Ultrastructural Changes of the Gastric Mucosa Induced by the *Helicobacter pylori* Infection

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The Helicobacter pylori colonisation of the gastric mucosa is not a disease in itself, but creates a favourable ground for the development of other clinical entities which affect the upper gastrointestinal tract. The chronic inflammation induced by Helicobacter pylori may lead to complications such as peptic ulcer, gastric cancer, and gastric lymphoma. The cases which tested positive for Helicobacter pylori were selected for an ultramicroscopy examination. During the ultrastructural examination, the authors highlighted the relation between the stage of the infection (gastritis) and the level of the carcinogenic transformation.

Keywords: Helicobacter pylori, gastric mucosa, gastric metaplasia, gastric cancer

The prevalence of the *Helicobacter pylori* infection has a wide geographical distribution from one region to another, in many developing countries over 80 to 90% of the population testing positive for *Helicobacter pylori* even at a young age [1], 2].

The gastric colonisation with *Helicobacter pylori* induces histological gastritis in all infected subjects, but only a small part develops clinical evidence of this colonisation. An estimated 30 to 40% of the *H. pylori* positive individuals are likely to develop an ulcerous disease, and another 1 to 2% have a risk of developing a gastric cancer located distally [31].

After the discovery of *Helicobacter pylori*, the initial studies estimated that around 95% of the duodenal ulcers and 85% of the gastric ulcers occurred after an *Helicobacter pylori* infection [4].

The chronic inflammation induced by *Helicobacter pylori* may lead to the loss of the normal architecture of the gastric mucosa with the destruction of the gastric glands and the replacement of normal mucosa by fibrous tissue and intestinal epithelium.

Approximately half of the *Helicobacter pylori* positive patients develop atrophic gastritis and intestinal metaplasia, beginning with the anatomical structure affected by the most severe inflammatory process.

The damaged glandular areas and the intestinal metaplasia extension become multifocal without specific symptoms. These changes increased the risk of gastric cancer by five to 90 times, depending on the extent and the severity of the atrophy [4].

The effect of this intervention on the prevention of gastric cancer was, however, less obvious. In several studies [3, 4], the eradication of *Helicobacter pylori* did not have a relevant effect on the incidence of gastric cancer, in the first years post-eradication.

The persistence of *Helicobacter pylori* causes a lifelong proinflammatory response associated with cell damage that initiates histological alterations: chronic gastritis, atrophic gastritis with achlorhydria, metaplasia, dysplasia. The permanent inflammatory process may lead to DNA damage, which includes multiple mutations that seem to initiate a neoplastic cascade.

The ultramicroscopic study was used to highlight the histological changes of the gastric mucosa infected with *Helicobacter pylori*.

Experimental part

Materials and methods

The clinical trial included 101 patients with dyspeptic symptoms who addressed the healthcare units for an upper gastrointestinal endoscopy. The 101 patients were divided into two groups:

- the first group of 52 patients included the subjects with *H. pylori* infection.

· the second group, composed of 49 patients, was the control group, comprising those patients in whom *H. pylori* could not be found, but who presented dyspeptic symptoms.

An ultrastructural study was performed on the biopsy material obtained by upper gastrointestinal endoscopy.

The transmission electron microscopy (TEM) study involved the following steps: collection, fixation, dehydration, infiltration and inclusion, encapsulation, shaping, cutting, contrasting, examination, and digital image recording and processing by a computer.

The collection of gastric mucosa fragments was carried out very quickly (1 to 2 min) (to prevent the installation of morphological and functional alterations in the cells), with a mean volume of about 0.3 mm^3; the fragments collected were preserved in ice.

Next, the collected fragments were fixed in glutaraldehyde solution of 2.7% concentration in 0.15M phosphate buffer, *p*H 7.2, for 2 h at 4p C.

The tissue fragments were then dehydrated in increasing concentrations of acetone solution chromatography, starting with a concentration of 50% and ending with 100% acetone, for 15 to 20 min in each solution.

The inclusion was made in a polyester resin called Westopal W.

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The samples were encapsulated in gelatine capsules and then maintained for 72 h in the oven at +60p C until the polymerisation of the synthetic resin.

The shaped blocks were then used to obtain ultra-fine sections, 70 nm thick, using a LKB III-type ultramicrotome.

The sections were contrasted with uranyl acetate and lead citrate. The contrasting sections were introduced and examined in the TESLA BS-500-type electron microscope and the selected images were captured sequentially and stored in the database by using the software of the MegaView III camera, Soft Imaging Analysis.

