Serum Antibodies to *Helicobacter pylori* and the CagA Antigen Do Not Explain Differences in the Prevalence of Precancerous Gastric Lesions in Two Chinese Populations with Contrasting Gastric Cancer Rates

Frank D. Groves, Guillermo Perez-Perez, Lian Zhang, Wei-cheng You, Stuart R. Lipsitz, Mitchell H. Gail, Joseph F. Fraumeni, Jr., and Martin J. Blaser

Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland 20892-7244 [F. D. G., W.-c. Y., M. H. G., J. F. F.;] Department of Internal Medicine, New York University School of Medicine, New York, New York 10016 [G. P.-P., M. J. B.;] Beijing Institute for Cancer Research and School of Oncology, Peking University, Beijing, People’s Republic of China 100034 [L. Z., W.-c. Y.;] Department of Biometry and Epidemiology, Medical University of South Carolina, Charleston, South Carolina 29425-0835 [F. D. G., S. R. L.;] and Veterans’ Affairs Medical Center, New York, New York 10010 [M. J. B.]

Abstract

Incidence and mortality rates for gastric cancer in rural People’s Republic of China differ greatly over short distances. In Shandong Province, we studied asymptomatic adult subjects from Bei Duan village (n = 196) in Linqu County (a high-risk area for gastric cancer) and from Shi Huang village (n = 192) in Cangshan County (a low-risk area for gastric cancer). The prevalence of advanced precancerous gastric lesions (APGL) was assessed by microscopic examination of endoscopic stomach biopsies. ELISAs were used to detect serum IgG to *Helicobacter pylori* whole-cell antigen and to the CagA protein. A logistic regression model was used to quantify the role of the two *H. pylori* seromarkers in explaining the differences in prevalence of APGL between the two villages after adjusting for age and sex.

The prevalence of APGL was much greater in Bei Duan than in Shi Huang. Although *H. pylori* seroprevalence by the whole-cell ELISA was similar in the two populations, seroprevalence of CagA was significantly greater in Bei Duan. Although age, sex, and both *H. pylori* seromarkers were associated with APGL in the logistic regression model, the effect of village of residence remained strong after adjustment for all four covariates. Only a relatively small proportion of the difference in prevalence of APGL between these two rural Chinese populations can be explained by differences in *H. pylori* or CagA seroprevalence.

Introduction

Extremely high gastric cancer mortality rates of 70 (per 100,000, age-adjusted to the world standard population) in men and 26 in women (1) are found in Linqu County of Shandong Province, compared with only 5 for males and 3 for females in Cangshan County, located only 200 miles away (2). *Helicobacter pylori* seroprevalence among adult males in 46 rural Chinese counties is highly correlated with gastric-cancer mortality (3), and *H. pylori* colonization has been linked to preneoplastic gastric lesions in Linqu (4).

Although *H. pylori* strains are heterogeneous (5), the majority possess cagA (6–9), which encodes CagA, an immunodominant protein antigen (10). In prospective cohort studies of *H. pylori*-positive subjects in The Netherlands and Hawaii, those who were CagA seropositive were twice as likely to develop atrophic gastritis (11), nine times as likely to develop intestinal metaplasia (11), and twice as likely to develop adenocarcinoma of the distal stomach (12), compared with those who were CagA seronegative. A similar cohort study in California indicated that the risk of developing gastric adenocarcinoma was ~3-fold higher among subjects carrying cagA + versus cagA- strains (13).

In an endoscopic survey in Shi Huang, a village in Cangshan, only 13.5% of subjects had intestinal metaplasia or dysplasia, compared with 45.1% in 14 villages in Linqu (14). In Shi Huang, 55% of subjects were seropositive for *H. pylori*, compared with 72% in Linqu (4). In view of the higher risks of gastric cancer associated with CagA + strains of *H. pylori* (11–13), we undertook a study to determine whether whole-cell seropositivity to *H. pylori* and/or CagA seropositivity could account for the higher prevalence of APGL in Linqu County than in Shi Huang. In particular, we compared findings in Shi Huang with those in Bei Duan, a village in Linqu County.

Subjects and Methods

Study Populations. Residents 35–64 years of age in Bei Duan village (population 1281) of Linqu County and in Shi Huang village (population 3271) of Cangshan County were invited to participate in the study. The mean ages of study participants were 50.7 (SE = 0.6) years in Bei Duan (52% male) and 48.9 (SE = 0.7) years in Shi Huang (51% male).

