Association between \textit{Helicobacter pylori} Infection and Pathological Changes in the Gastric Mucosa in Chinese Children

Yi Yu$^1$, Lin Su$^1$, Xinqiong Wang$^1$, Xiaojin Wang$^2$ and Chundi Xu$^1$

\section*{Abstract}

\textbf{Objective} \textit{H. pylori} infection in children has a high prevalence worldwide. The disease can cause progressive gastric mucosal inflammation, as verified in animal models. However, data from large-scale clinical studies are limited.

\textbf{Methods} We examined 1,634 Chinese children with upper gastrointestinal discomfort using endoscopy. The clinical and pathological data of the patients were analyzed.

\textbf{Results} A total of 524 (32.1\%) patients were infected with \textit{H. pylori}, and the prevalence of \textit{H. pylori} infection increased with age. The \textit{H. pylori}-infected patients exhibited a significantly higher prevalence of active inflammation (26.9\% vs. 4.1\%), lymphoid follicle formation (18.5\% vs. 4.6\%) and marked lymphocyte infiltration (19.7\% vs. 5.6\%). The \textit{H. pylori}-infected patients also exhibited a significantly higher prevalence of moderate to marked chronic superficial gastritis (41.9\% vs. 9.2\%) and moderate chronic atrophic gastritis (21.7\% vs. 2.6\%) than the uninfected patients (p<0.01).

\textbf{Conclusion} \textit{H. pylori} infection is associated with the degree of gastric mucosal inflammation and the severity of different types of chronic gastritis.

\textbf{Key words:} \textit{Helicobacter pylori}, gastritis, gastric mucosa, histopathology, child

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\section*{Introduction}

Approximately half of the global population is infected with \textit{Helicobacter pylori} (1). \textit{H. pylori} is typically acquired in early childhood and may persist for a lifetime if left untreated (2). \textit{H. pylori} infection is usually accompanied by gastritis that starts in childhood and progresses to severe inflammation (3). The infected gastric mucosa undergoes changes that may ultimately lead to atrophy, metaplasia and cancer at an older age (4). In animal models (5) and at the cellular/molecular level (6), researchers have studied the pathological changes that occur in patients with chronic gastritis and gastric cancer induced by \textit{H. pylori} infection. However, there is a lack of large-scale clinical studies of \textit{H. pylori}-infected children. Understanding these changes in \textit{H. pylori}-infected children of different ages may provide insight into the pathogenic processes/mechanisms of \textit{H. pylori}-induced diseases.

In this study, we analyzed pathological changes in 1,634 \textit{H. pylori}-infected Chinese children undergoing routine gastroscopic examinations at our hospital. We hoped to find an association between \textit{H. pylori} infection in children of different age groups and pathological changes in their gastric mucosa.

\section*{Materials and Methods}

\textbf{Ethics} This work was carried out in accordance with the Declaration of Helsinki of the World Medical Association and ap-
proved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine. Written consent was obtained from the guardians of all patients.

**Patients**

From January 2007 to December 2011, children who underwent upper gastrointestinal endoscopy at our department for upper gastrointestinal discomfort (e.g., abdominal distension, repeated abdominal pain, nausea, vomiting, belching, retrosternal pain and regurgitation) were included in this study. All of the patients had been difficult to diagnose using noninvasive examinations and cure with symptomatic treatment. The exclusion criteria included idiopathic inflammatory bowel disease, active gastrointestinal bleeding, a history of gastric surgery and the use of medications (H2 histamine blockers, antimicrobials, proton pump inhibitors, bismuth salts, nonsteroidal anti-inflammatory drugs, immunosuppressive drugs or steroids) within the previous four weeks. Following the criteria, a total of 1,634 children were included in this study. The patients were classified into four groups according to age: group A (≤3 years old, n=69), B (4-6 years old, n=313), C (7-10 years old, n=706) and D (11-18 years old, n=546).