Results and discussions

This study investigated patients divided into two groups according to the presence or absence of *H. pylori* in order to research the relationship between the gastric changes induced by *H. pylori* and gastritis or gastric cancer.

The research used an ultrastructural study to highlight the changes in the gastric mucosa from the infection stage (gastritis) to the level of gastric cancer.

The gender ratio was males : females = 2.25:1 (70:31). In most cases, chronic gastritis was found in the distal stomach, the most common being the antral location with a number of 29 cases, namely 55.7%.

The next locations in terms of frequency are the body and fornix of the stomach (the fundus), with an absolute value of 13 cases, or 25%. The multifocal atrophic gastritis is present in those patients that have a high incidence of gastric carcinoma, with an absolute value of 10 cases, respectively 19.2%.

It was found that 10 patients with chronic atrophic gastritis and *H. pylori* present had intestinal metaplasia lesions. On the other hand, the other 10 patients with chronic atrophic antral gastritis and metaplasia had developed varying degrees of intraepithelial dysplasia, which ultimately led to gastric cancer.

The main histological types of tumour were adenocarcinoma (50%), carcinoma (30%), and carcinoid (20%).

After examining the gastric mucosa samples, we noticed large differences from one patient to another regarding the degree of infection; the alterative and degenerative severity structural changes appeared to be directly proportional to the infection.

It was found that those patients with a mild stage infection had only isolated bacteria located on the mucosal surfaces.

The electron microscopic examination highlighted:

- a gastric epithelium with high cylindrical cells presenting short microvilli; an oval, euchromatic aspect in the third basal nuclei; several mitochondria with average electron density (fig 1);

- secretory granules in the Golgi complex; apical pole loaded with mucus; intact microvilli edges (fig 2).

The ultrastructural investigation allowed the capture of the impact and destruction of the epithelial cells manifesting in two ways:

Fig 1. The early stage of infection with *H. pylori*: gastric epithelial high cell with euchromatic nuclei, multiple mitochondria (X5880)

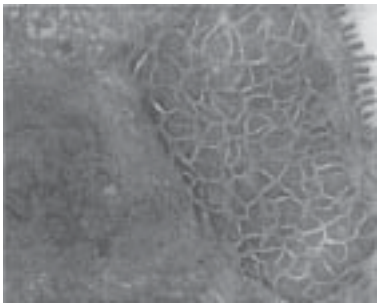

Fig 2. Intense metabolic activity of the epithelial cells: secretory granules in the Golgi complex, apical pole loaded with mucus, intact microvilli edges (X12600)

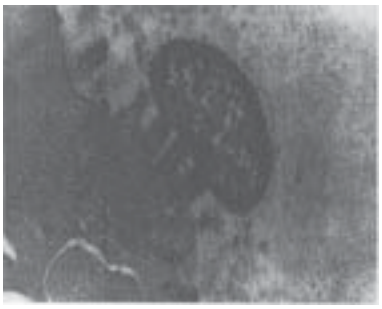

Fig 3. Attachment detail of *H. pylori* to the microvilli of the apical surface (X50400)

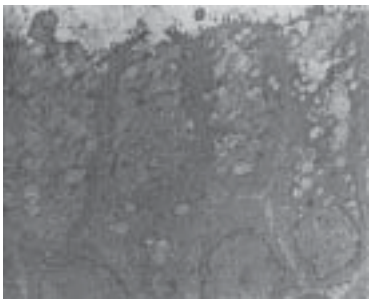

Fig 4. Early changes in the epithelial cells: thinning edges of the microvilli, incomplete maturation of the mucus granules (X5880)

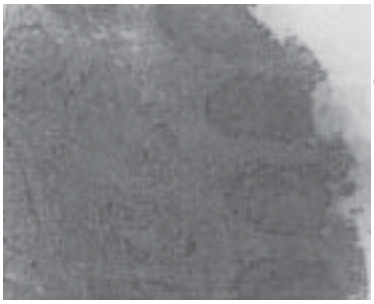

Fig 5. Dilation of the endoplasmic reticulum, loss of the oval shape, and distortion of the nuclear envelope (X6510)

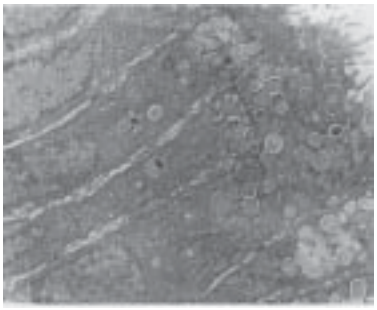

Fig 6. Intercellular spaces widening and the intense elimination of mucus in the lumen (X8400)

1.H. pylori attachment to the apical pole of the epithelial cells with the intercellular space widening and the release of significant mucosal secretions (fig 3)

As a result of this interaction, the first ultrastructural changes of the epithelial cells occur:

Thinning edges of the microvilli, incomplete maturation of the mucus granules which appear as having a low electron density (fig 4);

· early expansion of the endoplasmic reticulum, loss of the oval shape, distortion of the nuclear envelope (fig 5);

· intercellular spaces widening and the intense elimination of mucus in the lumen (fig 6).

