Helicobacter pylori status and cell proliferation activity in chronic antral gastritis

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Background: Helicobacter pylori is known to cause antral gastritis and multifocal atrophic gastritis. In addition to its inflammatory effect, H. pylori has a direct effect on gastric mucosa. Increased epithelial proliferation, which may be an early biologic change in the development of gastric carcinoma, can be measured using silver stain for nuclear organizer regions (AgNOR). Aim: To detect the relation between H. pylori colonization and AgNOR index. Methods: One hundred and twenty consecutive antral endoscopic biopsy specimens from patients with dyspepsia were examined for H. pylori colonization, polymorphonuclear infiltrate, mononuclear infiltrate, germinal center formation, mucus depletion and AgNOR index. Results: AgNOR indices were not significantly related to grades of H. pylori colonization and chronic and active inflammation. The index increased significantly (p=0.03; ANOVA) with increasing mucin depletion. Conclusion: H. pylori colonization and presence of gastric antral inflammation are not related to cell proliferation activity; the latter is associated with mucin depletion. [Indian J Gastroenterol 2001;20:50-52] Key words: AgNOR, cell proliferation index

Antral gastritis and multifocal atrophic gastritis are strongly related to environmental factors, especially in poor countries. Although there are other etiologic factors, Helicobacter pylori infection is the most important.1 Increased epithelial proliferation may be an early biologic change in the pathogenesis of gastric carcinoma.2 Silver stain for nuclear organizer regions (AgNOR) is an indicator of cell proliferation activity; the latter is directly proportional to the number of AgNOR dots in a nucleus.3 Ki-67 is an intranuclear antigen expressed particularly in the G1, S and G2 phases of cell cycle.3

Recent reports have suggested that, in addition to its inflammatory effect, H. pylori has a direct effect on gastric mucosa.4 We therefore looked for a relation between H. pylori colonization and AgNOR index in order to assess the direct effect of H. pylori on the proliferative activity of gastric mucosa.

Methods

One hundred and twenty consecutive endoscopic antral biopsy specimens examined in our department during 1998 were included in this study. Specimens showing atypia, dysplasia or intestinal metaplasia were excluded. All patients (aged 20-80 [mean 48.5] years; 72 women) had complaints of dyspepsia. There was no history of non-steroidal anti-inflammatory drug intake or antibacterial therapy in the previous 6 months. One to 3 (mean 1.2) biopsies were obtained from each patient.

Hematoxylin-eosin stained slides were re-examined to evaluate polymorphonuclear leukocyte infiltration, mononuclear inflammation and germinal center formation. H. pylori colonization was quantified. Twenty-nine specimens were stained with Ki-67 (DAKO), as described elsewhere.2 PAS-alcian blue stain was used to evaluate mucin depletion as evidence of chronic mucosal irritation.

H. pylori colonization, chronic inflammation and mucin depletion were categorized into 4 grades each: none (0), mild (1), moderate (2) and severe (3). Active inflammation was graded as none (0), mild (1) and severe (2). Presence of germinal center-forming lymphoid follicles was evaluated as absent (0) or present (1).

AgNOR studies were done on formalin-fixed and paraffin-embedded tissue sections. Briefly, 3-μm-thick sections were cut, dewaxed and hydrated in deionized water. Later, sections were treated with freshly prepared solution consisting of 1 volume 2% gelatin in 1% aqueous formic acid and 2 volumes of 50% silver nitrate in a dark room at room temperature for 30 minutes. The sections were then washed with distilled water, dehydrated through graded alcohols to xylene, and mounted in a synthetic medium. AgNOR dots were counted in 200 nuclei from the glandular neck region, and number of AgNOR dots per nucleus was termed as AgNOR index. Ki-67 index was computed in 29 specimens by counting Ki-67-positive nuclei in 200 cells from the glandular neck region.

The AgNOR indices in different grades of H. pylori colonization, chronic inflammation, active inflammation, lymphoid follicles and mucin depletion were compared statistically by ANOVA. Relationship between Ki-67 index and AgNOR index was studied using Pearson's correlation coefficient.

Results

On microscopic examination, there was mononuclear inflammatory infiltration, consisting mostly of plasma cells, in the form of germinal centers or aggregates.
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Polymorphonuclear leukocytes were also identified in some areas. Fibrosis and smooth muscle strands were observed in the lamina propria. *H. pylori* colonization was detected on the luminal surface and in intraglandular areas.

