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Modulation of Galectin-3 and Galectin 9 in gastric mucosa of patients with chronic gastritis and positive *Helicobacter pylori* infection

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ABSTRACT

Objectives: Galectins are mediators that play an important role in the inflammatory response and in this study we analyzed the expression of Galectins (Gal) −1, −3 and −9 in biopsies of the gastric antrum of patients with upper gastrointestinal symptoms. Methodology: 44 patients with upper digestive tract symptoms were evaluated, and underwent Upper Digestive Endoscopy examination. Sections of the gastric antrum were fixed in buffered formaldehyde at 4% in order to perform the anatomopathological examination and immunohistochemical analysis for Galectins-1, −3 and −9 expression. Fresh sections of gastric antrum were used for DNA extraction and evaluation of *Helicobacter pylori* (*H. pylori*). P values < 0.05 were considered statistically significant.

Results: Gal-1 was significantly more expressed on stroma than epithelium ($p < 0.0001$), whereas Gal-3 and Gal-9 were more expressed on epithelium ($p < 0.0001$). Gal-3 was found to be significantly higher in the stroma of patients with *H. pylori* infection, mainly on Cag-A positive *H. pylori* ($p < 0.0001$). Gal-9 was down modulated in stroma of patients with chronic gastritis.

Conclusion: Up modulation of Gal-3 expression was associated with *H. pylori* infection and down modulation of Gal-9 with the inflammatory process of chronic gastritis.

1. Introduction

Gastritis is inflammation of the gastric mucosa in response to a noxious agent [1]. Chronic gastritis is predominantly characterized by mononuclear inflammatory cells, whereas active gastritis is a chronic inflammatory process with a predominance of neutrophils [2]. One of the main etiopathogenesis of gastritis is the infection by *Helicobacter pylori* (*H. pylori*) [3]. This highly mobile Gram-negative spiral bacterium is found on the luminal surface of the gastric epithelium [4], and neutrophils are found in the epithelial glands and in the underlying lamina propria during infection, with an increase of lymphocytes, macrophages, eosinophils in the gastric mucosa [5]. Several studies suggest that *H. pylori* infection determines a chronic inflammation of the gastric mucosa, and that it may also persist for years and confer increased risk for development of gastric cancer [6,7].

Studies investigating endogenous lectins and their properties have been emphasized over the years, due to the immune modulating properties of these lectins as an inflammatory response, both in acute, chronic diseases and also in autoimmune disorders and cancer [8]. Galectins (Gals) are a family of lectins which have a high β-galactosidase affinity, and these proteins participate in various biological phenomena, such as cell differentiation, angiogenesis, cell interaction, apoptosis and inflammation [9,10]. Galectin-1 (Gal-1) seems to have an anti-inflammatory property once it can induce apoptosis of activated T cells, inhibit cytokines such as IL-2 and IFN-gamma, inhibit the production of iNOS by macrophages, as well as promote the migration of neutrophils [11,12]. In biopsies of patients with gastric ulcer and intestinal metaplasia, Gal-1 was highly expressed in the stroma and epithelial cells [13]. Likewise, Gal-1 was found to be over expressed both in the chronic gastritis and in gastric cancer, suggesting a strong

Abbreviations: Gal, Galectin; Gal-1, Galectin-1; Gal-3, Galectin-3; Gal-9, Galectin-9; H. pylori, Helicobacter pylori; UDE, Upper Digestive Endoscopy; BSA, bovine serum albumin; MN, mononuclear cells; PMN, Polymorphonuclear cells

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association of these roteins with chronic inflammatory processes and carcinogenesis [14]. Galectin-3 (Gal-3) has a wide functional activity depending on its location [15,16]. In the immune system, it seems to play an important proinflammatory activity, it promote the proliferation of T lymphocytes, and involved T cell/dendritic cell interaction [17]. In macrophages and neutrophils, Gal-3 is associated with chemotaxis, migration and phagocytosis. In neutrophils, Gal-3 seems to contribute to leakage and adhesion to laminins, thus facilitating the migration of these cells to the site of infection [18]. Moreover, Gal-3 also increases NADPH oxidase activity [19]. Intracellular expression of Gal-3 seems to be associated with inhibition of apoptosis, whereas extracellular expression was associated with induction of apoptosis [20,21]. Low expression of Gal-3 has been found in biopsies of patients with gastric cancer [22]. Gal-3 also seems to be an important adhesion molecule to *H. pylori*, some authors have observed that this adhesion may influence the severity of the disease [23]. *In vitro* studies suggest that *H. pylori* infection stimulates rapid secretion of Gal-3 in gastric epithelial cells [24]. On its turn, galectin-9 (Gal-9) was first identified as an eosinophil chemotactic factor [25]. Therefore, Gal-9 has the capacity to suppress Th1 and Th17 pattern inflammation [26]. A decrease in Gal-9 was observed in gastric cancer patients, as well as in breast cancer [27] [27]. However, the role of Gal-9 in the pathogenesis chronic gastritis needs to be investigated.

Here antral biopsies of patients with chronic gastritis, chronic active gastritis or control groups were analyzed and compared on the basis, expression of Gal-1, Gal-3 and Gal-9, and compared with presence or absence of *H. pylori* infection.

