# *HELICOBACTER PYLORI* GASTRIC INFECTION AND MAST CELLS - ULTRASTRUCTURAL STUDY

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**ABSTRACT. Background:** *Helicobacter pylori* infection results in a cascade of biochemical events in the gastric mucosa. In the inflammatory process, mast cells play a pivotal role as initiators and regulators of inflammation. As they play a key role in infection and immunity and because they are present in large numbers in the mucosa of the gastrointestinal tract, it is likely that mast cells participate in the pathophysiology of *Helicobacter pylori* associated gastroduodenal diseases. **Aims & Methods:** Our paper tried to investigate by ultrastructural examination the role of mast cells in the pathophysiology of *H. pylori*-associated gastritis. The study comprised 56 *H. pylori*-positive and 20 *H. pylori*-negative patients. During endoscopy, gastric antral and body biopsies were taken for the assessment of *H. pylori* and morphological examination. All samples were evaluated according to the Sydney system and mast cell density in both the corpus and antrum mucosa was analyzed by modified Romanovsky stain. Wilcoxon Matched Pairs test was used to determine the relationship between mast cells and other histopathological parameters. The mast cell density between *H. pylori* positive and negative groups was compared by Student test. The specimens from 12 infected patients were processed for ultrastructural examination, contrasted with acetate-uranyl and lead-citrate and studied with a JEM-1010 electron microscope. **Results:** In our study, mast cell density was similar in the corpus and antral mucosa ($p > 0.5$) but higher in the HP-positive group than in the HP-negative group ($p < 0.01$), both in the antrum and corpus. At the same time we observed that there was a direct correlation between the mast cell infiltration and the increased activity of the gastritis, as well as between the mast cell infiltration and HP density in the antrum and corpus ($p < 0.005$). There was no correlation between the mast cell infiltration and intestinal metaplasia or between the mast cell infiltration and gastric atrophy (p>0, 05). At the ultrastructural examination, we found many mast cells within the *lamina propria* of the *H. pylori*-positive subjects. In no infected patients the mast cells were normal, with regular shape nucleus and electron dense granules in the cytoplasm from the *lamina propria* of the antrum and corpus mucosa. The majority of the mast cells were present in sub epithelial areas, around blood vessels, in the vicinity of eosinophils and rarely around nerves. Hence, it is strongly suggested that the mast cells infiltrating the inflamed mucosa are activated and may induce tissue damage. **Conclusion:** All of the above supported the fact that mast cells play a role in the occurrence and development of the pathogenesis of HP infection. Mast cells can penetrate the basal membrane and move toward the interepithelial space. During this process, degranulation appears gradually, which results in the phenomena of vacuolation.The relationship between mast cells and HP infection and the effect of the mediators secreted by mast cells on the pathophysiology of HP associated gastritis needs further investigation. Thus, manipulating mast-cell adhesion may be an important strategy for controlling the outcome of the inflammatory response.

**Keywords:** *Helicobacter pylori* infection, chronic active gastritis, mast cells, electron microscopy

## INTRODUCTION

The discovery of *Helicobacter pylori* in 1983 by Warren and Marshall meant beyond any doubt a moment of reference in the medical world and it also modified deeply the etiopathogenesis, pathophysiology and therapy of gastritis and peptic disease (Warren et al., 1983).

The HP infection provokes a remarkable inflammatory process in the gastric mucosa and it implies a number of cells such as: neutrophils, eosinophils, lymphocytes and plasma cells (Abraham et al., 1997). This constellation of inflammatory responses contributes to the turn up of chronic active gastritis which can display different aspects (e.g. mainly antral gastritis, multi focal atrophic gastritis) (Kusters et al., 2006), and it can also remain clinicaly silent or it can progress towards the development of other digestive diseases like peptic ulcer, gastric carcinoma or gastric B cell lymphoma (McFarlane et al., 1997). Although peptic disease is the most studied issue concerning the HP infection, this bacterium is also implicated in the development of other disorders

as: MALT lymphoma, coronaritis, gastroesophageal reflux disease, iron deficiency anaemia, skin diseases and rheumatic diseases (Davydov et al., 2000, Zhang et al., 2005).

The association between chronic HP infection and cell multiplying alteration is well known from a long time ago (Alexandere et al., 1997). Moreover, HP can produce and release several bioactive factors that may directly affect the gastric mucosal cells (which produce chlorhidric acid) and enterochromaffin like cells (ECL) which produce gastrin and somatostatin (Moss et al., 1992). Although almost anyone can develop a form of gastritis which can be demonstrated by means of microscopic examination, only few people develop peptic ulcer or other disorders.