**Methods**

The endoscopic examinations (Fujinon 400, Fujifilm, Tokyo, Japan) were performed by clinicians who specialized in pediatric upper gastrointestinal endoscopy. At least four biopsy specimens were collected during endoscopy: one from the corpus, one from the incisura angularis and two from the gastric antrum. One biopsy sample from the antrum was used for an immediate rapid urease test. All biopsies were examined independently by two pathologists who were blind to the patients’ information.

The tissue samples were fixed overnight in buffered formalin, embedded in paraffin and sectioned into 3-mm-thick slices. The slices were stained with hematoxylin/eosin and modified Giemsa, then studied using light microscopy. A patient was considered to be uninfected by *H. pylori* if no *H. pylori* was detected in any of the biopsy specimens and a rapid urease test was negative. Conversely, a patient was considered to be *H. pylori*-infected if *H. pylori* was identified in any sample and a rapid urease test was positive. If the patient had only one positive test result, he/she was excluded from the study.

The following variables were analyzed in each of the samples: chronic inflammation (the presence of mononuclear cells), the activity and severity of gastritis (infiltration by neutrophils), glandular atrophy and intestinal metaplasia. The ordinal variables were graded using a visual analogue scale to generate a score (0=absent, 1=mild, 2=moderate and 3=marked), following the visual analog scale of the updated Sydney system. The severity of the lesions was classified according to the degree of infiltration in a sample of the most severe lesion in each patient. Lymphoid aggregates with germinal centers were designated as lymphoid follicles. Glandular atrophy was defined as a loss of the appropriate glands. Intestinal metaplasia was defined as the formation of intestinalized glands only in the foveolar region of the gastric glands (incomplete intestinal metaplasia) or involving the entire length of the original glandular unit (complete intestinal metaplasia).

**Statistical analyses**

Categorical data are expressed as numbers (%). The Cochran-Armitage trend test was used to test for trends in the *H. pylori* proportion across the four levels of age. The significance of the following parameters was measured using the chi-square test for patients with *H. pylori* infection and those without *H. pylori* infection: the activity of inflammation, degree of inflammation, infiltration of inflammatory cells, detection of lymphoid follicles (FLs) and pathological findings of the gastric mucosa. A p<0.05 indicated statistical significance. The SAS 9.12 software package (SAS, Cary, NC, USA) was used for all statistical analyses.

**Results**

**Diagnosis and *H. pylori* infection rate**

In total, 1,634 children were evaluated in our study (M/F: 865/769), with a mean age of 8.98 years. Most of the patients were clinically diagnosed with non-atrophic gastritis, while atrophic gastritis was rare. Special forms of gastritis were found to be increased with age, and reflux gastritis accounted for the majority of cases. Regarding the endoscopic appearance, nearly 60% of the patients exhibited erythematous/exudative gastritis, while raised erosive gastritis and rugal hyperplastic gastritis were rare (Table 1).

A total of 524 patients (32.1%) were found to be infected with *H. pylori*. The prevalence of *H. pylori* infection increased with age (Table 1); the Cochran-Armitage test indicated that the differences between the groups were statistically significant. (Z=-2.58, p=0.0098).

**Inflammation activity in the different groups**

Inflammation activity was detected in 26.9% of the *H. pylori*-infected patients, which was significantly higher than the 4.1% observed in the uninfected patients (χ²=182.01, p<0.001). The data were analyzed separately for each group. In Groups B, C and D, the *H. pylori*-infected patients exhibited a significantly higher prevalence of active inflammation than the uninfected patients (p<0.01). Furthermore, among the *H. pylori*-infected patients, the prevalence of active inflammation showed a tendency to increase with age. No such trend was found in the uninfected patients (Table 2).

**Association between *H. pylori* infection and gastric lymphoid follicle formation**

Lymphoid follicles were detected in 18.5% of the *H. pylori*-infected patients, which was significantly higher than the rate observed in the uninfected patients (4.6%) (χ²=
had mild lymphocyte infiltration, 66.4% had moderate lymphocyte infiltration and 5.6% had marked lymphocyte infiltration. Among the 524 H. pylori-infected patients, 13.9% exhibited a significantly higher prevalence of severe lymphocyte infiltration than the uninfected patients (all p<0.05, Table 3).