2. Materials and methods

This study was approved by the Research Ethics Committee (CEP) of the University of Uberaba (UNIUBE) under protocol CAAE-19666013.0.0000.5145. Forty-four patients with symptoms of the upper digestive tract were evaluated, and all of them underwent the Upper Digestive Endoscopy (UDE) followed by mucosal biopsies. One antral section was used for investigation of *Helicobacter pylori* by rapid urease test immediately after UDE. Another section of the gastric antrum was fixed in buffered formalin 4% for anatomopathological examination and immunohistochemical analysis. Finally, another, fresh, section of gastric antrum was used for DNA extraction for evaluation of *H. pylori*.

The anatomopathological examination was performed by a single subject and interpreted in accordance with the Sydney System of classification, whose changes/grading were proposed at the Houston meeting [1]. Out of the 44 patients, 11 patients had no inflammatory process and were grouped as controls, 18 patients had an inflammatory process with predominance of mononuclear cells (MN) and were classified as chronic gastritis, and 15 patients had an inflammatory infiltrate composed of polymorphonuclear (PMN) and mononuclear cells (MN), and were thus classified with active gastritis. The sample size of this studied has a confidence interval of 9.23% at a confidence level fixed at 95%.

2.1. Immunohistochemistry

Indirect immunohistochemistry was performed in order to evaluate the expression of galectin-1, −3 and −9. Deparaffinized sections were hydrated and treated with 3% hydrogen peroxide in methanol for 10 min for endogenous peroxidase inhibition, incubated for 30 min at 90 °C for antigen retrieval, and then incubated with PBS 2% BSA to reduce non-specific binding. Next, the sections were incubated with monoclonal antibody specific for human anti-galectin-1 (1:50; R & D Minneapolis, MM, USA; cod-AF1152), anti-galectin-3 (1:75; R & D; cod-AF1154,) and anti-galectin-9 (1:75; R & D; cod-AF2045). In the second step, a biotinylated Link System (LSAB-K0690, Dako, Carpinteria, CA, USA) was used according to manufacturer instructions. The reaction was visualized by incubating the sections with diaminobenzidine (Sigma, St. Luis, MO, USA) and counterstaining with hematoxylin.

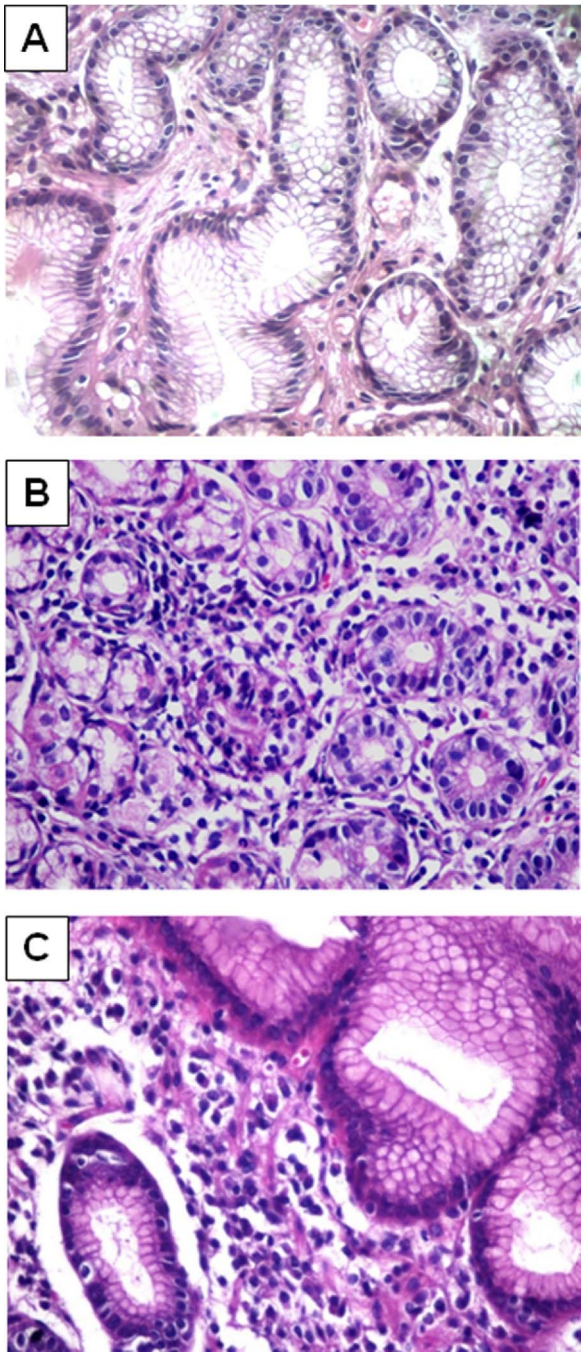

Fig. 1. Histological sections of gastric mucosa obtained of patients grouped according to the presence or absence of gastritis. A- Gastric mucosa obtained of patients without inflammatory exudate (control group- 400x)(Hematoxylin Eosin −HE); B- Gastric mucosa obtained of patients with inflammatory exudate of polymorphonuclear/mononuclear cells and neutrophil permeating the foveolar epithelium-(chronic active gastritis- 400x) (Hematoxylin Eosin −HE); C− Gastric mucosa obtained of patients with inflammatory exudate mononuclear cells (chronic gastritis- 400x) (Hematoxylin Eosin −HE).

2.2. DNA extraction

DNA extraction was performed from biopsy tissue samples from the antrum region obtained using the kit “QIAamp DNA Minikit” (QIAGEN GMbH, Hilden, Germany) according to the manufacturer’s specifications. Briefly, 20 µl of Proteinase K solution (20 mg/ml) was added to