

Chronic gastritis associated with *Helicobacter pylori*. Correlation between histological and bacteriological findings

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Summary. Biopsy specimens of gastric and duodenal mucosa from 326 patients were examined bacteriologically and histologically to determine the correlation between chronic gastritis and *H. pylori* colonization. *H. pylori* was identified in 111 (66.5%) patients with evidence of chronic gastritis and in 97 (82.2%) individuals who had gastritis associated with other pathology (gastric o duodenal ulcer, carcinoma o bulboduodenitis). The spiral bacteria was found more frequently in specimens with chronic superficial gastritis (88/107) and no significant difference was observed between the grade of activity of gastritis and *H. pylori* colonization. Giemsa stain was the most suitable method for detecting *H. pylori* in histological sections. By electron microscopy the microorganism was seen on the surface of the gastric mucosa, beneath the mucous layer, and more occasionally in intercellular junctions and the gastric pit.

Key words: Chronic gastritis, *H. Pylori*, Histology

Introduction

H. pylori has recently been recognized as an important factor in the pathogenesis of gastritis and peptic ulcer disease. Although the high association between this microorganism and the presence of histological gastric lesion suggests that *H. pylori* is a cause of chronic type B gastritis (Marshall and Warren, 1984; Aase et al., 1988), and although several studies have shown that its inoculation is able to produce inflammatory lesions (Krakowka et al., 1987; Lambert et al., 1987), the exact mechanism of tissue injury is now unknown.

Most studies show that *H. pylori* is not invasive, and

different toxic mechanisms have been proposed (Fumarola and Miragliota, 1988). Nevertheless, a recent work (Andersen and Holck, 1990) evidences a slight invasive capacity on observing *H. pylori* in the lamina propria.

In the present work we report the findings of a bacteriological, histological (light and electron microscope) and statistical study about the presence of *H. pylori* in 285 patients with chronic gastritis, distributing the patients in groups according to the type and grade of activity of gastritis.

Materials and methods

The prospective study was carried out in 326 patients (241 males and 118 females, mean age 56.3, range 15-87) referred for gastrointestinal tract endoscopy. Four biopsy samples were obtained from each patient; two specimens being taken from antral mucosa and two from other mucosal lesions observed in the endoscopy examination. The biopsies destined for microbiological study were placed in sterile tubes with 6 ml of thioglycolate solution.

Each biopsy was rubbed over a slide, stained by Gram and acridine orange methods and at once inoculated in Christensen's urea medium without agar. Culture was carried out in Brucella agar supplemented with 10% sheep blood and Skirrow's selective supplement and incubated in a microaerophilic atmosphere for 5-7 days. Identification of colonies was based on the morphology of the bacilli in the Gram stain, and positive reactions for catalase, oxidase and urease test.

Histological study was carried out with light microscopy (H-E stain, methenamine silver —Groocot technique— and Giemsa) and electron microscopy, following the usual processing protocols. Chronic gastritis was distributed according to Whitehead's classification into chronic superficial gastritis, chronic atrophic gastritis and chronic gastritis with intestinal

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metaplasia. The activity of gastritis was graded as follows:

- O: absence of polymorphonuclear leucocytes (PMN)
- I: 3 PMN per x400 field
- II: 3-6 PMN per x400 field
- III: more than 6 PMN per x400 field

Results

285 gastritis (167 single and 118 associated with other pathology) were diagnosed on histological examination. The distribution of these cases are shown in Table 1.

Signs of activity were observed in 162 patients; 89

Fig. 1. Growth of *H. pylori* on Brucella agar with 10% sheep blood.

