

Helicobacter pylori Prevalence and CagA Status Among Children in Two Counties of China with High and Low Risks of Gastric Cancer

WEI-CHENG YOU, MD, LIAN ZHANG, MD, KAI-FENG PAN, MD, JI JIANG, MD, PHD, YUN-SHENG CHANG, MD, GUILLERMO I. PEREZ-PEREZ, PHD, WEI-DONG LIU, MD, JUN-LING MA, MD, MITCHELL H. GAIL, MD, PHD, MARTIN J. BLASER, MD, JOSEPH F. FRAUMENI, JR, MD, AND GUANG-WEI XU, MD

BACKGROUND: Studies in adult populations in selected countries with widely varying rates of gastric cancer have shown a weak correlation between gastric cancer mortality rates and the prevalence of CagA+ strains of *H. pylori*. However, only limited data are available in ethnically homogenous populations with varying rates in the same region.

METHODS: We compared the prevalence of *H. pylori* in general and of CagA+ strains in particular among children in Shandong Province, China in areas at high (Linqu County) and low risk (Cangshan County) of gastric cancer. *H. pylori* status among children aged 3 to 12 years was determined by ¹³C-UBT, and CagA status was determined by enzyme-linked immunosorbent assay (ELISA). Because of the difficulty in obtaining blood from young children aged 3 to 4 years and from some children aged 5 years, CagA status was determined among part of children 5 years old and children 6 to 12 years old.

RESULTS: Among 98 children aged 3 to 12 years in Linqu, 68 (69.4%) was *H. pylori*-positive, as compared with 29 (28.7%) among 101 children in Cangshan. Among children positive for ¹³C-UBT, the proportion of the CagA+ strains were identified was 46 (88.5%) of 52 in Linqu and 13 (81.3%) of 16 in Cangshan, respectively.

CONCLUSIONS: The prevalence of *H. pylori* was nearly three times higher among children in Linqu than in Cangshan, which may contribute to the large differential in gastric cancer rates for two neighboring populations in Shandong Province.

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KEY WORDS: *H. pylori*, CagA, Children, Garlic, Gastric Cancer.

INTRODUCTION

The presence of CagA+ strains of *Helicobacter pylori* (*H. pylori*) in the human stomach may be associated with an increased risk for chronic atrophic gastritis and, possibly, gastric cancer (1-4). However, cross-sectional studies in adult populations in five countries with varying mortality rates for gastric cancer yielded only a weak association of gastric cancer and mortality rates with the prevalence of CagA+

strains of *H. pylori* (5). Only limited data are available on ethnically homogeneous populations in a particular region with varying rates, particularly with respect to early-life infection, which is suspected of playing an important initiating role in populations at high risk for gastric cancer (1,2,6). Linqu County in Shandong Province, China has one of the highest gastric cancer mortality rates in the world (70/10⁵ males and 25/10⁵ females per year), along with a high prevalence of precancerous lesions, including intestinal metaplasia (7). In contrast, Cangshan County, less than 200 miles away, has gastric cancer mortality rates that are much lower (5/10⁵ males and 3/10⁵ females per year), and the prevalence of intestinal metaplasia also is low (7). Linqu and Cangshan counties are both rural areas with a population of approximately 1 million in Shandong Province. The two counties have similarly ethnic, cultural, and socioeconomic characteristics except that Cangshan County is one of the world's leading agricultural producers of garlic (garlic production: 900,000 tons per year or 806 kg per person per year in 1996). Previously, we found that the prevalence of *H. pylori* infection was 73.7% among adults aged 35 to 64 in Linqu County, significantly higher than

From the National Cancer Institute (W-CY,MHG,JFF), Bethesda, MD; Beijing Institute for Cancer Research and School of Oncology (LZ,K-FP,Y-SC,J-LM,G-WX), Peking University, Beijing, China; Peking Union Medical College (JJ), Beijing, China; Vanderbilt University Medical Center (GIP-P,MJB), Nashville, TN; and Linqu Public Health Bureau (W-DL), Linqu, China.

Address correspondences to: Dr. Wei-cheng You, Division of Cancer Epidemiology and Genetics, National Cancer Institute, EPS Room 8030, Bethesda, MD, USA 20892-7244.

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that in Cangshan County (59.9%) (8). One limitation of the previous study was that we had no information on the prevalence of *H. pylori* among children in the two areas, because *H. pylori* infection in the childhood might have an important role in the risk of gastric cancer and its precursor. In this study, we compared the prevalence of *H. pylori* in general and of CagA+ strains in particular, among children aged 3 to 12 in these two counties.