As a result of our observations, we can state that the surface mucous cell is trying to protect itself from bacterial aggressions by mucosal hypersecretion. The increased

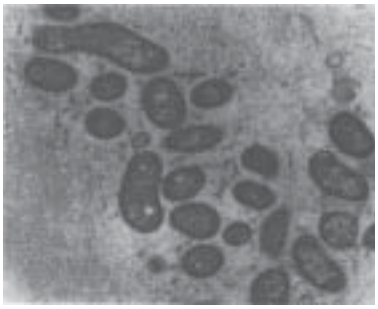

Fig 7. *H. pylori* in large numbers in the gastric mucus (X21000)

mucus secretion favours the bacteria that are very well adapted to the *p*H conditions, viscosity, and structural features of the mucus, multiply faster (fig 7), and appear as groups attached to the apical surface of the epithelium, forming "pedestals attachment" (fig 8).

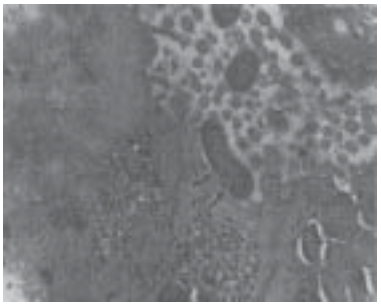

Fig 8. Attaching *H. pylori* by forming "pedestals attachment" (X21000)

Fig 9. Massive infection with *H. pylori* on the epithelium surface (X6000)

Fig 10. Area of lysis and cell necrosis induced by the cytotoxic action of *H. pylori* (X8400)

As a result of this continuous bacterial attack, more extensive erosions appear; at this level, *H. pylori* is found in large numbers. It was observed that in the beginning the erosions are rare, then they gradually deepen, become wider and multiply, and the number of bacteria present in the surface mucus greatly increases (fig 9). Gradually, areas of lysis and cell necrosis appear, leading to the capillary wall rupture and the disintegration of those cells which constitute the mucosal barrier (fig 10).

2.H. pylori penetrates the mucous cells' surface and dissociates the intercellular junctions

At first, the bacteria penetrate the superficial upper cells, after which, gradually, they penetrate deeper into the basal third of the epithelium. The intercellular spaces widen (fig 11), and the cells undergo progressive atrophy.

The electron microscopy revealed groups of bacteria "forcing" the intercellular junctions.

We proved the presence of bacteria in the lamina propria and the degenerative changes induced at this level: cell

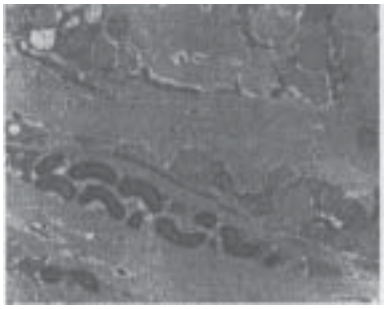

Fig 11. Enlarged intercellular spaces penetrated by bacteria (X12600)

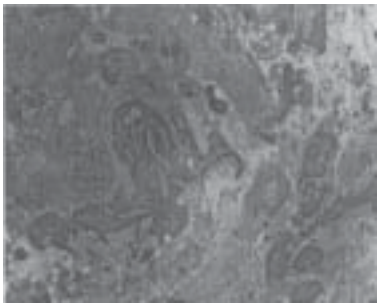

Fig 12. *H. pylori* present in the lamina propria: destruction of the basement membrane and alterations of the cellular components (X7140)

alterations, nuclear pyknosis, and the destruction of the parietal capillaries (fig 12).

The main etiologic agent of gastritis, *H. pylori,* is considered to be a non-invasive coloniser of the gastric mucosa. Coccoid forms of *H. pylori* have previously been described in gastric tissue sections using optical and electron microscopy [5].

The previous electron microscopy scanning studies suggested that this micro-organism has an invasive potential at the surface of the gastric epithelium and the intercellular junctions (Y. TANIGUCHI & al. [6]). The ability of bacteria to invade the epithelial cells may also be a defence mechanism such as a sanctuary, and, therefore, the infected patients have a high recurrence rate after the eradication therapy [12].