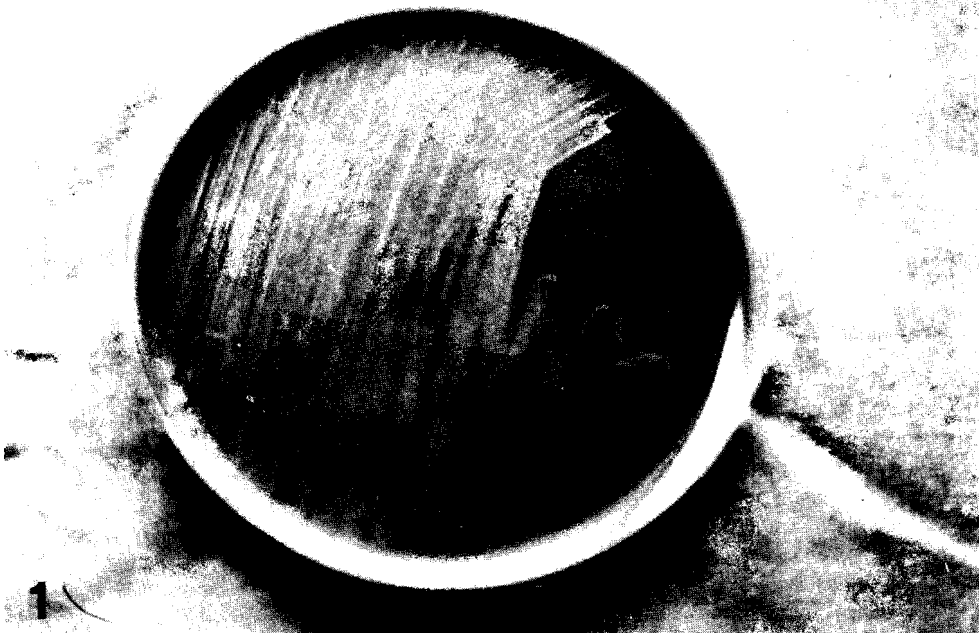
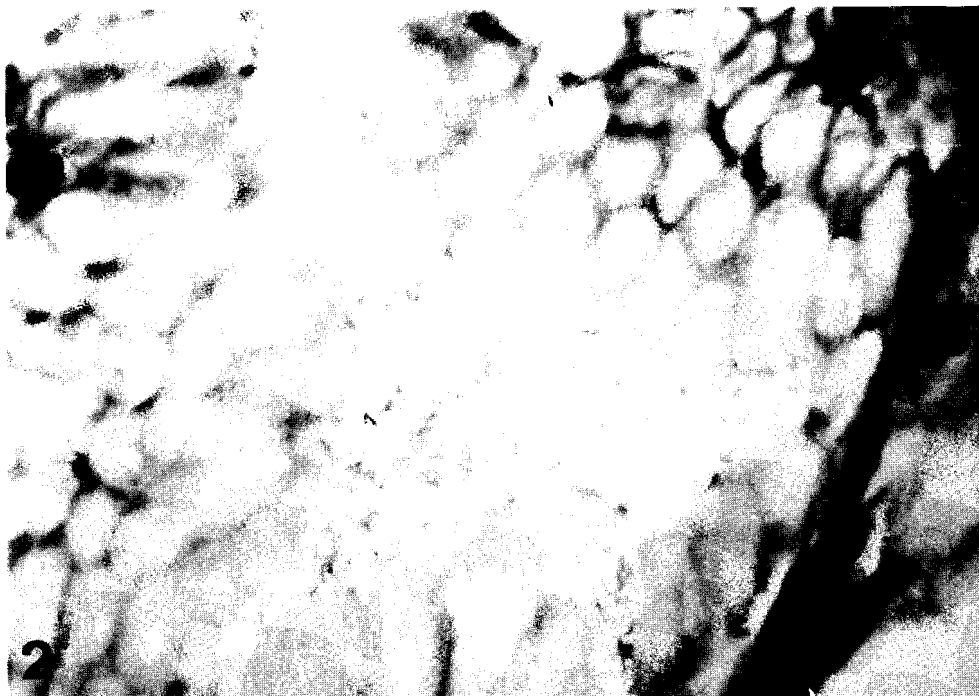


Fig. 2. Antral gastric mucosa with a moderate degree of chronic gastritis. Note presence of *H. Pylori* on the epithelial surface. Giemsa. $\times 400$



(31.2%) had a slight activity or grade I; 51 (17.9%) moderate activity or grade II; and in the remaining 22 (7.8%) the maximum grade of activity was observed. *H. pylori* colonization was present in 208/285 patients with histological evidence of gastric pathology, while this colonization could only be seen in 3 out of 41 patients with entirely normal gastric histology. Within

the group of patients with gastritis, and attending to the classification of Whitehead, *H. pylori* was more prevalent in chronic superficial gastritis (88/107), as shown in Table 1.

Microscopic visualization (Gram and acridine orange stain) was the most suitable microbiological technique for detecting *H. pylori* on the human antral



Fig. 3. Presence of *H. Pylori* on the surface of the gastric mucosa showing different shapes and sizes. $\times 12,000$



Fig. 4. Presence of spiral bacilli in the gastric epithelium inside the mucous layer surrounded by secretory vacuoles and necrotic tissue. $\times 4,500$

Chronic gastritis and *H. pylori***Table 1.** Correlation between histological type of single or associated gastritis and presence of *H. Pylori*.