MATERIALS AND METHODS

One village was selected at random from Linqu County and one from Cangshan County. The names of all children 3 to 12 years old were transcribed from the village resident roster, and 10 randomly selected children in each of the 10 single year categories aged 3 to 12 from 291 children in the village in Linqu County and 350 children in the village in Cangshan County were invited to participate in tests to detect the prevalence of *H. pylori* and of CagA+ strains. Eighty percent of the children came from one child family (three persons per family) in two counties. The study was approved by the Institutional Review Board of both the National Cancer Institute and the Beijing Institute for Cancer Research (BICR). All children had an informed consent form signed by their parents. *H. pylori* status was determined by the ¹³C-urea breath test (¹³C-UBT) and the CagA+ strains by the enzyme-linked immunosorbent assay (ELISA).

All children fasted overnight, and baseline samples of exhaled CO₂ were collected. Each child consumed 120 ml of a sweet starch paste to delay gastric emptying and then drank 10 ml of cold water containing 60 mg ¹³C-urea (> 99%) (Baylor Medical College, USA). Exhaled CO₂ was collected with the sampling tube 30 minutes later. The gas then was purified via a system for ¹³C analysis on an automated breath ¹³C analyzer (ABCA) (Europa Scientific). Any ¹³CO₂ concentration that exceeded the baseline pre-¹³C-urea breath concentration by more than six parts per 1000 was regarded as positive for *H. pylori* (9).

Details of the serologic assay for identifying CagA strains of *H. pylori* are described elsewhere (1). Briefly, approximately 5 ml of blood was collected from part of children aged 5 and children aged 6 to 12 years. Serum was separated and aliquoted, stored immediately at -20°C, and then stored at -70°C. Serum IgG antibody concentrations against CagA were measured using an ELISA test with a CagA recombinant antigen, as described in (1). A subject was determined to be positive for CagA if the ELISA absorbance reading for IgG was above 0.3, a cutoff based on examination of the distribution of values in a group of uninfected people and reference sera. All assays were done on coded samples in duplicate and repeated at least twice. The intra-assay and interassay variations were less than

10%, as estimated from positive and negative control sera. The chi-square test was used to evaluate the statistical significance of differences in the prevalence of *H. pylori* and CagA positivity among several subgroups.

RESULTS

H. pylori status was determined by ¹³C-UBT on 98 children (54 boys and 44 girls) in Linqu County and 101 children (53 boys and 48 girls) 3 to 12 years old in Cangshan County, respectively. Among children in Linqu, 68 (69.4%) were ¹³C-UBT-positive compared with 29 (28.7%) in Cangshan ($p < 0.01$). Figure 1 shows the number of children at age groups of 3 to 4, 5 to 6, 7 to 8, 9 to 10, and 11 to 12, and age-specific prevalence of *H. pylori* with the range of one standard deviation in Linqu and Cangshan counties. In Linqu, the prevalence of *H. pylori* rose from 52.6% at 3 to 4 years of age to 85.0% at 9 to 10 years of age before dropping to 66.7% at age 11 to 12 years. In contrast, the prevalence of *H. pylori* in Cangshan was persistently low (less than 30%) from 3 to 4 to 11 to 12 years of age (one-tailed trend test $p = 0.53$).

A total of 52 among 68 children in Linqu and 16 among 29 children in Cangshan with positive ¹³C-UBT tests were available for CagA tests. The seroprevalences of CagA+ strains among children with positive ¹³C-UBT tests were 46/52 = 88.5% in Linqu and 13/16 = 81.3% in Cangshan, indicating that a high proportion of *H. pylori* strains were CagA+ in both areas. However, the over-all both seroprevalences of CagA+ strains and positive ¹³C-UBT tests was 46/70 = 65.7% in Linqu and 13/59 = 22.0% in Cangshan, respectively (Table 1).

DISCUSSION

This survey in Shandong Province, China, revealed that the prevalence of *H. pylori* in children in Linqu, a county with an exceptionally high risk of gastric cancer, was more than twice as high as the prevalence in Cangshan, a nearby county with a comparatively low risk. The *H. pylori* prevalence rate rose from ages 3 to 10 among children in Linqu but remained low in Cangshan. In Linqu, more than half of the children had acquired *H. pylori* by 3 to 4 years of age, a prevalence similar to that reported in Peru and Colombia (10,11), where the rates of gastric cancer are elevated also, suggesting that early childhood acquisition of *H. pylori* may increase the risk of developing gastric cancer (12).