Gastric Histopathology. Health officials visited each person 35–64 years of age in village rosters and offered a consent form to invite participation in gastric-cancer screening. All residents were invited except those who were ill or disabled. Participants were given a brief physical examination, their medical histories were recorded, and sera were collected by venipunc-

---

1 The abbreviations used are: CagA, cytotoxin-associated gene A; APGL, advanced precancerous gastric lesions; BICR, Beijing Institute for Cancer Research.
ture. Subjects who had significant blood-clotting disorders, high blood pressure, liver disease, or chronic obstructive pulmonary disease were excluded from further examination. Subjects in both villages underwent endoscopy in 1994, with biopsies taken from up to seven standard sites in the stomach (in Bei Duan) or from a subset of up to four standard sites (in Shi Huang). Because subjects in Shi Huang had at most four biopsies, only these four standard sites were used in comparing the histological scores of subjects from the two villages. There were 747 eligible adults in Shi Huang village, 224 (30%) of whom consented to participate and underwent endoscopy. In- sufficient biopsy material was obtained from 10 of these subjects, and 196 (92%) of the remaining 214 also had serology and interview data and were included in the present analysis (2). Of the 292 adults 35–64 years of age in Bei Duan village, 277 were invited to participate in the study, and 263 of these were interviewed and examined; two subjects failed this physical examination. Endoscopic examinations were completed on 239 of the remaining 261 subjects (86%) of those recruited; 218 had sufficient tissue samples, and 192 also had serology and interview data and were included in the present analysis.

Biopsies were preserved in 10% neutral buffered formalin, embedded in paraffin, and sectioned at the BICR (Beijing, People’s Republic of China). Each slide was reviewed independently according to the protocol of the Chinese Association of Gastric Cancer (15) by three senior pathologists at BICR; these pathologists were not aware of the serostatus or village of residence of the subject. Any discrepancies were resolved by consensus. The lesions of interest, in increasing order of severity, were as follows according to established diagnostic criteria (16): (a) superficial gastritis; (b) chronic atrophic gastritis; (c) intestinal metaplasia; and (d) dysplasia. Each biopsy was given a global diagnosis based on the most advanced lesion, and then each subject was assigned a global diagnosis based on the most advanced lesion among any of the four biopsies. We use the term “APGL” to denote intestinal metaplasia or dysplasia. In a separate quality-control study, two senior pathologists from China and the United States with expertise in gastric cancer [Drs. Ji-You Li (BICR) and Pelayo Correa (Louisiana State University, Baton Rouge, LA)] examined 270 slides selected from subjects in the larger cohort from which the study population in Bei Duan was drawn. This sample consisted of 200 randomly selected subjects, 30 cases with presumptive diagnoses of gastric carcinoma and 40 cases with presumptive diagnoses of dysplasia. Drs. Li and Correa achieved consensus on 267 (98.9%) of the slides; diagnoses for the remaining three slides were either mild or borderline dysplasia (17).

**Helicobacter pylori Serology.** Serological testing was performed by personnel who were not aware of the histological diagnoses. Serum samples were tested for the presence of IgG to the *H. pylori* whole-cell antigens by a previously described ELISA (18, 19). The pooled antigen was derived from an equal mixture of sonicates from five strains from the United States (84-180, 84-182, 84-183, 86-63, 86-86) and used at a concentration of 1 μg/well. Previous studies showed little difference in sensitivity and specificity between whether a United States or Chinese antigen pool was used (19, 20). All serum samples were diluted 1:80 and examined in duplicate wells on 2 different days. Appropriate blanks and positive and negative controls were included in each assay as described (18). We define “*H. pylori* seropositive” as a positive whole-cell antigen ELISA.

CagA status was determined by ELISA to detect serum IgG antibodies to ORV220, a 65-kDa recombinant CagA truncated protein purified from *Escherichia coli*, and positivity was defined as A ≥ 0.30/nm as validated previously (21). Samples were diluted 1:100 and examined in duplicate wells on 2 different days.

