 (57.7)</td>
<td>186 (67.4)</td>
<td>33 (32.0)</td>
<td>101 (51.8)</td>
</tr>
<tr>
<td>Turkmen</td>
<td>56 (20.6)</td>
<td>71 (13.5)</td>
<td>19 (13.4)</td>
<td>33 (11.9)</td>
<td>32 (31.1)</td>
<td>28 (14.4)</td>
</tr>
<tr>
<td>Turk</td>
<td>27 (9.9)</td>
<td>49 (9.4)</td>
<td>15 (10.6)</td>
<td>25 (9.1)</td>
<td>10 (9.7)</td>
<td>21 (10.1)</td>
</tr>
<tr>
<td>Others</td>
<td>20 (7.4)</td>
<td>19 (3.6)</td>
<td>8 (5.6)</td>
<td>6 (2.2)</td>
<td>11 (10.7)</td>
<td>12 (6.1)</td>
</tr>
<tr>
<td>Education, N (%)</td>
<td>46 (16.9)</td>
<td>143 (27.3)</td>
<td>22 (15.5)</td>
<td>80 (29.0)</td>
<td>18 (17.5)</td>
<td>48 (24.6)</td>
</tr>
<tr>
<td>Some education</td>
<td>226 (83.1)</td>
<td>381 (72.7)</td>
<td>120 (84.5)</td>
<td>196 (71.0)</td>
<td>85 (82.5)</td>
<td>147 (75.4)</td>
</tr>
<tr>
<td>No formal education</td>
<td>169 (62.1)</td>
<td>340 (64.9)</td>
<td>94 (66.2)</td>
<td>171 (62.0)</td>
<td>58 (56.3)</td>
<td>134 (68.7)</td>
</tr>
<tr>
<td>Tobacco smoking, N (%)</td>
<td>103 (37.9)</td>
<td>184 (35.1)</td>
<td>48 (33.8)</td>
<td>105 (38.0)</td>
<td>45 (43.7)</td>
<td>61 (31.3)</td>
</tr>
<tr>
<td>Never</td>
<td>178 (65.4)</td>
<td>414 (79.0)</td>
<td>98 (69.0)</td>
<td>219 (79.4)</td>
<td>64 (62.1)</td>
<td>154 (79.0)</td>
</tr>
<tr>
<td>Ever smoker</td>
<td>94 (34.6)</td>
<td>110 (21.0)</td>
<td>44 (31.0)</td>
<td>57 (20.6)</td>
<td>39 (37.9)</td>
<td>41 (21.0)</td>
</tr>
<tr>
<td>Optum, N (%)</td>
<td>13 (4.8)</td>
<td>28 (5.3)</td>
<td>7 (4.9)</td>
<td>17 (6.2)</td>
<td>5 (4.9)</td>
<td>8 (4.1)</td>
</tr>
<tr>
<td>Negative*</td>
<td>259 (95.2)</td>
<td>496 (94.7)</td>
<td>135 (95.1)</td>
<td>259 (95.8)</td>
<td>98 (95.1)</td>
<td>187 (95.9)</td>
</tr>
<tr>
<td>H. pylori ELISA, N (%)</td>
<td>18 (6.6)</td>
<td>43 (8.2)</td>
<td>11 (7.7)</td>
<td>20 (7.3)</td>
<td>6 (5.8)</td>
<td>19 (9.7)</td>
</tr>
<tr>
<td>Positive*</td>
<td>254 (93.4)</td>
<td>481 (91.8)</td>
<td>131 (92.3)</td>
<td>256 (92.7)</td>
<td>97 (94.2)</td>
<td>176 (90.3)</td>
</tr>
</tbody>
</table>

*All gastric adenocarcinomas (n = 272), including cardia (n = 142) and noncardia (n = 103) adenocarcinomas, as well as 27 adenocarcinomas of mixed or unspecified site (with 53 controls).

*Defined as recognizing antibodies to ≥4 antigens.*
Table 2. Conditional logistic regression: OR and 95% CI for seropositivity to antigens and risk of gastric adenocarcinoma and cardia and noncardia adenocarcinoma subsites

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Controls N (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole cell ELISA-Pos.</td>
<td>254 (93.4)</td>
<td>2.2 (1.5–3.7)</td>
<td>2.2 (1.5–3.7)</td>
</tr>
<tr>
<td>HP0231-Pos.</td>
<td>133 (48.9)</td>
<td>0.7 (0.5–1.1)</td>
<td>0.7 (0.5–1.1)</td>
</tr>
<tr>
<td>HP0305-Pos.</td>
<td>144 (52.9)</td>
<td>0.9 (0.7–1.2)</td>
<td>0.9 (0.7–1.2)</td>
</tr>
<tr>
<td>HP0152-Pos.</td>
<td>176 (64.7)</td>
<td>1.3 (1.1–1.7)</td>
<td>1.3 (1.1–1.7)</td>
</tr>
<tr>
<td>HP0151-Pos.</td>
<td>247 (89.7)</td>
<td>2.4 (1.5–4.2)</td>
<td>2.4 (1.5–4.2)</td>
</tr>
<tr>
<td>CagM-Pos.</td>
<td>169 (62.1)</td>
<td>1.2 (0.9–1.6)</td>
<td>1.2 (0.9–1.6)</td>
</tr>
</tbody>
</table>