Mast cells are inflammation initiators and regulators, but their exact role in the stomach is still unclear (Raica, 1995; Santacroce et al., 2000). Mast cells are probably best known as the main effectors in immunoglobulin E-mediated immediate hypersensitivity reactions, but they are also involved in other types of hypersensitivity reactions, inflammation, mucus secretion, smooth muscle contraction and wound healing (Czkwianianc et al., 2007). The cytoplasmic granules of mast cells contain preformed mediators such as: histamine, heparin, proteases, and so on. Activated mast cells undergo a severe degranulation process and then produce some newly formed mediators like prostaglandin D2, leukotrienes C4, D4 and E4, platelet activating factor, interleukins-8, 4, 6 (Nakajima et al., 2004).

The research during the last couple of years revealed that HP infection produces a chronic active gastritis which is characterized by a striking infiltrate of the gastric epithelium and the underlying *lamina propria* by neutrophils, T and B lymphocytes, macrophages, and mast cells (Lopes et al., 2006). Mast cells, usually responsible for the immune response balance, may be important effector cells in the pathogenesis of gastritis.

The aim of our paper was to investigate by structural and ultra structural examination the presence and the possible role of the mast cells in the patho-physiology of adult *H. pylori*-associated gastritis.

## MATERIALS AND METHODS

We studied 76 patients from Oradea Clinical County Hospital with dyspeptic complaints. All of the patients underwent upper digestive endoscopy examination and they were tested to establish the presence of HP infection. 56 patients were HP positive and 20 of them were HP negative.

The examination was performed using a Olympus Exera CLE 145 videoendoscop. During endoscopy, gastric antral and body biopsies were taken for the assessment of *H. pylori* and morphological examination.

### *Histological Examination*

Biopsy specimens were fixed in Carnoy liquid and then processed, oriented on edge, embedded in paraffin, cut in sequential 5 μm sections and stained by a modified Romanovsky stain. The obtained pieces were examined using a light microscope Carl Zeiss Ergaval type (ob. ×40, ob. ×100, ob. ×200).The density of neutrophils and mononuclear cells in the gastric mucosa was graded on a scale from 0 (absent) to 3 (marked infiltration) according to the updated Sydney system. All mast cell counts (cells/mm$^2$) were performed in at least 5 consecutive fields and by a single observer who was unaware of the status or group (HP positive or negative).

### *Electron Microscopy*

Two specimens (one from the antrum and one from corpus) from 12 infected patients were fixed in 2.5 % phosphate buffered 0.1M glutaraldehide sol. (pH 7.4) and then postfixed with 1% osmic acid in phosphate buffer (pH 7.4)**.** After rinsing in distilled water the pieces were dehydrated with acetone**,** embedded in Vestopal W and then thin sections were cut (70nm), stained with uranyl acetate and lead citrate**.** Afterwards, they were studied with a JEM-1010 transmission electron microscope (JEOL, Tokyo, Japan).

### *Statistics*

Student t test was used to compare the mast cell density between the groups of studied subjects and Wilcoxon Matched Paired test - used for the correlation between the mast cells and other histological parameters. All the differences were regarded significant at $p < 0.05$.

## RESULTS AND DISCUSSION

In our research, we obtained some statistically significant results which can sustain and complete the data existing in literature.

So, in our study, mast cell density was similar in the corpus and antral mucosa ($p > 0.5$), but higher in the HP-positive group than in the HP-negative group ($p < 0.01$), both in the antrum and corpus. At the same time, we observed that there was a direct correlation between the mast cell infiltration and the increased activity of the gastritis, as well as between the mast cell infiltration and HP density in the antrum and corpus ($p < 0.005$). There was no correlation between the mast cell infiltration and intestinal metaplasia or between the mast cell infiltration and gastric atrophy ($p>0. 05$).

Other data published in literature also demonstrates the existence of a high number of mast cells in gastric inflammation secondary to HP infection and correlates the results with the degree of inflammation (Nakajima et al, 1997; Mysorekar et al., 2003).