**Statistical Analysis.** Differences between the two villages for the prevalence of APGL, as well as for the *H. pylori* whole-cell and CagA serostatus, were tested using the continuity-adjusted two-tailed χ² test with one degree of freedom (22). The effect of village of residence on the prevalence of APGL was modeled by unconditional logistic regression (23), adjusting for sex, age group (<50 versus ≥50 years), and *H. pylori* whole-cell and CagA serostatus. Ninety-five percent confidence intervals are shown in parentheses (Table 1). Unreported data from Linqu County indicates that the intraclass correlation for APGL is

---

**Table 1** Cross tabulation of APGL, sex, village, and *H. pylori* and CagA Serostatus

<table>
<thead>
<tr>
<th></th>
<th>Number and percentage of males with APGL</th>
<th>Number and percentage of females with APGL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bei Duan (n = 99)</td>
<td>Shi Huang (n = 100)</td>
</tr>
<tr>
<td><strong>APGL status</strong></td>
<td><strong>Number</strong></td>
<td><strong>% (95% CI)</strong></td>
</tr>
<tr>
<td><strong>Whole-Cell</strong></td>
<td>+ (136)</td>
<td>28/78 (64–91)</td>
</tr>
<tr>
<td></td>
<td>− (82)</td>
<td>8/22 (9–36)</td>
</tr>
<tr>
<td></td>
<td>+ (60)</td>
<td>9/50 (27–73)</td>
</tr>
<tr>
<td></td>
<td>− (60)</td>
<td>23/85 (72–99)</td>
</tr>
<tr>
<td><strong>CagA</strong></td>
<td>+ (181)</td>
<td>37/69 (56–81)</td>
</tr>
<tr>
<td></td>
<td>− (137)</td>
<td>17/31 (19–44)</td>
</tr>
<tr>
<td><strong>Whole-Cell</strong></td>
<td>+ (133)</td>
<td>6/46 (19–73)</td>
</tr>
<tr>
<td></td>
<td>− (20)</td>
<td>7/54 (27–81)</td>
</tr>
<tr>
<td><strong>CagA</strong></td>
<td>+ (19)</td>
<td>11/34 (18–51)</td>
</tr>
<tr>
<td></td>
<td>− (118)</td>
<td>21/66 (49–82)</td>
</tr>
<tr>
<td><strong>Whole-Cell</strong></td>
<td>+ (32)</td>
<td>17/38 (24–52)</td>
</tr>
<tr>
<td></td>
<td>− (138)</td>
<td>28/62 (48–76)</td>
</tr>
<tr>
<td><strong>CagA</strong></td>
<td>+ (113)</td>
<td>54/55 (45–64)</td>
</tr>
<tr>
<td></td>
<td>− (275)</td>
<td>45/45 (36–55)</td>
</tr>
</tbody>
</table>

a Seropositivity status and number of subjects in each stratum (in parentheses).
b APGL status is indicated by + or −, and number of subjects in each stratum (in parentheses).
c Continuity-adjusted χ² test.
d CI, confidence interval.
only 0.019.4 Thus there was no need to adjust the analyses for the presence of more than one subject in some households.

Results

The seroprevalence of *H. pylori* colonization, as determined by the whole-cell ELISA, did not differ significantly between the two villages (54/99 = 55% in Bei Duan versus 52/100 = 52% in Shi Huang for males; 59/93 = 63% versus 53/96 = 55% for females). However, CagA seropositivity was higher in Bei Duan than in Shi Huang (49/99 = 49% versus 30/100 = 30% for males, *P* = 0.008; 51/93 = 55% versus 39/96 = 41% for females, *P* = 0.05). Altogether, 71% and 58% (*P* = 0.009) of the subjects in Bei Duan and Shi Huang villages, respectively, were seropositive by either the whole-cell or the CagA assay (data not shown). Many subjects who were seronegative by the whole-cell ELISA were seropositive for CagA, and it was among this subset that the differences in CagA seroprevalence between Bei Duan and Shi Huang, both among males (13/45 = 29% versus 5/48 = 10%, *P* = 0.05) and among females (11/34 = 32% versus 4/43 = 9%, *P* = 0.02), were most pronounced (data not shown). Among the subjects who were *H. pylori* seropositive by the whole-cell ELISA, the differences in CagA serology were small (67% versus 48% in males, *P* = 0.08; 68% versus 66% in females).

Table 1 is a cross-tabulation of the prevalence of APGL with *H. pylori* and CagA serostatus in the two villages. The prevalence of APGL was much higher in Bei Duan than in Shi Huang, particularly among males (55% versus 11%, *P* < 0.0001), but also among females (38% versus 14%, *P* = 0.0003). Within each village and *H. pylori* serostatus, the frequency of APGL was higher in CagA-positive subjects. Subjects in Bei Duan had a higher proportion of APGL, regardless of their *H. pylori* or CagA status. The proportion of subjects with APGL in each village was similar among those who were *H. pylori* positive but CagA negative and those who were *H. pylori* negative but CagA positive.

