# *Helicobacter pylori* Antibodies in Relation to Precancerous Gastric Lesions in a High-Risk Chinese Population[1]

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## Abstract

***Helicobacter pylori* infection is a major cause of gastritis and may be a key risk factor for stomach cancer, but its role in the process of gastric carcinogenesis is not well understood. Herein, we examine *H. pylori* prevalence in relation to demographic and lifestyle factors and to severity of precancerous lesions in an area of China with one of the highest rates of stomach cancer in the world. *H. pylori* serum IgG antibody positivity was assayed among 2646 adults, ages 35–64, participating in a population-based gastroscopic screening survey in the high-risk area. The prevalence of positivity was evaluated according to gastric histology, environmental and lifestyle variables determined by interviews during the screening, and level of serum pepsinogens. The odds of advanced precancerous lesions (intestinal metaplasia and dysplasia) of the stomach among those with antibody positivity were estimated by logistic regression. Seventy-two % of the population was *H. pylori* antibody-positive, with nonsignificant variation by sex, age, income, education, family size, and cigarette smoking habits. *H. pylori* positivity was higher among those who ate sour pancakes, a fermented indigenous staple that is a risk factor for gastric dysplasia and stomach cancer in this population. The prevalence of *H. pylori* varied most notably, however, with gastric pathology. The percent of *H. pylori* positivity increased from 55 to 60 to 87% among those with superficial (nonatrophic) gastritis, mild chronic atrophic gastritis, and severe chronic atrophic gastritis, respectively, before falling to 78% among those with intestinal metaplasia or dysplasia. *H. pylori* antibody positivity also was strongly correlated with serum pepsinogen concentrations, particularly pepsinogen II, but knowledge of *H. pylori* status did not markedly improve serological identification of advanced precancerous lesions above that provided by pepsinogen ratios alone. The findings suggest that *H. pylori* infection contributes to the process of gastric carcinogenesis, particularly during the early stages, in this high-risk area.**

## Introduction

*Helicobacter pylori* are bacteria colonizing the human stomach adjacent to gastric epithelial cells. The infection has been detected throughout the world, and appears to be a major cause of active gastritis and peptic ulcer disease (1). *H. pylori* infection also may be involved in the etiology of stomach cancer because elevated serum *H. pylori* antibody positivity has been reported among persons who subsequently developed this cancer (2). It is believed that stomach cancer generally arises from a series of progressive changes, with normal tissue being transformed to CAG,[3] followed by IM and then dysplasia before cancer onset (3), and that *H. pylori* infection may influence early and perhaps later stages of this progression. To help assess the role of *H. pylori* in the process of gastric carcinogenesis, we examined serum antibodies to the bacteria among individuals with precancerous gastric lesions detected via a population-based gastroscopic screening survey in an area of Shandong Province, China, with one of the highest rates of stomach cancer in the world (4).

## Materials and Methods

A total of 3433 subjects participated in this gastroscopic screening study, representing 83% of eligible residents ages 35–64 in 14 villages selected at random within four townships of Linqu county, a rural area in Shandong Province, in northeast China, in 1989. Details of this study are described elsewhere (4). The endoscopic examination included collection of seven biopsies from standard locations: two in the body, one in the angulus, and four in the antrum. In two of the villages, an eighth biopsy was taken within 2 cm of the cardia along the lesser curvature.

Histological diagnoses were made according to criteria proposed by the Chinese Association of Gastric Cancer (4). Slides were reviewed by senior pathologists at the Beijing Institute for Cancer Research, with a sample reviewed by experts on stomach pathology in China and the United States, and

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[3] The abbreviations used are: CAG, chronic atrophic gastritis; IM, intestinal metaplasia; OR, odds ratio; SG, superficial gastritis.

*Table 1* Prevalence of H. pylori antibody positivity according to precancerous lesion status

| Most advanced gastric lesion | *n* | % *H. pylori* positive | OR[a] | 95% CI[b] |
|---|---|---|---|---|
| SG | 42 | 54.8 | 1.0 | |
| Mild CAG | 980 | 59.8 | 1.3 | 0.7–2.4 |
| Severe CAG | 219 | 86.8 | 5.9 | 2.8–12.3 |
| IM | 859 | 78.8 | 3.5 | 1.8–6.7 |
| Dysplasia | 546 | 78.0 | 3.6 | 1.9–6.9 |

[a] OR associated with *H. pylori* IgG antibody positivity for each lesion relative to SG. ORs from logistic regression model adjusted for age, sex, intake of sour pancakes, water source, and cigarette smoking.
[b] CI, confidence interval.