**Inflammatory cell infiltration**

The H. pylori-infected patients had a significantly higher prevalence of mild and moderate neutrophil granulocyte infiltration in the gastric mucosa compared with that observed in the uninfected patients (26.3% vs. 3.8%; 0.6% vs. 0.4%; \( \chi^2 = 185.55; p<0.001 \)). In each group, the H. pylori-infected patients also exhibited a higher prevalence of lymphoid follicles compared with that observed in the uninfected patients (26.3% vs. 3.8%; 0.6% vs. 0.4%; \( \chi^2 = 185.55; p<0.001 \)). In each group, the H. pylori-infected patients also exhibited a significantly higher prevalence of severe lymphocyte infiltration than the uninfected patients (all p<0.05, Table 5).

**Association between H. pylori infection and the pathological findings of the gastric mucosa**

Among the 1,634 patients, 1,572 (96.2%) were diagnosed with chronic superficial gastritis and 62 (3.8%) were diagnosed with chronic atrophic gastritis. The H. pylori-infected patients exhibited a significantly higher prevalence of moderate (39.1% vs. 8.0%) and marked (2.8% vs. 1.1%) chronic superficial gastritis compared with the uninfected patients.
tritis or intestinal metaplasia were observed. Epidemiological surveys have found the prevalence of 
H. pylori infection to be 7-33% in Europe, 48-78% in South America, 37.5-66% in Asia and 87% in South Africa (10). The prevalence observed in the present study (32.1%) is lower than that reported in the inland area of China as well as in some developing countries (10, 11), likely for two reasons. First, the known risk factors for H. pylori infection are age, a low socioeconomic status, limited living space, sharing of beds, a low parent education level, pollution of daily used water and H. pylori infection in family members (especially the mother) (12-14). Shanghai is one of the most developed cities in China, and its living conditions, parental education levels and hygienic conditions are comparatively good. Consequently, the risk of H. pylori cross-infection is low in Shanghai. Second, some patients may have taken antibiotics or proton pump inhibitors before the endoscopic ex-
amination, which potentially produced false-negative results (15), as demonstrated by the detection of IgG to H. pylori in the serum samples of some apparently ‘uninfected’ patients. Additionally, we found that the prevalence of H. pylori infection increased with age, confirming that H. pylori is first acquired primarily in early childhood and may persist for a lifetime if left untreated and that the accumulated risk of H. pylori infection increases with age.

In each age group, the H. pylori-infected patients also had a higher prevalence of active inflammation than the uninfected patients. In Group A (<3 years old), the H. pylori-infected patients had a higher prevalence of active inflammation than the uninfected patients; however, this difference was not significant, likely due to the insufficient statistical power resulting from the small samples. In the H. pylori-
infected patients, the prevalence of active inflammation showed a trend of increasing with age, which was not found in the uninfected patients. Our findings suggest that, in all age groups, H. pylori infection is closely associated with the occurrence of active gastric mucosal inflammation.

The normal gastric mucosa contains no lymphoid follicles. The formation of lymphoid follicles results from the host immune response following H. pylori infection. The lymphatic system in children is active. As a chronic antigen, H. pylori can stimulate the gastric mucosa to elicit specific immune responses. In the early stage of the immune response, inflammatory cell infiltration primarily involves neutrophil granulocytes. The subsequent chronic stage primarily involves the infiltration of lymphocytes, which aggregate into germinal centers and form lymphoid follicles. In the current study, the H. pylori-infected patients had a higher prevalence of lymphoid follicle formation than the uninfected patients, although the prevalence remained moderate in both groups. This finding confirms that lymphoid follicle formation is a highly specific but not sensitive indication of H. pylori infection in children (16). Kon et al. also observed that the detection rate of nodular gastritis is positively associated with the pathological score of H. pylori-related gastritis (17). Indeed, lymphoid aggregates and follicles are more