Density of AgNOR dots decreased progressively from the glandular neck region to the surface (Fig). There was no significant difference in AgNOR index in relation to grades of *H. pylori* colonization, and of chronic and active inflammation (Table). The index increased significantly (p=0.03) with increasing grades of mucin depletion. There was no difference in AgNOR index in the 82 specimens with lymphoid follicles (6.3 [1.6]) as compared to the 38 cases without (6.0 [1.8]).

There was no correlation between Ki-67 and AgNOR indices (p=0.31).

**Discussion**

Early and frequent infection with *H. pylori* is common in populations with poor socioeconomic status; the prevalence in our population is around 80%. Persistent infection with *H. pylori* has been implicated in gastric carcinogenesis. The long-term effects of this infection progress from antral gastritis, through multifocal atrophic gastritis and intestinal metaplasia to gastric carcinoma. However, not all patients with *H. pylori* gastritis develop gastric carcinoma, and additional factors like infection at early age, vitamin C deficiency, dietary nitrite intake and genetic predilection have been incriminated.

The mechanism of irritation of the gastric mucosa by *H. pylori* is not clear. Neutrophils attracted by the organism release myeloperoxidase, which produces hypochlorous acid, yielding monochloramine in the presence of ammonia. Both hypochlorous acid and monochloramine cause gastric mucosal and endothelial damage. Several studies claim that *H. pylori* activates lymphocytes in peripheral blood and stimulates the release of cytokines including tumor necrosis factor and interferon-α. Fan *et al* reported that cytokines released from T lymphocytes increase cell proliferation in vitro. Hatz *et al* concluded that some products of these microorganisms pass over the epithelium and initiate chemotaxis and granulocyte activation.

Additionally, several studies suggest a direct effect of *H. pylori* on the gastric mucosa. Stachura *et al* implicated nitric oxide radicals synthesized by nitric oxide synthase enzyme released by *H. pylori*. Megraud *et al* claim that cytotoxicity is the result of direct contact of ammonia synthesized by urease enzyme because of the adhesion of the bacteria to epithelial surface. Recently, Tamura *et al* emphasized that ammonia made by the microorganism hastens the synthesis of superoxide.

Chronic irritation by *H. pylori* increases proliferation of cells and makes them more sensitive to mutation. This is concordant with the increased cell proliferation model in gastric carcinogenesis. Fan *et al* observed an increase in Ki-67 labeling by flow cytometry in gastric mucosa cells treated with *H. pylori* and interpreted this as the direct effect of the organism on gastric mucosa. On the other hand, Wagner *et al* reported that *H. pylori* caused in vitro inhibition of DNA synthesis.

Correa *et al* reported an increase in AgNOR index during acute inflammation, which declined with *H. pylori*-eradication therapy. We found an insignificant increase in AgNOR indices with increasing degree of active inflammation. We found no difference between *H. pylori*-positive and -negative cases. This discordance is probably because only 2 of 29 *H. pylori*-positive cases had active inflammation in their series. In our series, 32% of *H. pylori*-negative cases had active inflammation. Cahill *et al* also found significant difference in cell proliferation activity in antral biopsies with and without *H. pylori*. However, in their study, *H. pylori*-negative cases had normal antral mucosa. In contrast, Yabuki *et al* did not find any correlation between proliferation activity and *H. pylori* colonization.
The increase in AgNOR index with increasing mucin depletion that we observed suggests increased proliferation of cells by chronic irritation with inability to synthesize enough neutral mucin. The depletion starts at the glandular neck region and decreases progressively towards the surface.

Interestingly, there was no correlation between Ki-67 and AgNOR indices. Ki-67 protein can be detected in any cell that is not in G0 phase. AgNOR protein disappears in mitoses during prophase and reappears in telophase. Although quantitative analysis of cell proliferation rate and DNA content is not possible, AgNOR index is proportional to proliferation activity and DNA content. Therefore, AgNOR technique is a better indicator than Ki-67 immunostaining to evaluate proliferation activity.

In conclusion, our data suggest that H. pylori colonization and gastric antral inflammation do not affect cell proliferation activity. The increased cell proliferation in these patients is associated with mucin depletion.

References


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