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Alteration of E-Cadherin Expression in Gastric Mucosa: Role of Intestinal Metaplasia and *Helicobater pylori* Infection

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Abstract. *Background: Although E-cadherins have been involved in gastric carcinogenesis, their role in precancerous lesions, such as intestinal metaplasia, is still unclear. This study aimed to assess the role of both intestinal metaplasia and H. pylori infection on E-cadherin expression in gastric mucosa. Patients and Methods: Twenty-one consecutive patients with intestinal metaplasia were enrolled to assess E-cadherin expression in metaplastic areas. Twenty further patients without intestinal metaplasia, with and without H. pylori, were enrolled to evaluate the role of the infection on E-cadherin expression. All patients underwent upper endoscopy and gastric biopsies were taken for histological and immunohistochemical assessment. Results: A substantial reduction of E-cadherin expression in metaplastic areas was observed in 14 (67%) of the 21 patients, similarly in H. pylori-infected and uninfected patients (64% vs 71%, p=0.3). In the group without intestinal metaplasia, no reduction in E-cadherin expression was detected either in infected patients or in those without H. pylori infection. Conclusion: The data showed that intestinal metaplasia is associated with E-cadherin down-regulation, whereas H. pylori infection does not seem to play a direct role in this process.*

Despite a progressive reduction of incidence in previous decades, gastric cancer remains the second cause of cancer-related deaths in the world (1). Gastric carcinogenesis is a multistep process including both phenotypic and genotypic alterations (2). Defined phenotypic changes, starting with active chronic gastritis and progressing to atrophy, metaplasia and dysplasia, have been proposed to be involved in a cascade of events culminating in gastric cancer (3). In detail, intestinal metaplasia is widely recognised as being the most prevalent precursor of intestinal-type gastric carcinoma (4, 5). Among several molecular changes, abnormalities of both E-cadherin expression and related molecules – including α-, β- and γ-catenins – have been detected in gastric cancer (6-8). Indeed, cadherins are a family of transmembrane proteins which play a pivotal role in the epithelial intercellular adhesion exerting an invasion-suppressor function (9). Noteworthy, E-cadherin down-regulation seems to correlate with invasiveness of the tumour and with poor survival of patients (10, 11). Nevertheless, controversial data are available regarding the role of E-cadherin in precancerous lesions. A down-regulation of E-cadherin expression has been recently found in intestinal metaplasia (12, 13), whilst no significant alterations emerged from other studies (10,14). Moreover, all these studies were performed on surgical specimens of patients operated for gastric cancer, whereas no data are available in intestinal metaplasia without concomitant neoplastic lesions.

Among environmental factors involved in gastric carcinogenesis, the role of *Helicobacter pylori* infection is of increasing interest (15). This bacterium has been recognised as a definite type carcinogen (16) and it has been shown to significantly increase the risk of gastric cancer development (5). Indeed, several changes in gastric mucosa – such as epithelial cell hyperproliferation, free oxygen radical formation, genetic alterations and ascorbic acid reduction – have been described in infected patients (17-19). Moreover, *H. pylori* infection has recently been shown to cause a significant reduction in E-cadherin expression in the gastric mucosa of patients with either dyspepsia or peptic ulcer (20), although another study failed to confirm such a finding in early gastric cancer (10).

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Based on these controversial data, we designed the present study in order: a) to assess the E-cadherin expression in patients with intestinal metaplasia without either dysplasia or gastric cancer, and b) to further evaluate the role of *H. pylori* infection in the E-cadherin expression of gastric mucosa.

Patients and Methods

Patients. Consecutive patients with dyspeptic symptoms referred for upper endoscopy were considered for enrolment in the study. Two distinct groups of patients were selected. In order to assess the E-cadherin expression in intestinal metaplasia, a first group of patients with presence of intestinal metaplasia in the antrum without concomitant evidence of both dysplasia in the stomach or neoplastic lesions in the upper gastrointestinal tract, irrespective of *H. pylori* status, was selected. To evaluate the role of *H. pylori* on E-cadherin expression in gastric mucosa, a second group of patients, without intestinal metaplasia in any site of the stomach, was selected. For both groups, patients were excluded if they had taken proton pump inhibitors, H_2-receptor antagonists, antibiotics or NSAIDs in the four weeks preceding the study, as well as those previously treated for *H. pylori* infection. Patients with either liver impairment or kidney failure were also excluded.