### Table 5. Degree of Lymphocyte Infiltration in H. pylori-infected and Uninfected Patients

<table>
<thead>
<tr>
<th>Group</th>
<th>H. pylori-infected</th>
<th>Uninfected</th>
<th>( \chi^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Moderate</td>
<td>Marked</td>
<td>Mild</td>
</tr>
<tr>
<td>A</td>
<td>1(5.9%)</td>
<td>11(64.7%)</td>
<td>5(29.4%)</td>
<td>14(26.9%)</td>
</tr>
<tr>
<td>B</td>
<td>13(15.3%)</td>
<td>62(72.9%)</td>
<td>10(11.8%)</td>
<td>55(24.1%)</td>
</tr>
<tr>
<td>C</td>
<td>28(12.1%)</td>
<td>154(66.4%)</td>
<td>50(21.5%)</td>
<td>73(15.4%)</td>
</tr>
<tr>
<td>D</td>
<td>31(16.3%)</td>
<td>121(63.7%)</td>
<td>38(20.0%)</td>
<td>57(16.0%)</td>
</tr>
</tbody>
</table>

### Table 6. Occurrence and Severity of Chronic Superficial Gastritis in H. pylori-infected and Uninfected Patients

<table>
<thead>
<tr>
<th>Group</th>
<th>H. pylori-infected</th>
<th>Uninfected</th>
<th>( \chi^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild</td>
<td>Moderate</td>
<td>Marked</td>
<td>Mild</td>
</tr>
<tr>
<td>A</td>
<td>10(71.4%)</td>
<td>4(28.6%)</td>
<td>0(0%)</td>
<td>45(93.75%)</td>
</tr>
<tr>
<td>B</td>
<td>50(61.0%)</td>
<td>29(35.4%)</td>
<td>3(3.7%)</td>
<td>208(92.9%)</td>
</tr>
<tr>
<td>C</td>
<td>126(57.3%)</td>
<td>91(41.4%)</td>
<td>3(1.4%)</td>
<td>418(91.7%)</td>
</tr>
<tr>
<td>D</td>
<td>105(56.8%)</td>
<td>72(38.9%)</td>
<td>8(4.3%)</td>
<td>302(88.0%)</td>
</tr>
</tbody>
</table>

(\( \chi^2 =235.30, p<0.001 \)). In each group, the H. pylori-infected patients exhibited a significantly higher prevalence of moderate and marked chronic superficial gastritis than the uninfected patients (Table 6). The H. pylori-infected patients also exhibited a higher prevalence of chronic atrophic gastritis compared with the uninfected patients (4.4% vs. 3.5%), although both groups were in the minority. In particular, the prevalence of moderate chronic atrophic gastritis was significantly higher in the H. pylori-infected patients than in the uninfected patients (21.7% vs. 2.6%). Additionally, the uninfected patients predominantly presented with mild chronic atrophic gastritis compared with the H. pylori-infected patients (97.4% vs. 78.3%; \( \chi^2 =6.09; p=0.014 \)). No marked chronic atrophic gastritis or intestinal metaplasia was observed.

### Discussion

H. pylori infection is prevalent in China, partly due to the traditional eating style (sharing food from the same plates) observed in the country. A survey of children in Gansu Province (China) conducted in 2009 revealed a prevalence of H. pylori infection of 72.3% (8) compared with a prevalence of only 13.1% in children in Hong Kong (9). Recent epidemiological surveys have found the prevalence of H. pylori infection to be 7-33% in Europe, 48-78% in South America, 37.5-66% in Asia and 87% in South Africa (10). The prevalence observed in the present study (32.1%) is lower than that reported in the inland area of China as well as in some developing countries (10, 11), likely for two reasons. First, the known risk factors for H. pylori infection are age, a low socioeconomic status, limited living space, sharing of beds, a low parent education level, pollution of daily used water and H. pylori infection in family members (especially the mother) (12-14). Shanghai is one of the most developed cities in China, and its living conditions, parental education levels and hygienic conditions are comparatively good. Consequently, the risk of H. pylori cross-infection is low in Shanghai. Second, some patients may have taken antibiotics or proton pump inhibitors before the endoscopic ex-

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prevalent in infected patients and decrease following the eradication of the infection (18). Our results also showed that the prevalence of *H. pylori*-infected patients decreases with age.