Unlike several previous studies that only showed immunoreactivity in the lamina propria, our study highlighted an intact form of *H. pylori* with aggressive potential, infiltrating the lamina propria (fig 12).

Several previous studies have also shown that the presence of micro-organisms in the epithelium or on the gastric glands gives the patient increased resistance to the eradication therapy and numerous relapses after treatment [7]. The presence of *H. pylori* in the lamina propria could be an indicator of a more aggressive infection and could be used as a surrogate marker for the resistance to the eradication therapy.

Our study illustrates how *H. pylori* attaches to the apical pole of the epithelial cells of the mucosa and acts through several mechanisms. Initially, *H. pylori* attaches to the apical pole of the cells in an approximately horizontal position relative to the surface epithelium and driven tight against the cell, followed by the occurrence of the first ultrastructural changes in the epithelial cells. These changes are described by other authors [9].

We have shown that on the mucous membrane layer of the stomach, the bacteria multiply, explaining why *H. pylori* is found in larger groups or isolated. This observation is consistent with other studies demonstrating that *H. pylori* multiplies in the mucous layer adjacent to the epithelial surface where bacterial growth is favoured by the nutritional conditions [10].

The images in figures 7 and 8 allow us to consider that the mucus exocytosis is a first step in case of aggression. Previously, I. MICOTS & al. [11] noted that *H. pylori* deeply weakens the exocytosis mechanism (in addition to the

cytotoxic effect), allowing the mucosal cell injury by various aggressive stimuli.

It was estimated that during the direct action of the micro-organism on the cell membrane, the structures are exposed to high concentrations of cytotoxins, ammonia generated by the bacterial urease, proteases, and phospholipases, resulting in inevitable cell damage. The hydrophobicity of the mucosa coating is compromised, with consequent reduction of its viscosity and thickness of the hydrogen ions, and facilitates backscatter. Overall, hydrophobicity compromises the protective layer of the mucosa, with consequent reduction of its thickness and viscosity and facilitates the backscatter of hydrogen ions [12, 13]. These favour the development of inflammation and/or ulceration [14], 15].

Similarities between the bacterial and affected cells' structures, which we presented in figure 5, were also noticed in the case of the enteropathogenic *Escherichia coli* [16, 17]. The previous ultrastructural studies have highlighted *H. pylori*'s ability to adhere to the epithelial cells [18-21]). It should be noted that adherence is important for the potentiation of the inflammatory response.

Another mechanism by which *H. pylori* affects the gastric mucosa is by gradually breaking the intercellular junctions, widening the intercellular spaces and loosening the epithelial cohesion.

In our study, we observed the formation of erosions by the separation of the chains of the epithelial cells composing the basement membrane, especially in severe infections, with the inflammation and degeneration of the cells, resulting in marked denudation of the basement membrane.

In agreement with other authors [22], we found that the degenerative intraepithelial changes are common.

On the other hand, we believe that the epithelial lesions induced by bacteria lead to a compensatory increase in the mucous cell proliferation. Consequently, these vulnerable cells may be exposed to the mutagens from the stomach and play a decisive role in the chain of events leading to gastric carcinoma [23].

In relation to the action mechanism of *H. pylori* that "forces" the intercellular junctions, we assume that this could explain the increased risk of upper gastrointestinal bleeding associated with the *H. pylori* infection, which is reported by other authors [24].

H. pylori induces the alteration of the gastric mucosa (highlighted histochemically and with the aid of electrons) in two ways: by its attachment to the apical pole of the gastric epithelial cells in an approximately horizontal position relative to the surface and by the dissociation of the intercellular junctions by bacteria which then penetrate through the surface mucosal cells.

The *H. pylori* infection may cause structural and ultrastructural gastric mucosal changes identified by histochemical, immunohistochemical, and electron examinations. The evolution corresponds to a typical gastric carcinogenic sequence: active chronic gastritis, atrophic gastritis, metaplasia, dysplasia, and adenocarcinoma.

Conclusions

Helicobacter pylori multiplies in the gastric mucus and can be found in large groups or isolated. This bacterium decreases the mucus exocytosis and produces gastric mucosal lesions.

The *Helicobacter pylori* infection determines the degeneration of the gastric epithelium and mucosal cell proliferation, alterations which may provide a basis for the gastric carcinoma development. *Helicobacter pylori*, by its action upon the intercellular junctions, increases the risk of upper gastrointestinal bleeding.

Note: All authors contributed equally for the manuscript.

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