ALIMENTARY TRACT AND PANCREAS

Prevalence of *Helicobacter pylori* in southern Indian controls and patients with gastroduodenal disease

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Abstract The spiral organism *Helicobacter pylori* has been causally implicated in the genesis of various gastroduodenal diseases. Since these diseases are common in southern India, this study was undertaken to determine the prevalence of *H. pylori* in the gastric mucosa of asymptomatic adults and patients with various gastroduodenal diseases. *H. pylori* was detected in the gastric mucosa of 25 of 30 (83.3%) normal volunteers. Prevalence rates in the disease groups were also high, and included 38 of 41 patients with duodenal ulcer (92.6%), 13/16 with gastric ulcer (81.3%), and 85/119 subjects (71.4%) with non-ulcer dyspepsia. Light microscopic examination of the gastric mucosa provided the best method of detecting *H. pylori*. *H. pylori* colonization was significantly associated with histological abnormalities, mainly chronic atrophic gastritis (147) and superficial gastritis (11), while only three of 161 *H. pylori* positive patients had histologically normal antral mucosa. Ultrastructural examination revealed changes in the apical complex of the gastric mucosal cells in response to bacterial adherence, with mucus depletion and cellular damage. Bacteria were also noted disrupting the tight junctions and entering the intercellular spaces. The high prevalence of *H. pylori* infection may explain the high incidence of gastritis, duodenal ulceration and gastric cancer in this population. However, in this population, the prevalence of infection in asymptomatic individuals was nearly as high as that in duodenal ulcer, underlining the need for further study to identify the differences in host response or bacterial pathogenicity that lead to the development of ulcer in only some individuals.

Key words: gastritis, *Helicobacter*, non-ulcer dyspepsia, peptic ulcer.

INTRODUCTION

There is considerable interest in the causal relationship between colonization of the gastric mucosa by *Helicobacter pylori*, a spiral, micro-aerophilic organism, and chronic atrophic gastritis, peptic ulcer disease and gastric cancer. There is a high prevalence in southern India of chronic gastritis (20%), peptic ulcer (6.5 per 1000) and gastric carcinoma (incidence 11.3 per 100 000) and the association of a bacterial infection with these diseases, which are of importance in public health, would have implication for strategies of control and therapy. There are only a few reports of *H. pylori* from developing countries that suggest a higher prevalence and an earlier age of acquisition of infection than in developed countries. This study was therefore designed to determine the prevalence of *H. pylori* in a variety of upper gastrointestinal diseases and in asymptomatic volunteers.

METHODS

A total of 206 individuals were enrolled in this study, of whom 176 were consecutive patients who underwent upper gastrointestinal endoscopy, for conditions other than gastric carcinoma and portal hypertension, as part of their routine clinical work-up. Thirty healthy, asymptomatic adult volunteers from the rural population around Vellore also underwent an upper gastrointestinal endoscopy after informed consent. Based on symptoms, clinical findings and endoscopic appearance, the 176 patients were divided into 91 patients with duodenal ulcer, 16 with gastric ulcer, and 119 with non-ulcer dyspepsia. The age range and median age are shown in Table 1.

In the first 100 patients studied, a variety of techniques were used to standardize the methodology. At endoscopy, one biopsy bit from the body and two from the antrum were processed for histologic examination after fixation in Bouin's solution and paraffin embedding. Sections were stained with haematoxylin-eosin (H&E), Alcian Blue-PAS, Warthin Starry silver and a modified Giemsa stain. A rapid urease test was done on one antral biopsy bit in 1 mL ursus broth at room temperature and the test read at 2 h. One biopsy bit each from the body and antrum were cultured on freshly prepared Brain Heart infusion agar with 7-10% sheep blood and selected antibiotics. Colonies of *H. pylori* were identified by standard micro-molecular methods.
Table 1: Age range and median age of subjects in this study

<table>
<thead>
<tr>
<th>Group</th>
<th>Age range (years)</th>
<th>Median age (years)</th>
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<tbody>
<tr>
<td>Controls</td>
<td>21-51</td>
<td>30</td>
</tr>
<tr>
<td>Non-ulcer dyspepsia</td>
<td>12-70</td>
<td>34</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>20-71</td>
<td>45</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>24-54</td>
<td>37</td>
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In all further subjects, two biopsy pieces from the antrum were processed for histologic examination after staining with H&E and the modified Giemsa technique. All biopsies were coded and examined without knowledge of final clinical categorization by three pathologists (S.P., M.M., S.V.). Gastritis was histologically classified according to the Whitehead scheme. All biopsies from five patients who were positive for _H. pylori_ by rapid urease test and two controls were available for electron microscopic examination. These biopsies were fixed in 2% glutaraldehyde, post-fixed in osmium tetroxide and embedded in araldite. Areas for ultrathin sections were selected after examining survey sections (1 micron stained with toluidine blue) and examined in a Philips EM 201C after staining with aqueous uranyl acetate and lead citrate.