Our previous studies of adults participating in a gastric screening program in these areas revealed that the prevalence and severity of precancerous lesions were much greater in Linqu than in Cangshan (7). In Linqu, *H. pylori* has been identified as a risk factor for chronic atrophic gastritis and intestinal metaplasia (13). In addition, a recent follow-up

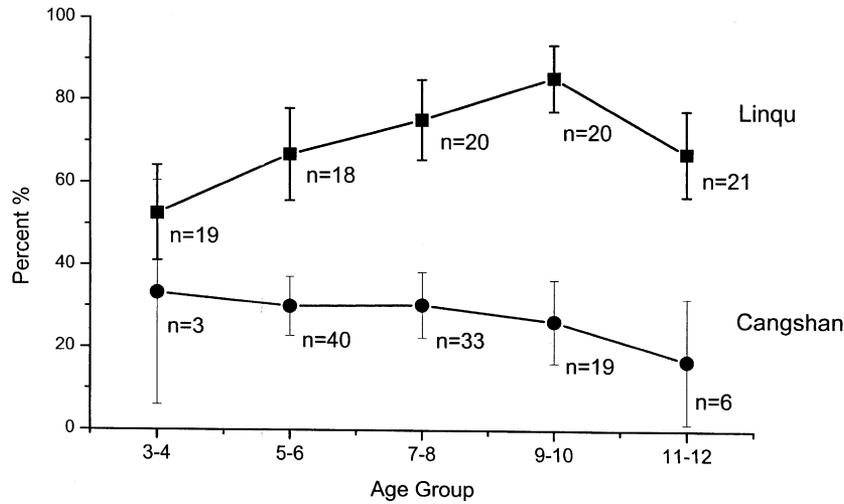


FIGURE 1. Prevalence of *H. pylori* Among Children in Linqu and Cangshan Counties

study in Linqu indicated that *H. pylori* positivity at baseline increases the risk of progression to dysplasia and subsequent gastric cancer (14). The significantly higher prevalence of *H. pylori* among children in Linqu than in Cangshan may explain why the gastric atrophy arises earlier and is more extensive and severe in Linqu than in Cangshan.

H. pylori strains differ in their virulence, with CagA+ strains inducing a more intense gastritis and a greater risk of atrophic gastritis (1-4). Although the prevalences of *H. pylori* and precancerous gastric lesions among adults were higher in Linqu than in Cangshan (7,8), our present study showed that the great majority of ¹³C-UBT-positive children in both areas were CagA positive as well. The similar proportions of CagA positivity among those children in both counties with *H. pylori* suggest that it is difficult to distinguish a special role in advanced gastric lesions between the over-all prevalence of *H. pylori* and the prevalence of CagA+ *H. pylori* because both were higher in Linqu than that in Cangshan. In a recent study in Europe, subjects with CagA+ *H. pylori* infection had a lower pepsinogen I:II ratio, a potential marker for precancerous gastric lesions, than

subjects with CagA- infection (15). In addition, other cytotoxin strains, such as VacA s1 or m1, often co-expressed with CagA+, should be studied (3). Because it was difficult to draw the blood from young children, we missed the information on CagA status among 16 out of 68 children in Linqu and 13 out of 29 children in Cangshan. Because the prevalence of CagA+ *H. pylori* infection in children of ¹³C-UBT-positive are high (81 to 89%), the missing information in part of children with ¹³C-UBT-positive might not substantially influence our findings.

It is not clear why the *H. pylori* prevalence curve has an unusual pattern among children in this garlic farming village in Cangshan, which is consistently lower than in Linqu. It is noteworthy that adult residents of this village consume an average of 27 grams (~ 10 to 13 cloves) of fresh garlic per day as compared with 2.7 grams (~ 1 clove) in Linqu (7,16). Because garlic is the major vegetable consumed in this area, the high intake fresh garlic by children may contribute to the lower *H. pylori* prevalence in this village in Cangshan. Garlic can inhibit the growth of *H. pylori* in vitro (17), and an inverse association has been found in Cangshan between garlic intake and *H. pylori* positivity (14).

We used only the ¹³C-UBT assay to determine *H. pylori* status among very young children. Concerns may be raised about the comparability of ¹³C-UBT status, which measures the current infection, with the ELISA assay used in classifying CagA status in children, which measures the past or current infection. However, the concordance between the ¹³C-UBT and ELISA results was 115/129 = 89.1%, and the sensitivity and specificity of the ELISA assay compared to the ¹³C-UBT "gold standard" were 56/65 = 86.2% and 59/64 = 92.2%, respectively (see Table 1). Another limitation was the relatively small sample size. Large confidence intervals for age-specific prevalence were observed in both regions, and in Cangshan the proportion of CagA positive children was based on only 16 *H. pylori* positive children.

TABLE 1. *H. pylori* CagA status among children aged 5 to 12 years in Linqu and Cangshan

		ELISA assays		Total
		CagA+	CagA-	
Linqu				
¹³ C-UBT	+	46	6	52
<i>H. pylori</i>	-	4	14	18
Total		50	20	70
Cangshan				
¹³ C-UBT	+	13	3	16
<i>H. pylori</i>	-	1	42	43
Total		14	45	59

Because the prevalence of *H. pylori* infection in children was nearly three times higher in Linqu than in Cangshan, those limitations are unlikely to have substantially influenced our results.

In conclusion, the prevalence of *H. pylori* among children was nearly three times as high in Linqu as in Cangshan, two neighboring counties in Shandong Province, which may contribute to the substantial difference in the risk of developing gastric cancer in these populations.

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