HELICOBACTER PYLORI AND CHRONIC GASTRITIS		
HISTOLOGICAL STUDY		PRESENCE OF HELICOBACTER
I.- HISTOLOGICALLY DEMONSTRATED GASTRITIS	285	208 (72.9%)
a) Single	167	111 (66.5%)
1.-Superficial chronic gastritis	107	88 (82.2%)
2.-Atrophic chronic gastritis	21	8 (38.5%)
3.-Atrophic chronic gastritis with intestinal metaplasia	39	15 (38.4%)
b) Associated with other pathologies	118	97 (82.2%)
1.-Gastroduodenal ulcer	96	79 (82.3%)
2.-Stomach carcinoma	10	6 (60%)
3.-Bulboduodenitis	12	12 (100%)
II.-WITH NO HISTOLOGICALLY DEMONSTRATED ALTERATIONS	41	3 (7.3%)
TOTAL PATIENTS	326	

mucosa. Bacterial culture was successful in 90.7% with small, smooth, translucent and slightly haemolytic colonies (Fig. 1). The urease test revealed a sensitivity of 82.9% and a specificity of 100%.

On histological examination, the presence of this microorganism was observed in preparations stained with H-E, silver stain and Giemsa. Spiral bacilli were only found in the gastric epithelium; in no case was observed in areas of intestinal metaplasia. Colonization displayed a focal distribution; the bacilli being found in the luminal portion in close connection with the superficial epithelial cells, or immersed in the mucous layer. Occasionally, the bacilli were seen in the intercellular junctions (Fig. 2). Ultrastructurally, the surface of the bacteria was smooth in aspect, with globose ends about 0.5 µm in diameter and 2.5 µm in length. Rounded electron-opaque formations of different sizes were also observed, perhaps corresponding to polyphosphate granules (Fig. 3). The bacilli were seen to be encased in the deepest part of the mucous layer together with necrosed material in contact with the epithelial surface of the mucous-secreting cells (Fig. 4), or in the glandular lumen. The epithelial cells displayed numerous secretory vacuoles, a decrease in the number of microvilli, basal nuclei of different sizes with an evident nucleolus, and intercellular oedema. Occasionally, it was possible to observe cells with a decrease in secretory capacity.

Discussion

The results of the present study confirm the high prevalence described previously of *H. pylori* in the gastric mucosa of patients with gastric pathology (Gustavsson et al., 1987; Aase et al., 1988).

Of the 213 patients in which the presence of *H. pylori* was demonstrated, 98.6% showed histological gastroduodenal alterations, pointing to the significant

association of this microorganism with digestive pathology and in turn corroborating the results of other authors (Jones et al., 1984; McNulty and Watson, 1984; Price et al., 1985; Goodwin et al., 1987).

With respect to the gastritis, there are differences between the different histological patterns, colonization being more frequent in superficial chronic gastritis. Marshall and Warren (1984) reported a positive correlation between microorganisms and the presence of a marked inflammatory infiltration of the gastric mucosa, considering *H. pylori* as a marker of active chronic gastritis. The results of our study are in disagreement with these observations since the bacterium was detected in 48.8% of the samples without activity as compared with 66.6% in which the presence of neutrophil PMN cells was demonstrated, suggesting that the activity of the gastric inflammation is not correlated exclusively with the presence of *H. pylori*.

Ultrastructurally, the epithelial cells showed the features described by Goodwin et al. (1986), such as a decrease in the number of microvilli and in the mucin content. Unlike Camilleri et al. (1989) bacilli inside cytoplasmic vacuoles were not observed. *H. pylori* was preferentially located in the neighbourhood of the epithelial surface and although *H. pylori* in the lamina propria was seen, as reported by Andersen and Holck (1990), this finding was uncommon.

We believe that the presence of *H. pylori* in endoscopic samples of antral mucosa in routine clinical practice is easy to detect with the Giemsa method and may be of great interest in routine diagnosis and also in the evaluation of the response to treatment, since active therapy against *H. pylori* leads to the eradication of this microorganism and, in turn, remission or curing of the histologically observed lesions (Melikian et al., 1986; Rokkas et al., 1988).

All the foregoing seems to clearly show that gastritis represents a response of the mucosa to a challenge by this bacterial species.

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