Helicobacter pylori in Dyspeptic Children and Adults: Endoscopic, Bacteriologic and Histologic Correlations

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Using different bacteriological (urease test, Gram staining and culture) and histological (Steiner staining and modified Giemsa staining) techniques, we searched for the presence of Helicobacter pylori in the gastric antrum of 200 dyspeptic Brazilian patients (106 females and 94 males aged 19 days to 81 years). The presence of bacteria was then correlated with the endoscopic and histological findings. H. pylori was present in 59.5% of the population studied. In Brazil, colonization occurs early, involving 37% of the dyspeptic population by 20 years of age. The presence of H. pylori in the gastric antrum was strongly associated with duodenal ulcer (P<0.001) and a normal endoscopic examination did not exclude the possibility of colonization of the gastric antrum by H. pylori. The most sensitive test was the preformed urease test (89%). We conclude that more than one diagnostic method should preferably be used for the detection of H. pylori and that the presence of H. pylori is closely correlated with active chronic gastritis (P<0.001).

Key words: Helicobacter pylori - gastric ulcer - duodenal ulcer - adults - children

Helicobacter pylori has been associated with gastritis, peptic ulcer, non-ulcerous dyspepsia and, more recently, has been considered as a potential risk factor for the development of gastric cancer (The Eurogast Study Group 1993). Despite these observations, however, the clinical significance of H. pylori continues to be obscure.

Many studies have been published in the international literature (Marshall & Warren 1984, Price et al. 1985, Von Wulffen et al. 1986, Lamouliatte et al. 1987, Itoh et al. 1987, Marcheggiano et al. 1987, Gnarpe et al. 1988), whereas information about this topic is scarce in Brazil (Coelho et al. 1987, Ferrari et al. 1989). Questions such as ethnic variations, incidence and associated digestive pathologies, especially in the younger age ranges, continue to be unexplained. On this basis, the objective of the present study was to determine the incidence of H. pylori in the gastric antrum of dyspeptic children and adults using different bacteriological and histological techniques, and to correlate the presence of bacteria with endoscopic and histological findings.

MATERIALS AND METHODS

Patients - The study was conducted on 200 consecutive patients with complaints related to the upper digestive tract who were referred to three endoscopy services located in the municipality of Rio de Janeiro for diagnosis. The sample consisted of 106 females and 94 males ranging in age from 19 days to 81 years. All the patients were inquired if they were taking antibiotics or bismuth compounds during ten days before the procedure.

Endoscopy and collection and transport of gastric biopsies - All patients or the persons responsible for them gave informed consent to participate in the study. All patients were examined after a fast of more than 6 hr. Children were examined with an Olympus GIF XP10 fibroscope under general inhalation anesthesia (halothane-nitrogen protoxide and oxygen). The adults were examined with an Olympus XQ fibroscope after anesthesia of the oropharynx by local spraying with 10% lidocaine hydrochloride and sedation with a combination of the ansiolytic

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drug diazepam (0-10 mg) and of the hypna-
algesic drug meperidine (50 mg) administered intravenously.

After judicious evaluation of the esophage-
gastroduodenal tract, four biopsy fragments of gas-
tric antral mucosa were taken 1 and 2 cm from the
pylorus of children and adults, respectively. The
fragments were immersed in the following solu-
tions: urea buffered with an indicator (0.05 ml)
for the presumptive test for the presence of H. py-
lori by the method of Hazell et al. (1987); 20%
glucose (0.5 ml), the transport medium for bacte-
riological analysis; 10% formalin, a histological
preserver (two fragments).

Bacteriology - The preformed urease test
(PFUT) was started in the endoscopy room and
monitored for 24 hr in order to observe urease ac-
tivity, revealed by the change in color of the pH
indicator. The transport medium containing the
biopsy fragment was maintained under refrigera-
tion at 4°C in an isothermal container and sent to
the laboratory for processing within less than 3
hr. The fragment was inoculated by rolling onto
the surface of the selective medium (Goodwin
et al. 1985) and incubated at 37°C in a jar with a
humid microaerophilic atmosphere (Gas Pak BBL
70304 envelope, an H2 and CO2 generator, with
no catalyst). After inoculation, a smear was ob-
tained from the same fragment for Gram bac-
terioscopy.

The jar was kept under observation for seven
days and was opened on the 3rd and 7th days. Growth was characterized on the basis of colony
morphology, Gram stain, and the production of
urease, catalase, and oxidase according to Czinn
et al. (1989).