Our ultrastructural study revealed that in no infected patients the mast cells were normal, with regular shape nucleus and electron dense granules in the cytoplasm. Found in the *lamina propria* of the antrum and corpus mucosa, the number of mast cells from each sample examined was different (mean-8) (fig.1). The majority of the mast cells were present in sub epithelial areas, around blood vessels, in the vicinity of eosinophils and rarely around nerves.

In infected patients, we observed the mast cells migrating towards the bacteria from the mucosal surface (fig.2), also, we found mast cells with electron

dense granules in the cytoplasm, with speckled appearance, targeted granules with a central electron-dense material surrounded by a clear space and clear vacuoles that showed complete degranulation (fig.3).

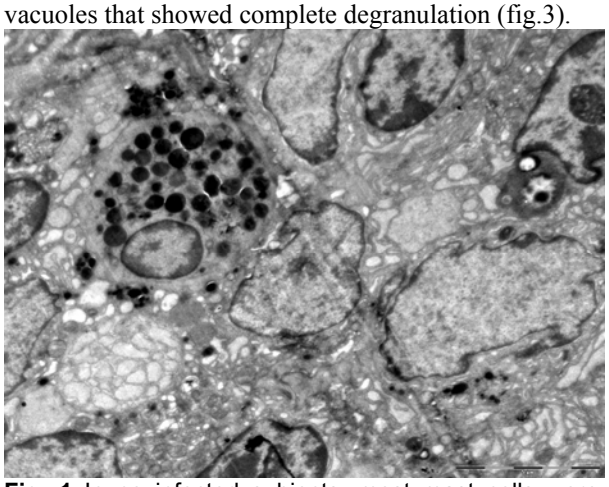

**Fig. 1** In no infected subjects, most mast cells were normal with regular shape nucleus and electron dense granules in the cytoplasm (Barr=5 μm)

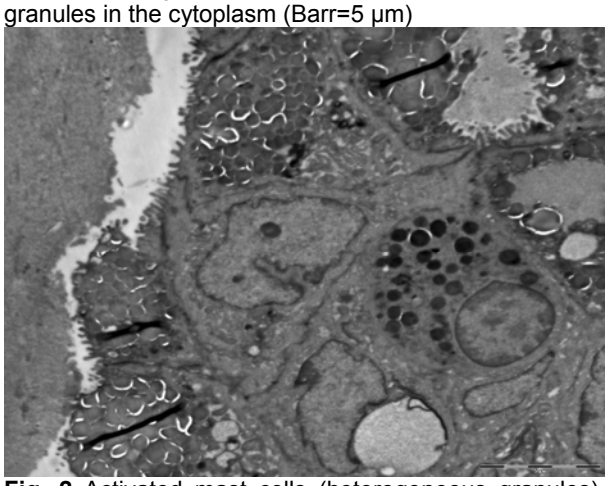

**Fig. 2** Activated mast cells (heterogeneous granules) infiltrating the gastric mucosa and migrating towards the lumen and towards HP (Barr=3 μm)

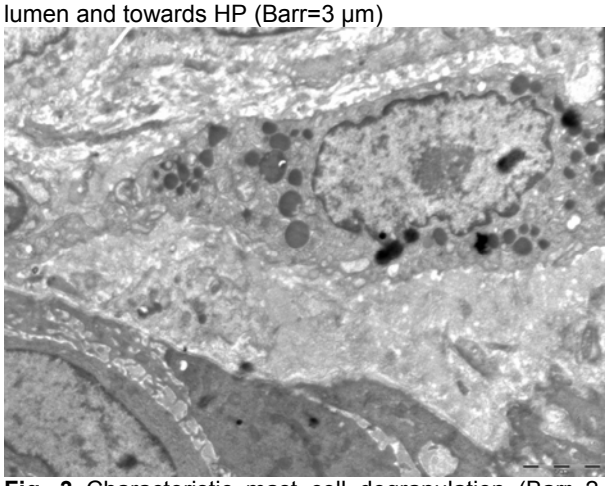

**Fig. 3** Characteristic mast cell degranulation (Barr=2 μm)

Some of the mast cells showed beside the signs of degranulation, irregular contour of the nucleus with a lot of heterochromatin, denoting a possible sufferance due to excessive demands on the course of HP infection (fig.4).

During the HP infection, mast cells promote the migration of inflammatory cells from the blood vessels towards *lamina propria* (releasing immunoregulatory cytokines), but they might also be recruited, in their turn, by factors produced by the activated inflammatory cells (fig.5).