Table 2: Estimates of odds ratios from logistic regression model of risk factors for advanced precancerous gastric lesions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate models</th>
<th>Full model</th>
<th>Reduced models</th>
<th>Stratified by sex</th>
<th>Stratified by village</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude odds ratios</td>
<td>Adjusted odds ratios</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (male versus female)</td>
<td>1.4 (0.9–2.2)</td>
<td>0.9 (0.4–2.1)</td>
<td></td>
<td>2.3 (1.2–4.2)</td>
<td>0.8 (0.3–2.1)</td>
</tr>
<tr>
<td>Village (Bei Duan versus Shi Huang)</td>
<td>6.2 (3.7–10.3)</td>
<td>3.8 (1.8–8.1)</td>
<td>10.1 (4.6–22.2)</td>
<td>3.9 (1.8–8.3)</td>
<td></td>
</tr>
<tr>
<td>Age group (≥50 versus &lt;50 years)</td>
<td>1.9 (1.2–2.0)</td>
<td>2.1 (1.3–3.4)</td>
<td>1.7 (0.9–3.5)</td>
<td>2.4 (1.2–5.0)</td>
<td>1.8 (1.0–3.4)</td>
</tr>
<tr>
<td>CagA (A≥0.30/nm versus A&lt;0.30/nm)</td>
<td>2.6 (1.6–4.1)</td>
<td>2.0 (1.1–3.5)</td>
<td>2.4 (1.1–5.3)</td>
<td>1.6 (0.7–3.9)</td>
<td>1.9 (1.0–3.6)</td>
</tr>
<tr>
<td>Sex-village interaction term</td>
<td>3.2 (2.0–5.1)</td>
<td>2.3 (1.4–4.0)</td>
<td>2.2 (1.0–4.7)</td>
<td>2.4 (1.1–5.5)</td>
<td>2.5 (1.3–4.8)</td>
</tr>
<tr>
<td></td>
<td>2.6 (0.9–7.6)</td>
<td></td>
<td></td>
<td>2.0 (0.9–7.6)</td>
<td>1.9 (0.9–9.3)</td>
</tr>
</tbody>
</table>

a Intestinal metaplasia and dysplasia.

b Referent group italicized.

c *P* = 0.08 for sex-village interaction.

Discussion

As anticipated from previous comparisons of Shi Huang village in Cangshan with 14 villages in Linqu (14), the prevalence of APGL was greater in the village of Bei Duan in Linqu than in Shi Huang, and the same was true of CagA seropositivity. The finding of similar whole-cell *H. pylori* seropositivity in the two villages was unexpected in view of the higher seroprevalences found in other villages in Linqu County (4), although the trend was in the expected direction. Perhaps Shi Huang is somewhat atypical in this respect because the 95% confidence interval on the seroprevalence was 46–61%. Also, because of the rather low response rate in Shi Huang (196/747, 26%, included in the final study), the Shi Huang subjects may not be representative of the eligible population in that village. If agreement to participate were associated with being infected with *H. pylori* (e.g., those with symptoms more likely to agree), then the population seroprevalence would be biased upward.

Whole-cell seropositivity and CagA seropositivity were independent risk factors in models also containing age and sex, both in Bei Duan and in Shi Huang. Thus, both indicators of APGL presence were associated with increased risk of gastric cancer, which is consistent with data elsewhere (11–13). Nonetheless, the effect of village on risk was virtually the same in univariate analyses as in analyses that were adjusted for age, sex, whole-cell seropositivity, and CagA seropositivity. Thus, these factors do not account for the >6-fold greater odds of APGL in Bei Duan than in Shi Huang. Other factors, such as the high consumption of garlic in Shi Huang (2) or the lower *H. pylori* prevalence among children in Shi Huang (24) may help explain the difference. The interaction of male sex with residence in Bei Duan village, although not statistically significant, suggests an uncontrolled risk factor, such as tobacco smoking.

a M. Gail, personal communication.
(25), which is disproportionately more common among men in Bei Duan.

References


Gastric Cancer Rates
Gastric Lesions in Two Chinese Populations with Contrasting Gastric Cancer Rates

Frank D. Groves, Guillermo Perez-Perez, Lian Zhang, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/11/10/1091

Cited articles
This article cites 20 articles, 11 of which you can access for free at:
http://cebp.aacrjournals.org/content/11/10/1091.full#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/11/10/1091.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.