Histology - Staining with haematoxylin and
eosin (H & E) was used for the diagnosis and class-
ification of gastritis (Warren 1983) and the meth-
ods of Steiner and Steiner (1949) and modified
Giemsa (Gray et al. 1986) were used for visualiza-
tion of spiral forms.

Statistical analysis - Data were analysed by the
chi-square test for the determination of associa-
tion between variables using contingency tables and
Yates correction when necessary.

RESULTS

Endoscopy of the esophagegastroduodenal tract
revealed as main diagnosis 62 normal examinations,
59 cases of chronic gastritis, 48 cases of peptic ulcers, 11 cases of duodenitis, 10 cases of
esophagitis, 5 cases of gastric erosion, and 5 mis-
cellaneous examinations.

The presence of H. pylori was determined by
bacteriological techniques (PFUT, Gram staining
and culture) and/or histological techniques (Steiner
staining and modified Giemsa staining) in the gas-
tric antrum of 119 patients (59.5%), with an inci-
dence of 67.8% in the population older than 20
years and an incidence of 37.0% in the under 20
group and a similar distribution among males an
females (Table I). H. pylori was detected in all age
ranges studied, except for that of less than 30 days
(Table II).

When we calculated the correlation between
endoscopic data and presence of H. pylori in the
gastric antrum, the microorganism was found to
be present in high percentages in most of the up-
der digestive pathologies diagnosed (Table III). H.
pylori was significantly associated with duodenal
ulcer (P<0.001; X2 = 15.79) and with gastric ulcer
(P<0.01; X12 = 6.66) but not with gastritis
(P>0.05; X2 = 0.02).

Comparison of the bacteriological and histo-
logical methods used to demonstrate the presence
of H. pylori (Table IV) showed a clear superiority
of the preformed urease test (89%). The rate ob-
tained by histological staining was similar to that
obtained by culture.

Histology of gastric biopsies stained with H &
E was highly correlated with the presence of H.
pylori in patients with active chronic gastritis
(91.4%) and less markedly correlated with chronic
gastritis (47.1%). Both correlations were statisti-
cally significant at the P<0.001 level (X2 = 83.72
for active chronic gastritis and X2 = 12.68 for
choronic gastritis), as shown in Table V.

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution of patients with <em>Helicobacter pylori</em> in the gastric antrum by age and sex</td>
</tr>
<tr>
<td>Population</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Adult Less than 20 years</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
TABLE II

Distribution of patients with Helicobacter pylori in the gastric antrum by age range

<table>
<thead>
<tr>
<th>Age range</th>
<th>No.</th>
<th>Present</th>
<th>%</th>
<th>Absent</th>
<th>%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>100.0</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 30 days - 1 year</td>
<td>1</td>
<td>25.0</td>
<td>3</td>
<td>16</td>
<td>75.0</td>
<td>4</td>
</tr>
<tr>
<td>&gt; 1-6 years</td>
<td>5</td>
<td>23.8</td>
<td>16</td>
<td>12</td>
<td>76.1</td>
<td>21</td>
</tr>
<tr>
<td>&gt; 6-12 years</td>
<td>10</td>
<td>45.4</td>
<td>12</td>
<td>2</td>
<td>54.5</td>
<td>22</td>
</tr>
<tr>
<td>&gt; 12-19 years</td>
<td>4</td>
<td>66.6</td>
<td>2</td>
<td>6</td>
<td>33.3</td>
<td>6</td>
</tr>
<tr>
<td>20-29 years</td>
<td>4</td>
<td>40.0</td>
<td>6</td>
<td>1</td>
<td>60.0</td>
<td>10</td>
</tr>
<tr>
<td>30-39 years</td>
<td>25</td>
<td>62.5</td>
<td>15</td>
<td>2</td>
<td>37.5</td>
<td>40</td>
</tr>
<tr>
<td>40-49 years</td>
<td>20</td>
<td>74.0</td>
<td>7</td>
<td>4</td>
<td>25.9</td>
<td>27</td>
</tr>
<tr>
<td>50-59 years</td>
<td>22</td>
<td>73.3</td>
<td>8</td>
<td>3</td>
<td>26.7</td>
<td>30</td>
</tr>
<tr>
<td>60-69 years</td>
<td>20</td>
<td>76.9</td>
<td>6</td>
<td>1</td>
<td>23.1</td>
<td>26</td>
</tr>
<tr>
<td>70-79 years</td>
<td>7</td>
<td>70.0</td>
<td>3</td>
<td>2</td>
<td>30.0</td>
<td>10</td>
</tr>
<tr>
<td>80-89 years</td>
<td>1</td>
<td>33.3</td>
<td>2</td>
<td>1</td>
<td>66.7</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>59.5</td>
<td>81</td>
<td>40.5</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