The profound alterations induced in the *lamina propria* by the bacterial infection and by the inflammatory response of the host may also affect mast cells with the disruption of the cell membrane and irreversible degeneration of the cell components (fig.6).

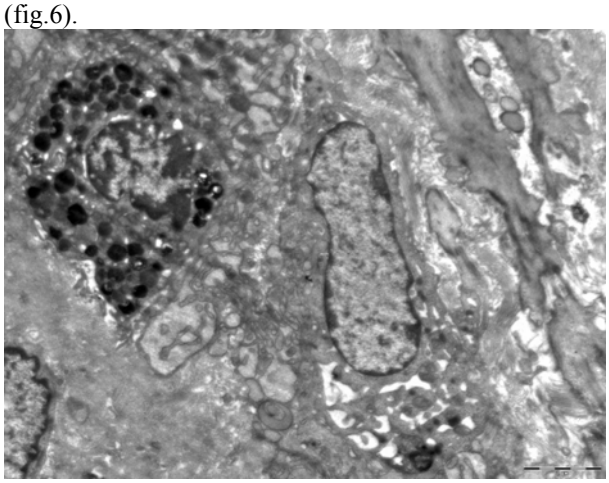

**Fig. 4** Irregular contour of the nucleus with a lot of heterochromatin (Barr=2 μm)

**Fig. 5** Infiltration of lamina propria with eosinophils and mast cells, oedema and erythrocyte infiltration (Barr=5 μm)

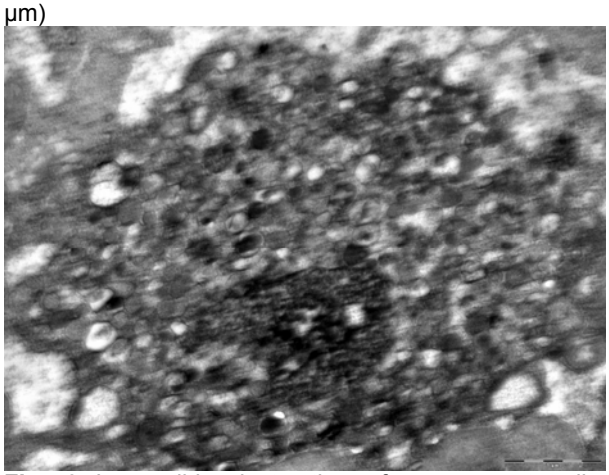

**Fig. 6** Irreversible destruction of some mast cells probably due to the intense inflammatory response and depletion of cell resources

Mast cells are important components of the cellular infiltration in the course of gastric mucosa inflammation with *H. pylori* infection. Mast cells actively participate in the induction of epithelial cells apoptosis (Hofman et al., 2007) and enhancement of the inflammatory process by their influence on: vascular dilatation and increasing blood flow capacity (Velin et al., 2006), migration of inflammatory cells and inflammatory infiltration development (Moorchung et al., 2006).

Kurose was the first who demonstrated that *H. pylori* extracts could induce mast cells degranulation. He observed that this phenomenon appeared after 10 minutes from the mesentery exposition to an aqueous extract of H. pylori. Activated mast cells release also pro-inflammatory factors, which may increase vascular flow capacity (Kurose et al., 1994).

Mast cells also produce TNF-alpha, platelet activating factor, IL-8, which, in turn, are chemotactic factors for neutrophils (Gionchetti et al., 1994). In the patients studied up to the present, the increase in mast cell density was correlated with the intensity of polimorphonuclear and mononuclear cell infiltration. Therefore, it is very possible that mast cells are acting an active part in the recruitment of mononuclear cells into the mucosa (both in the epithelium and in the *lamina propria*) (Maciorkowska et al., 2004).

Our study provides evidence for the involvement of mast cells in the pathogenesis of gastritis, but further studies are needed to establish whether they contribute to regulate inflammation, stimulate tissue turn-over and ulcer healing and participate in independent gastrin hypersecretion.

## CONCLUSIONS

The number of mast cells is significantly increased in the gastric mucosa infected with HP; ultrastructurally they show characteristic changes of high activity and sometimes severe alterations.

Our data suggest that the mast cells, usually responsible for the immune response balance may be important effectors in the associated gastritis and contribute to the chronic inflammation.

Further studies are needed to determine their exact role and their limits in dealing with severe infection.

Manipulating mast cell adhesion may be an important strategy for controlling the outcome of the inflammatory response.

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