*Table 2* Prevalence of H. pylori antibody positivity according to serum pepsinogen levels

| Pepsinogen (μg/liter) | *n* | % *H. pylori* positive | OR[a] | 95% CI[b] |
|---|---|---|---|---|
| I | | | | |
| ≥100 | 1090 | 82.0 | 1.0 | |
| 75–99 | 682 | 71.4 | 0.5 | 0.4–0.6 |
| 50–74 | 638 | 59.6 | 0.2 | 0.2–0.3 |
| <50 | 309 | 61.5 | 0.2 | 0.2–0.3 |
| II | | | | |
| <7.5 | 517 | 29.8 | 1.0 | |
| 7.5–12.4 | 545 | 56.7 | 4.0 | 3.0–5.3 |
| 12.5–17.4 | 569 | 87.0 | 20.0 | 14–28 |
| 17.5–24.4 | 557 | 91.4 | 34.0 | 23–50 |
| ≥24.5 | 531 | 91.2 | 37.5 | 25–55 |
| III | | | | |
| ≥10 | 507 | 25.6 | 1.0 | |
| 7.5–9.9 | 484 | 53.7 | 3.5 | 2.7–4.6 |
| 5.0–7.4 | 988 | 88.9 | 23.2 | 17–31 |
| 3.0–4.9 | 542 | 95.0 | 54.8 | 35–85 |
| <3 | 198 | 84.9 | 18.0 | 11–28 |

[a] OR associated with *H. pylori* positivity of each pepsinogen level relative to the baseline level. ORs are adjusted for age, sex, and gastric pathology.
[b] CI, confidence interval.

a consensus diagnosis was made. The presence or absence of SG (nonatrophic), CAG, IM, or dysplasia was recorded for each biopsy. CAG was graded as mild or severe if atrophy affected less than or more than 50% of the glands located in the gastric mucosa, respectively. Each subject was assigned a global diagnosis based on the most severe lesion found in any of the biopsies. Details of the pathology procedures and classification criteria, along with photographs of SG, CAG, IM, and dysplasia, are provided elsewhere (4).

Information on demographic factors (age, education, occupation, and family income), water supply, diet, tobacco and alcohol use, and other variables was collected through a questionnaire interview, described in detail elsewhere (5).

Approximately 15 ml of blood were collected from each fasting subject. Serum was separated and aliquoted, stored immediately at −20°C, and then moved into a −70°C freezer at Beijing Institute for Cancer Research. From the serum specimens we measured concentrations of pepsinogen and antibodies to *H. pylori*. Serum concentrations of pepsinogen I and II were measured by RIA at the Department of Veterans Affairs laboratory in Los Angeles, as described in detail elsewhere (6).

*H. pylori* strains cultured from gastric biopsies of two patients in Linqu County were used to provide an antigenic preparation for serology. The two strains were grown on blood agar plates for 48 h and then harvested in distilled water. The cell suspensions were sonicated six times for 30 s each. The protein concentrations were measured by the Markwell *et al.* modification of the Lowry method (7) and diluted to 10 μg/ml, and the soluble material from the two strains was pooled for an ELISA procedure (8).

Serum *H. pylori* IgG and IgA antibodies concentrations were then measured, and each subject was determined to be positive for *H. pylori* infection if the ELISA absorbance reading for IgG was above 1.0, a cutoff based on examination of the distribution of such readings in relation to a group of uninfected persons and reference sera. The details were as follows: the microtiter plates were coated with 100 μl/well of antigen preparation, incubated at room temperature overnight, and blocked by PBSTTG (0.001 mg/ml thimersol/0.05% Tween 80/1.0 mg/ml gelatin) for 3 h. The sera were diluted at 1:100 for IgA and 1:800 for IgG. The plates were filled with 100 μg/well dilution of test sera and incubated at 37°C for 1 h. Plates were washed 3 times, and 100 μg of peroxidase-conjugated goat antihuman IgG were added to each well (1:2000 dilution; Tago Inc., Camarillo, CA). After incubation at 37°C for 1 h, the plates were washed three times, and 100 μg of developing solution consisting of 1 mg of 2,2′-azino-di-(3-ethylbenzthiasoline-6-sulfonic acid) per ml in McIlvain's buffer (pH 4.6) with 0.16% $H_2O_2$ was added to each well. Approximately 10 min later, each plate was read at 414 nm. All assays were done on coded samples in duplicate and repeated at least twice. The intra-assay and interassay variation were less than 10%, as estimated with positive and negative control sera.