TABLE III

Correlation between endoscopic data and presence of Helicobacter pylori in the gastric antrum

<table>
<thead>
<tr>
<th>Endoscopic data</th>
<th>Population</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
<td>Less than 20 years</td>
</tr>
<tr>
<td>Normal</td>
<td>17/34 (45.9%)</td>
<td>7/25 (28.0%)</td>
</tr>
<tr>
<td>Chronic gastritis</td>
<td>30/46 (65.2%)</td>
<td>4/13 (30.7%)</td>
</tr>
<tr>
<td>Active duodenal ulcer</td>
<td>14/14 (100.0%)</td>
<td>1/1 (100.0%)</td>
</tr>
<tr>
<td>Healed duodenal ulcer</td>
<td>7/7 (100.0%)</td>
<td>1/2 (50.0%)</td>
</tr>
<tr>
<td>Active gastric ulcer</td>
<td>10/11 (90.9%)</td>
<td>0/1</td>
</tr>
<tr>
<td>Healed gastric ulcer</td>
<td>8/9 (88.8)</td>
<td>-</td>
</tr>
<tr>
<td>Active gastric/duodenal ulcers</td>
<td>2/2 (100.0%)</td>
<td>-</td>
</tr>
<tr>
<td>Healed gastric/duodenal ulcers</td>
<td>1/1 (100.0%)</td>
<td>-</td>
</tr>
<tr>
<td>Duodenitis</td>
<td>4/7 (57.1)</td>
<td>3/4 (75.0)</td>
</tr>
<tr>
<td>Gastric erosions</td>
<td>3/4 (75.0)</td>
<td>1/1 (100.0)</td>
</tr>
<tr>
<td>Esophagitis (grades II/III)</td>
<td>1/4 (25.0)</td>
<td>2/6 (33.3)</td>
</tr>
<tr>
<td>Gastric neoplasia</td>
<td>1/2 (50.0)</td>
<td>-</td>
</tr>
<tr>
<td>Gastric polyps</td>
<td>0/1</td>
<td>-</td>
</tr>
<tr>
<td>Gastric lymphnode hyperplasia</td>
<td>-</td>
<td>1/1 (100.0)</td>
</tr>
<tr>
<td>Hiatus hernia</td>
<td>1/1 (100.0)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>99/146 (67.8)</td>
<td>20/54 (37.0)</td>
</tr>
</tbody>
</table>

*: patients with H. pylori/total no. of patients (percentage)

DISCUSSION

When the sample studied was divided into populations older than 20 and younger than 20, the rates of H. pylori colonization were 67.8 (99/146) and 37.0% (20/50), respectively. The percentage obtained for the adult population, although slightly lower than that detected by other Brazilian investigators (Coelho et al. 1987, Ferrari et al. 1989), was much higher than that obtained in developed countries (Marshall & Warren 1984, Price et al. 1985, Von Wulffen et al. 1986, Lamouliate et al. 1987, The Eurogast Study Group 1993). This difference was more marked in the under 20 group (37.0%) for which Hill et al. (1986) and Drumm et al. (1987) detected rates of 15.7% (6/39) and 10.4% (7/67), respectively.

It should be pointed out that in Brazil there is early colonization of the gastric antrum, that probably occurs more frequently among preschoolers...
TABLE IV

Performance of the different methods used for the detection of *Helicobacter pylori* in gastric biopsies according to origin

<table>
<thead>
<tr>
<th>Method</th>
<th>Population</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult = 99*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Performed urease test</td>
<td>91</td>
<td>91.9</td>
</tr>
<tr>
<td>Gram staining</td>
<td>76</td>
<td>76.7</td>
</tr>
<tr>
<td>Steiner/modified Giemsa stainingb</td>
<td>62</td>
<td>62.6</td>
</tr>
<tr>
<td>Culture</td>
<td>60</td>
<td>60.6</td>
</tr>
</tbody>
</table>

* : total number of patients with *H. pylori*.  
b : Steiner staining was used for the initial 119 patients and detected *H. pylori* in 68% (51/75), whereas modified Giemsa staining was used for the remaining 81 patients and detected *H. pylori* in 54.5% of them (24/44).