Statistical significance was determined with the Chi-squared test and, where applicable, the Spearman rank correlation test.

**RESULTS**

_H. pylori_ was detected in 84 of the first 100 patients with upper gastrointestinal symptoms. Histologic examination gave the maximum yield for _H. pylori_ (83 patients), with 49 patients in whom both antral and body biopsies were positive, 32 positive in antral biopsies only and two in the body only. Culture yielded only 30 positive biopsies, all of which were positive in histology and 28 in urease test. The urease test was positive in only 65 cases, in one of which histologic examination did not yield a positive diagnosis. A comparison of the different histologic staining techniques used showed that although the organism could be identified by H&E stain, identification was easier by special stains, especially when few organisms were present. The Warthin-Starry silver technique and the modified Giemsa method gave similar results, although the silver stain was technically more difficult and the control of deposits which could give rise to false positive results was a matter of concern. It was concluded that the modified Giemsa technique was the best stain for routine use and that antral biopsies gave the maximum yield. The addition of body biopsies and the urease test increased the positive diagnostic yield by only 3%

**Prevalence of _H. pylori_ in different diagnostic groups**

_H. pylori_ was detected in antral biopsies of 136 of the 176 patients (77.3%) and 25 of the 30 volunteer asymptomatic controls (88.3%). This difference is not statistically significant ($\chi^2 = 0.254$). The prevalence of 92.6% in patients with duodenal ulcer was also not statistically significantly different from that in the controls ($\chi^2 = 0.724$) or other groups of diagnosis (Table 2).

**Relationship of _H. pylori_ to histological grading of gastritis**

The prevalence of chronic atrophic gastritis was very high in antral biopsies (171, 206 subjects, 83%) while the change was found in only 8/100 (8%) gastric body biopsies (Table 3). The prevalence of _H. pylori_ was highest in antral biopsies with chronic atrophic gastritis (147/171, 86%). Forty-five of the gastric body biopsies were histologically normal while only 14 of the antral biopsies were normal.

In the 100 subjects where both gastric body and antral biopsies were available, the histologic changes in the gastric body were correlated with the prevalence of _H. pylori_ in both the gastric body and the antrum (Table 4). The prevalence of gastritis was highest in the gastric body when _H. pylori_ was present in both the sites.

**Ultrastructural changes associated with _H. pylori_ colonization**

In sections where _H. pylori_ were found in the vicinity of the gastric mucosal surface cells, there were prominent microvilli with expanded glycocalyx which appeared to entrap the organisms (Fig. 1). In other cells where the organisms were in the process of adherence to the cell, there was vesiculation and distortion of microvilli and loss of microvilli prior to adhesion. _H. pylori_ adhered to the cells either with the longitudinal axis parallel or at an angle to the cell membrane or end-on.

At the site of bacterial attachment the cell membranes had cup-like (end-on attachment) or sinuous (long axis attachment) indentations and occasional peduncle-like projections (Fig. 2). When a cell had large numbers of bacteria attached to it, the apical border showed dome-shaped bulging (Fig. 3). The mucus granules in the cells were depleted. Dilated rough endoplasmic reticulum, loss of secretory granules and increase in lysosomes were indicators of cell damage. In severely affected cells, there was nuclear and cytoplasmic lysis (Fig. 4). The bacteria were often seen to lie close to the intercellular junction and disrupt the tight junctions gaining entry into the intercellular spaces (Fig. 5). _H. pylori_ were not found within epithelial cells or in lamina propria cells although a few polymorphonuclear leukocytes in the lumen engulfed organisms.