Prevalence rates of *H. pylori* positivity were calculated according to gastric mucosa pathology, serum pepsinogen levels, and interview-derived demographic and lifestyle data. Associations between *H. pylori* positivity and risk of precancerous gastric lesions and serum pepsinogen levels also were measured by ORs. ORs adjusted for sex, age, cigarette smoking, and other dysplasia risk factors (5) were obtained by logistic regression techniques (9).

## Results

Serum *H. pylori* antibody positivity, serum pepsinogen, gastric pathology, and questionnaire data were available for a total of 2646 participants (78% of people screened), 1408 males and 1238 females. Among these individuals, almost all (98%) had CAG detected in at least one of the biopsy sites. The most advanced diagnosis was SG for 1.5%, mild CAG for 37.3%, severe CAG for 8.2%, IM for 32.5%, and dysplasia for 20.6%. As reported previously (4), the prevalence of the advanced lesions (IM and dysplasia) rose steadily with age. Males had a slightly higher prevalence of IM, a 1.6-fold higher rate of dysplasia, and a 3-fold higher rate of stomach cancer than females.

Overall, 72% of the individuals had positive IgG serum antibodies to *H. pylori*. The prevalence of *H. pylori* antibody positivity was slightly higher among females (75%) than males (68%), among those with no (74%) *versus* some schooling (71%), and among those with larger family size (73, 72, and 69% positivity for those with ≥6, 4–5, or ≤3 family members, respectively), but these differences were not statistically significant. *H. pylori* positivity also did not vary consistently by age or income. Furthermore, drinking water source was not significantly associated with *H. pylori* infection, although positivity was 88% among the small proportion who obtained water from ponds or ditches compared to 73% among persons whose water came from deep wells and 72% among those who

*Table 3* Prevalence of precancerous lesions according to H. pylori antibody positivity and serum pepsinogen I:II ratio

| *H. pylori* positivity | Pepsinogen I:II | *n* | % of subjects with | | | | |
|---|---|---|---|---|---|---|---|
| | | | SG | Mild CAG | Severe CAG | IM | Dysplasia |
| No | ≥10 | 377 | 3.7 | 57.6 | 3.2 | 24.1 | 11.4 |
| | 5–9.9 | 334 | 1.8 | 54.8 | 4.2 | 21.3 | 18.0 |
| | <5 | 57 | | 15.7 | 3.5 | 45.6 | 35.0 |
| Yes | ≥10 | 130 | 3.1 | 44.6 | 10.0 | 25.4 | 16.9 |
| | 5–9.9 | 1138 | 1.4 | 34.9 | 12.5 | 33.5 | 17.8 |
| | <5 | 683 | 0.4 | 20.4 | 6.0 | 42.3 | 30.9 |

used surface wells, the most common water source. *H. pylori* prevalence was not significantly related to cigarette smoking status or blood type, two risk factors for dysplasia in this population, but was mildly and significantly elevated (76 *versus* 70%; $P < 0.05$) among persons who ate *versus* those who did not eat sour pancakes.

Table 1 shows that the prevalence of *H. pylori* antibody positivity varied markedly by histological status. Positivity was lowest (55%) among those with SG, rose to 60% among those with mild CAG, and peaked at 87% among those with severe CAG, before falling below 80% with more advanced lesions. The ORs for each of the advanced lesions remained significantly increased after adjusting for water source as well as sex, age, cigarette smoking, and sour pancake consumption.

The prevalence of *H. pylori* IgG antibody positivity also was strongly associated with serum pepsinogen levels, particularly pepsinogen II (Table 2; analyses for this table were based on 2719 individuals with both *H. pylori* and pepsinogen data). Among persons with relatively low (<7.5 μg/liter) pepsinogen II levels, only 30% were *H. pylori* antibody positive, but 87–91% of those with pepsinogen II levels of 12.5 μg/liter or greater were positive. Table 3 shows the prevalence of the precancerous lesions according to *H. pylori* positivity and pepsinogen I:II ratio, shown elsewhere and in this population to be a marker for advanced gastric lesions (6). As indicated in the Table 3, the pepsinogen ratio was a clear predictor of advanced gastric lesions, with considerably higher percentages of IM and dysplasia among subjects with pepsinogen I:II ratios below 5, whereas the added information on *H. pylori* positivity did not measurably enhance prediction base on the pepsinogen index alone.

## Discussion

This large population-based cross-sectional survey in an area with one of the highest rates of stomach cancer in the world enabled assessment of the relation between *H. pylori* infection, as determined by serum IgG antibodies, and histologically determined precancerous gastric lesions. The large majority of adults in the 14 randomly selected villages participated in the survey, so the findings reflect population patterns and not trends limited to subjects who have gastric complaints or are otherwise ill.