TABLE V

Correlation between gastric biopsies stained with H & E and presence of *Helicobacter pylori* according to origin

<table>
<thead>
<tr>
<th>Histology data</th>
<th>Population</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt; 20 years</td>
<td>&lt; 20 years</td>
</tr>
<tr>
<td>Normal</td>
<td>4/27 (14.8*)</td>
<td>4/26 (15.3)</td>
</tr>
<tr>
<td>Chronic gastritis</td>
<td>19/40 (47.5)</td>
<td>6/13 (46.1)</td>
</tr>
<tr>
<td>Active chronic</td>
<td>76/79 (96.2)</td>
<td>10/15 (66.6)</td>
</tr>
<tr>
<td>gastritis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>99/146 (67.8)</td>
<td>20/54 (37.0)</td>
</tr>
</tbody>
</table>

* : patients with *H. pylori*/total no. of patients (percentage).

(23.8%) and schoolchildren (45.4%) and tends to increase with age, eventually reaching rates higher than 70%. The maintenance of a chronic status for consecutive years may represent a risk factor for the development of gastric cancer, as suggested by the Eurogast Study Group (1993).

When the endoscopic data of the present patients were correlated with the presence of *H. pylori* in the gastric antrum, the bacterium was found to be present in high percentages in most of the digestive pathologies diagnosed, particularly duodenal ulcers (100%) and gastric ulcers (83.3%), in agreement with data reported in the literature. In the under 20 population, the incidence of *H. pylori* was higher in duodenal pathologies (ulcer and duodenitis).

Endoscopic diagnosis of gastritis was not well correlated with the presence of *H. pylori* (65.2% in the over 20 population, 30.7% in the under 20 population, and 57.6% in the total sample), but the gastric mucosa is subjected to aggression of a different nature, such as alcohol and medications. In this respect, Brazilian rates (Coelho et al. 1987, Ferrari et al. 1989) are also higher than international rates (Marshall & Warren 1984, Itoh et al. 1987).

An interesting result was the presence of *H. pylori* in 38.7% of the patients with normal endoscopy, a rate lower than those reported by other Brazilian authors (Coelho et al. 1987, Ferrari et al. 1989) and by Marshall and Warren (1984) and Marchegiano et al. (1987), but higher than most of those reported by international investigators, possibly representing an early phase of evolving digestive pathology with still undetectable major macroscopic alterations.

Among the diagnostic methods used for the detection of *H. pylori*, the performed urease test had a higher positivity rate (89.0%), followed by Gram staining (73.9%), histological staining (63.0%) and culture (62.1%). The preformed urease test is quite safe since the inclusion of sodium azide prevents the multiplication of contaminants, only permitting the action of preformed urease (in a constitutive manner) on the substrate.

Gram staining is also a good method, but the use of the same biopsy fragment after inoculation for the preparation of the smear causes impover-
ishment of the material and a consequent reduction in the number of microorganisms, impairing the results of the test or leading to negative results.

Comparison of the histological staining techniques used showed that silver staining was more effective (68.0%, 51/75) than modified Giemsa (54.5%, 24/44), in agreement with data reported by Gustavsson et al. (1987).

The fact that the positivity rate of culture was lower than that obtained with the other methods may have been due to the excessive presence of contaminants, to an escape from the micro-aerophilic atmosphere by poor jar sealing, or in some cases to inhibition of the antimicrobial agents present in the selective medium.

It should be pointed out that the distribution of H. pylori in the gastric antrum may be heterogeneous or even focal (Lamouillette et al. 1987, Morris & Nicholson 1987), explaining the discrepant results obtained with different methods. Thus, it is recommended that the microorganisms be searched for in more than one biopsy and using several techniques, or at least two different methods. It is also important to certify that the patients are not using antimicrobials or bismuth compounds. We suggest the PFUT and either the Gram staining or the histological staining methods for routine.

All techniques employed, except culture, tended to be less effective for biopsies from the under 20 population. We propose that the smaller, more friable biopsy of infants and young individuals, with a thick mucous layer may explain this fact.

In the overall sample computation, histological analysis of the gastric biopsies (H & E) showed a close correlation of the microorganism with active chronic gastritis (91.4%) and a less marked correlation with chronic gastritis (47.1%). To justify the differences observed between the populations studied here or even between our data and those reported by others with respect to the presence of the bacteria in active chronic gastritis or in chronic gastritis, it is important to remember the experiments carried out on volunteers (Marshall et al. 1985, Morris & Nicholson 1987) who, after going through the acute phase with polymorphonuclear infiltrates in the gastric mucosa (active gastritis), developed chronic gastritis. Thus, the search for the bacteria may have occurred during distinct phases of colonization by the microorganism.

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