**DISCUSSION**

This study documents the high prevalence of colonization of the gastric antrum (78%) and body (52%) by _H. pylori_ in the population of southern India where atrophic
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Table 2  Prevalence of H. pylori in different diagnostic groups.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>No. with H. pylori</th>
<th>% Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>30</td>
<td>25</td>
<td>83.3</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>41</td>
<td>38</td>
<td>92.6</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>16</td>
<td>13</td>
<td>81.3</td>
</tr>
<tr>
<td>Non-ulcer dyspepsia</td>
<td>119</td>
<td>85</td>
<td>71.4</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>161</td>
<td>78.1</td>
</tr>
</tbody>
</table>

The prevalence of H. pylori in the different diagnostic categories was not significantly different among the categories or compared to that in the normal control group.

Gastritis, peptic ulcer, and gastric carcinoma have a high prevalence. Such a high prevalence of infection in the general population in the present study is not surprising. In southern India, a high prevalence of colonization of the lumen and mucosa of the gastrointestinal tract by a variety of aerobic and anaerobic microbes has already been documented and the prevalence of Campylobacter pylori is known to be high in this population. Several other studies have also shown an association between H. pylori prevalence and low socio-economic status and poor environmental hygiene. Prevalence studies in developed and developing countries using the detection of IgG antibodies against H. pylori showed that while in all countries there is an age-related increase in acquisition of the organism, it is much higher in tropical developing countries.

![Figure 1](image.png)

Apical border of surface mucus cell with H. pylori entrapped by the microvilli with prominent glycocalyx (x 29000).

Table 3  Relationship of H. pylori colonization to histological changes in gastric antral and body mucosa.

<table>
<thead>
<tr>
<th></th>
<th>Antral biopsies</th>
<th>Body biopsies</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>H. pylori +ve (%)</td>
</tr>
<tr>
<td>Normal histology</td>
<td>14</td>
<td>21.4</td>
</tr>
<tr>
<td>Superficial gastritis</td>
<td>21</td>
<td>52.3</td>
</tr>
<tr>
<td>Chronic atrophic gastritis</td>
<td>171</td>
<td>88.9</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>78.1</td>
</tr>
</tbody>
</table>

Table 4  Histologic appearance of gastric body mucosal biopsies.

<table>
<thead>
<tr>
<th>H. pylori present in body biopsy (n = 52)</th>
<th>H. pylori absent in body biopsy (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>H. pylori present in antral biopsies</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>(n = 82)</td>
</tr>
<tr>
<td>H. pylori absent in antral biopsies</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(n = 18)</td>
</tr>
</tbody>
</table>
The prevalence of *H. pylori* in 176 patients with upper gastrointestinal symptoms (77.3%) was not significantly different from that in 30 asymptomatic volunteer controls (83.3%). However, most (77.3%) of the controls had histologic evidence of gastritis, and the prevalence of *H. pylori* correlated well with histologic gastritis. The prevalence of the organism in antral biopsies correlated with the prevalence of atrophic gastritis in biopsies from the body of the stomach. Close association of the bacteria with...
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The cell surface with degenerative changes in the cells and formation of tight junctions were the major findings in histopathological examination which confirms earlier reports.1 19 Controversy exists over the role of H. pylori in symptomatic upper gastrointestinal disease,20-25 although it is widely believed that this organism is central to the development of duodenal ulcers. Volunteer studies, therapeutic trials and animal experiments suggest that H. pylori may be causally associated with gastritis and may be a factor in the causation and perpetuation of peptic ulcer.26-28 However, the high prevalence of this organism in asymptomatic volunteers reported here and in other studies29-37 suggests that there are either variations in the host response to this infection, or strain variations in the organism, which determine whether duodenal ulcers occur. In this respect, it is necessary to better understand the virulence and pathogenicity attributes of the organism.38 This is particularly so in view of the significant association between the prevalence of the organism and histologic evidence of antral gastritis. A variety of metabolic products and a cytotoxin have been described in H. pylori which may also be important in the pathogenesis. Further work is necessary to establish their role in disease states.

As mentioned above, histological gastritis is very often found in even asymptomatic subjects with H. pylori infection. This gastritis may be one reason for the high incidence of gastric cancer in this population. Such an association would have considerable preventive and therapeutic implications, but can be proved only by well-planned prospective epidemiologic studies. Our study suggests that antral biopsy stained with a modified Giemsa stain has a high diagnostic yield. However, this still involves a major invasive procedure and plans are under- way to further validate less invasive procedures in a topical population to conduct such studies.

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REFERENCES