Because almost none of the 3433 individuals examined had entirely normal gastric biopsies (4), we were limited to comparing *H. pylori* antibody positivity among those with at least some mucosal abnormality. The serological detection of *H. pylori* bacteria has been confirmed in biopsies from a sample of individuals,[4] but we used serological rather than tissue diagnoses because only serological results were available on the entire cohort of study participants.

The prevalence of *H. pylori* positivity rose steadily from SG to mild CAG and then severe CAG (55, 60, and 87%, respectively), suggesting that *H. pylori* infection is particularly associated with the most severe lesion. The prevalence rates of *H. pylori* infection were also elevated for IM (78%), again indicating that persistent infection may influence the transition to more advanced gastric lesions (10). However, the prevalence of *H. pylori* positivity for IM and dysplasia was lower than for CAG, which is consistent with reports from other populations that *H. pylori* does not thrive as well when gastric lesions become more advanced (11–13). Recent studies indicate that cagA strains produce several inflammatory cytokines, causing more intense gastritis (14), but we did not have strain-specific *H. pylori* data in this population.

The modes of transmission of *H. pylori* infection are unknown, although it has been suggested that *H. pylori* may be spread by the fecal-oral route (1). In developing countries, *H. pylori* infection is often acquired in childhood. In a survey in southern China, antibody positivity was found in up to 31% of children ages 1–5, then rose through adolescence and early adulthood (15). Our data pertained only to adults, but in a separate survey using $^{13}$C-area breath test similar method, we found that among 98 children in one village in this county, *H. pylori* prevalence rates were as high as 50% at ages 3–4 and over 70% at ages 7–12. Therefore, in our study population, most *H. pylori* infections arise in early childhood, with little additional acquisition at older ages. In southern China, the prevalence of infection was higher in urban than in rural areas, consistent with reports that domestic crowding may be a risk factor, and we found an increasing rate of *H. pylori* positivity with increasing family size. (16–19). In Peru, it has been reported that water source is related to infection with *H. pylori* (19). Our findings also revealed that antibody levels varied by source of drinking water, with persons who drank relatively unclear water (ditch or pond) having a higher positivity rate, but the differences were not statistically significant. In contrast to others (20), we found no clear link between *H. pylori* infection and educational level or family income, but the variation in socioeconomic indicators among the rural villagers was quite limited.

We have reported previously (6) that serum pepsinogen levels were related to risk of precancerous lesions in Linqu. The present analysis shows that *H. pylori* serum antibody positivity was closely related to levels of serum pepsinogen levels, particularly pepsinogen II, even after controlling for age, sex, and gastric pathology. Serum pepsinogen have also been found to be correlated with *H. pylori* antibodies among children in Costa Rica and randomly selected adults in Italy (20, 21). In addition,

[4] D. G. Frank, L. Zhang, J. Y. Li, W. C. You, L. Zhao, W. D. Liu, C. Rabkin, G. I. Perez-Perez, M. J. Blaser, and W. J. Blot. Diagnosis of *Helicobacter pylori* infection in a Chinese population at high risk of stomach cancer: comparison of two serologic tests *versus* histology, submitted for publication.

pepsinogen II levels have been reported to markedly diminish following eradication of *H. pylori* infection (22), suggesting that serum pepsinogen levels can be altered as a consequence of infection. Serum pepsinogen, particularly low pepsinogen I:II ratios, and *H. pylori* antibody positivity represent markers of increased stomach cancer risk (23, 24). We hoped to measure their independent effects upon severe CAG, IM, or dysplasia, but were hindered by the strong correlation between pepsinogen levels and *H. pylori* positivity. Using both markers did not enhance our ability to discriminate among the more severe gastric lesions when compared to the pepsinogen ratios alone. In a recent survey of British patients who received gastroscopic examinations and had a very low pepsinogen ratio (I:II $< 1.5$), all six who were *H. pylori* antibody positive also had CAG, compared to 1 of 3 who were *H. pylori* negative, but the small numbers limit generalization of the findings (24). In the United States, *H. pylori* positivity and gastric atrophy (as measured by low pepsinogen I) have been reported to be independently associated with risk of stomach cancer (22).

In conclusion, an increased risk of severe CAG and more advanced precancerous gastric lesions was associated with *H. pylori* antibody positivity in a Chinese population at exceptionally high risk of stomach cancer. The prevalence patterns indicate that *H. pylori* infection may enhance primarily the transition from SG to mild and then severe CAG, consistent with an important role during the early stages of gastric carcinogenesis. Further evaluation of the determinants of *H. pylori* infection is planned in this high-risk population along with an assessment as to whether antibacterial therapy can inhibit CAG or its progression to more advanced precancerous lesions.

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