Adjusted for education (illiterate vs. otherwise), ethnicity (Turkmen vs. non-Turkmen), tobacco and opium consumption (ever vs. never), and wealth score as continuous variable.
with a predominance of cardia adenocarcinomas (31). The Taihang mountain region of China also has concurrent high incidence of ESCC and GCC. (32) Previous studies of H. pylori seropositivity in the Taihang Mountains have shown modest, but statistically significant, increased risks for both gastric cardia and noncardia adenocarcinoma (7). This is in contrast to other parts of the world, particularly Western countries, where H. pylori is strongly associated with noncardia adenocarcinoma but is either not associated or inversely associated with cardia adenocarcinoma risk (6, 33–35). A recent meta-analysis found a positive association between H. pylori infection and gastric cardia cancer in geographic regions with high incidence of gastric cardia cancer (36). Our results from Northeastern Iran are similar to those of studies conducted in the Taihang Mountains. We note there are other similarities between these regions, including low SES, historically poor diets and both showing including increased risks conveyed by poor oral health (28, 37).

Other environmental or dietary factors, such as low iron availability, could lead to higher expression of CagA, VacA, urease, HopQ, and flagellar proteins and enhance the virulence of H. pylori (38). Similar, but inverse, effects occur when zinc is limited (39). Dietary habits such as high consumption of salt, proteins, or nitrite have also been linked to the pathogenicity of H. pylori (40). Furthermore, conditions in the stomach environment, such as altered pH, could facilitate colonization of the bacteria. Taken together, these results suggest that differences in associations between populations could be due to a number of environmental factors (41).

Our results may lend support to the hypothesis that there may be two distinct types of cardia cancer, one resembling noncardia adenocarcinoma, and associated with H. pylori-related atrophic gastritis (such as this study and in the Taihang Mountains), and another which arises from nonatrophic gastric mucosa and is associated with gastroesophageal reflux disease, like esophageal adenocarcinoma (such as is typically seen in Western studies; refs. 42, 43). These differences may be due to differences in the genetics of H. pylori in these populations compared with other geographic regions. We would expect, but have not tested, that based on genetic studies of seven modern H. pylori populations, our population would likely carry hpAsia2, which differs from hpEastAsia and hpEurope (44). Detailed comparisons of the H. pylori genetics in Iran, China, and Western countries will be necessary to address this hypothesis.

Alternatively, these differences could be due to host genetics. Currently, there is only limited information on the genomes of the people inhabiting this portion of Iran and our study included multiple distinct ethnic groups, including Turksmen of central Asian descent and non-Turksmen that are primarily of Indo-European descent. We note that recent reports from GWAS studies have identified some differences in genetic predisposition for gastric

### Table 3. Phi correlation coefficients between CagA, VacA, NapA, and GroEL among controls

<table>
<thead>
<tr>
<th></th>
<th>CagA</th>
<th>VacA</th>
<th>GroEL</th>
<th>NapA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CagA</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VacA</td>
<td>0.27 (<em>P &lt; 0.001</em>)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GroEL</td>
<td>0.17 (<em>P &lt; 0.001</em>)</td>
<td>0.13 (<em>P &lt; 0.05</em>)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>NapA</td>
<td>-0.02 (<em>P = 0.590</em>)</td>
<td>0.04 (<em>P = 0.341</em>)</td>
<td>0.32 (<em>P &lt; 0.001</em>)</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 4. Association between combinations of CagA with VacA seropositivity and risk of gastric adenocarcinoma and its subtypes

<table>
<thead>
<tr>
<th></th>
<th>Controls (N=17)</th>
<th>Adjusted* OR (95% CI)</th>
<th>Controls (N=48)</th>
<th>Adjusted* OR (95% CI)</th>
<th>Controls (N=196)</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CagA or VacA</td>
<td>17 (6.3)</td>
<td>Reference</td>
<td>17 (6.3)</td>
<td>Reference</td>
<td>196 (72.3)</td>
<td>Reference</td>
</tr>
<tr>
<td>VacA only</td>
<td>47 (17.0)</td>
<td>4.3 (2.1–7.9)</td>
<td>27 (19.0)</td>
<td>1.9 (0.9–3.8)</td>
<td>166 (60.1)</td>
<td>1.2 (0.7–2.0)</td>
</tr>
<tr>
<td>CagA only</td>
<td>112 (42.1)</td>
<td>1.6 (0.8–3.3)</td>
<td>54 (19.6)</td>
<td>1.2 (0.5–3.1)</td>
<td>78 (35.1)</td>
<td>2.3 (1.5–3.4)</td>
</tr>
<tr>
<td>Both CagA and VacA</td>
<td>297 (66.9)</td>
<td>2.8 (1.4–5.3)</td>
<td>166 (60.1)</td>
<td>1.6 (0.7–3.8)</td>
<td>107 (54.9)</td>
<td>3.1 (1.6–5.9)</td>
</tr>
</tbody>
</table>