Although it is noninvasive, *H. pylori* can release antigens to stimulate various cells to produce inflammatory chemokines. These chemokines induce infiltration of the gastric mucosa by immune cells (neutrophil granulocytes, lymphocytes, macrophages) and thus elicit an inflammatory response in the mucosa (19, 20). In our study, the *H. pylori*-infected patients showed a significantly higher level of infiltration of neutrophil granulocytes (a sign of active inflammation) and lymphocytes (a sign of chronic inflammation) in the gastric mucosa compared with the uninfected patients. Researchers from Brazil (11), Japan (21) and Greece (22) have also reported that *H. pylori* infection is associated with an intense inflammatory response in the gastric mucosa. In the current study, the *H. pylori*-infected patients had more mild to moderate infiltration of neutrophil granulocytes and moderate to marked infiltration of lymphocytes than the uninfected patients. This difference suggests that *H. pylori* infection is closely associated with the activity and severity of gastric mucosal inflammation. Previous studies have shown that the eradication of *H. pylori* can reduce the severity of inflammation in patients with chronic gastritis (23), thus corroborating our findings.

In our study, there was no marked infiltration of neutrophil granulocytes and only few cases of marked infiltration of lymphocytes in both the *H. pylori*-infected and uninfected patients. This may be due to the unique immune response observed in children that resulted in limited polymorphonuclear neutrophil/monocyte infiltration of the gastric mucosa and maintained epithelial integrity (16, 24). There were no patients with a normal gastric mucosa in our study. This can be explained by our inclusion criteria: only symptomatic children were selected. We did not find that the severity of gastric mucosal inflammation increases with age. Theoretically, the severity of gastric mucosal inflammation may be correlated with the course of infection, which is unknown in such a retrospective survey. Therefore, we believe that providing further follow-up of this cohort is essential in order to clarify the relationship between the course of *H. pylori* infection and the severity of gastric mucosal inflammation.

In our study, a significantly higher proportion of *H. pylori*-infected patients had moderate/severe chronic superficial gastritis and moderate chronic atrophic gastritis compared with the uninfected patients. These differences suggest that *H. pylori* infection is closely associated with the type and severity of chronic gastritis. Generally, the patients in the present study had a low prevalence of chronic atrophic gastritis, and no patients had severe chronic atrophic gastritis or intestinal metaplasia. Researchers from different countries have reported a varied incidence of gastric atrophy (0-72%) and intestinal metaplasia (0-21%) in children (11, 25-28). Genetic and environmental factors other than *H. pylori* infection may explain the striking differences.

Hoepler et al. also observed that mucosal atrophy and intestinal metaplasia may develop over time after *H. pylori* infection (29). Vannella et al. studied adults with atrophic gastritis and observed reversal of atrophy in 50% of the patients 2-8 years after the eradication of *H. pylori* (30). These results and our findings suggest that *H. pylori* infection and its course are associated with the development of chronic atrophic gastritis.

In Group A (<3 years old), the *H. pylori*-infected patients showed a trend toward more severe neutrophil granulocyte infiltration, a higher prevalence of active inflammation, a higher positive rate of lymphoid follicles and more severe chronic superficial gastritis than the uninfected patients. However, these differences were not statistically significant. Additionally, in each age group, there were no significant differences in the rate of chronic atrophic gastritis between the *H. pylori*-infected patients and uninfected patients. These findings are likely due to the limited sample size of patients in Group A and those with chronic atrophic gastritis in this study, resulting in insufficient statistical power.

In conclusion, we found a prevalence of *H. pylori* infection of 32.1% among children admitted to our hospital and a tendency of an increasing prevalence with age. We also found that the *H. pylori*-infected patients had a significantly higher prevalence of inflammatory cell infiltration, active inflammation and lymphoid follicles than the uninfected patients. *H. pylori* infection was also closely associated with the severity of different types of chronic gastritis. Therefore, we suggest that children with repeated upper gastrointestinal discomfort should be examined for *H. pylori* infection and, if confirmed, be administered anti-*H. pylori* treatment as early as possible to prevent further progression of injury.

The authors state that they have no Conflict of Interest (COI).

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