*Conditional logistic regression, adjusted for education (illiterate vs. otherwise), ethnicity (Turksmen vs. non-Turksmen), tobacco and opium consumption (ever vs. never), and wealth score as continuous variable.
cancer between Asian and Caucasian subjects (45). We did not observe significant difference in \textit{H. pylori} virulence factors between or cancer risk between ethnicities in our population, therefore it seems less likely that population differences are a primary reason of our findings with regard to \textit{H. pylori} seropositivity and risk of gastric cardia adenocarcinoma.

Surprisingly, GroEL and NapA were inversely associated with risk of gastric noncardia adenocarcinoma in our study, which has not previously been reported (refs. 16–19; Table 5). GroEL is a heat shock protein, which mediates protein folding (46, 47) especially misfolded proteins under stress conditions (25). It is also associated with the adhesion of \textit{H. pylori} to human gastric epithelial cells (48) and the induction of inflammatory responses (49). NapA is neutrophil-activating protein and antagonizes oxidase stress (50) and mediates the binding of \textit{H. pylori} to the host cell and stomach mucus (51). A study of GNCA in the Taihung Mountains did not observe inverse associations for these antigens, but did report an inverse association for CagM (19).

Because CagA and VacA have been associated with higher risk of gastric adenocarcinoma in most previous studies (52), we assume that their association with this cancer is established (13). Therefore, we investigated whether a combination of seropositivity to these two antibodies increases risk beyond seropositivity to each one. Our results show that subjects seropositive for both had the largest OR for gastric noncardia adenocarcinoma, but the association in the cardia was more modest than when examining CagA alone. This difference may be due to chance, but deserves further exploration when other GCC data sets become available.

In conclusion, in our population-based case–control study of gastric adenocarcinoma risk in Northeastern Iran, we found that seropositivity to CagA \textit{H. pylori} antigen was associated with increased risk of both noncardia and cardia gastric adenocarcinoma, whereas seropositivity to VacA was also associated with an increased risk of gastric noncardia cancer. We also found that seropositivity to GroEL and NapA may be associated with a lower risk of noncardia gastric adenocarcinoma. Further investigations of the association of individual antibodies, using the multiplex method, with risk of gastric adenocarcinoma in different regions of the world are needed to assess the robustness of these findings.

Table 5. Association of seropositivity to \textit{H. pylori} antigens and gastric adenocarcinoma in different populations

<table>
<thead>
<tr>
<th>Study area/design</th>
<th>CagA</th>
<th>VacA</th>
<th>GroEL</th>
<th>NapA</th>
<th>Others antigens with significant associations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iran/case-control</td>
<td>3.5</td>
<td>2.7</td>
<td>0.4</td>
<td>0.4</td>
<td>None</td>
</tr>
<tr>
<td>China, Shanghai/</td>
<td>3.3</td>
<td>2.1</td>
<td>1.2</td>
<td>1.3</td>
<td>Omp, HP0305, HpaA</td>
</tr>
<tr>
<td>nested control</td>
<td>(17)</td>
<td>(10)</td>
<td>(7)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td>Germany/case-control</td>
<td>5.6</td>
<td>2.1</td>
<td>1.4</td>
<td>1.4</td>
<td>HcpC, Catalse, HP0305, CagB, HyaA</td>
</tr>
<tr>
<td>(18)</td>
<td>(3-2.9)</td>
<td>(1-3.35)</td>
<td>(2.2-5.8)</td>
<td>(5.9-2.2)</td>
<td></td>
</tr>
<tr>
<td>Sweden/case-control</td>
<td>9.2</td>
<td>2.1</td>
<td>1.5</td>
<td>1.1</td>
<td>HcpC and HP0305; all others except HP0305</td>
</tr>
<tr>
<td>(10)</td>
<td>(3.5-75.8)</td>
<td>(1.3-2.7)</td>
<td>(1.0-2.3)</td>
<td>(8.0-1.4)</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age, education, ethnicity, tobacco and opium consumption, and wealth score.

*Adjusted for age, sex, education, family history of gastric cancer, smoking, and alcohol drinking.

*Adjusted for age, sex, area of residence, SES, use of tobacco, level of fruit and vegetable consumption, and number of siblings.

*Adjusted for age at blood draw, sex, ever smoking, alcohol, and body mass index.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions


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References


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Correction: Multiplex *H. pylori* Serology and Risk of Gastric Cardia and Noncardia Adenocarcinomas

In this article (Cancer Res 2015;75:4876–83), which appeared in the November 15, 2015 issue of Cancer Research (1), the name of the fourth author was misspelled. The correct name is Michael Pawlita. The authors regret this error.

Reference


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Multiplex *H. pylori* Serology and Risk of Gastric Cardia and Noncardia Adenocarcinomas

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