Endoscopic procedure. After overnight fasting, all patients underwent upper endoscopy and 3 biopsies were taken in the antrum and 3 in the gastric body. Two biopsies from the antrum and 2 from the gastric body were used for histological assessment and for immunohistochemical analysis. The remaining two biopsies (one each from the antrum and gastric body) were used to carry out a rapid urease test (CP-test, Yamanouchi, Milan, Italy). *H. pylori* infection was considered present when both the histological assessment on Giemsa staining revealed the presence of bacteria and the rapid urease test was positive, as suggested in current guidelines (21).

Immunohistochemical staining. Immunohistochemistry was carried out by the avidin-biotin-peroxidase method. Contiguous sections, 4-5 μm thick, were obtained and stained with a specific antibody for E-cadherin detection 1:1000, Transduction Laboratories, Lexington, KY, USA), as described elsewhere (10). Briefly, sections were dewaxed, retrieved by microwave and rehydrated. Endogenous peroxidase activity and non-specific bindings were blocked by incubation with 3% hydrogen peroxide and nonimmune serum, respectively. Slides were incubated sequentially with primary mouse monoclonal antibody, at a dilution of 1:150, overnight at 4˚C with a biotinylated goat anti-mouse secondary antibody for 30 min and peroxidase-conjugated streptavidin for 10 min. The chromogen DAB test was performed to localise the positive staining by microscope. Negative control sections were prepared by substituting primary antibody with mouse IgG.

For the interpretation of data in the first group of patients with intestinal metaplasia, we applied the method previously described by Ma *et al.* (13). In detail, E-cadherin stain was evaluated by comparing the staining pattern of the intestinal metaplastic areas with that of the adjacent normal gastric mucosa. The expression of E-cadherin in the metaplastic area was considered 'normal' when both the intensity and the frequency of the cell membrane stains were equivalent to those found on the bordering nonmetaplastic gastric mucosa, 'reduced' when the staining was less than the adjacent mucosa, and 'negative' in the absence of staining.

For the interpretation of data in the second group of patients without intestinal metaplasia, we employed the method described by Terres *et al.* (20). In brief, the E-cadherin expression was graded as 'strong' or 'weak', based on the intensity of staining in the whole section.

Interpretation of the immunohistochemical stains was performed blindly by two independent observers. All sections for which the two observers disagreed were re-evaluated and, after discussion, a final agreement was achieved.

Statistical analysis. Data between patient subgroups were compared by using the Fisher's exact test with Yate's correction for small numbers. A p value less than 0.05 was considered statistically significant.

Results

E-cadherin expression and intestinal metaplasia. The study enrolled 21 patients with intestinal metaplasia in the antral mucosa. There were 10 males with a mean age 58 ± 7 years. At endoscopy no gastric lesion was observed, whilst two patients showed duodenal erosions. *H. pylori* infection was detected in 14 (67%) of the patients. Intestinal metaplasia was graded as complete type in all cases. The intensity of staining in the areas with intestinal metaplasia appeared to be substantially reduced in comparison to the staining of adjacent areas without intestinal metaplasia (Figure 1). In detail, a distinct reduction of E-cadherin expression in the metaplastic areas was observed in 14 (67%) of the 21 patients, whilst in the remaining 7 cases E-cadherin expression was equivalent in metaplastic and nonmetaplastic areas. No case of completely negative staining on metaplastic areas was observed in our series. Sub-grouping these patients on the basis of the presence of *H. pylori* infection, we found that E-cadherin expression in the metaplastic area was reduced in 9 out of 14 infected patients and in 5 out of 7 uninfected cases, without a significant difference between the two groups (64% *vs* 71%, $p=0.3$).

E-cadherin expression and H. pylori infection. The study enrolled 20 patients without intestinal metaplasia in the gastric mucosa. There were 8 males with mean age 55 ± 11 years. At endoscopy no gastric lesion was observed, whilst one patient showed duodenal ulcer. *H. pylori* infection was detected in 9 (45%) patients. *H. pylori* infection was confined in the antrum in 11 patients, whilst it also involved the gastric body in the remaining 9 patients. All patients with *H. pylori* infection showed chronic active gastritis, whilst a normal mucosa was observed in those patients without infection. The expression of E-cadherin was graded as 'strong' in all patients, irrespective of *H. pylori* status (Figure 2).

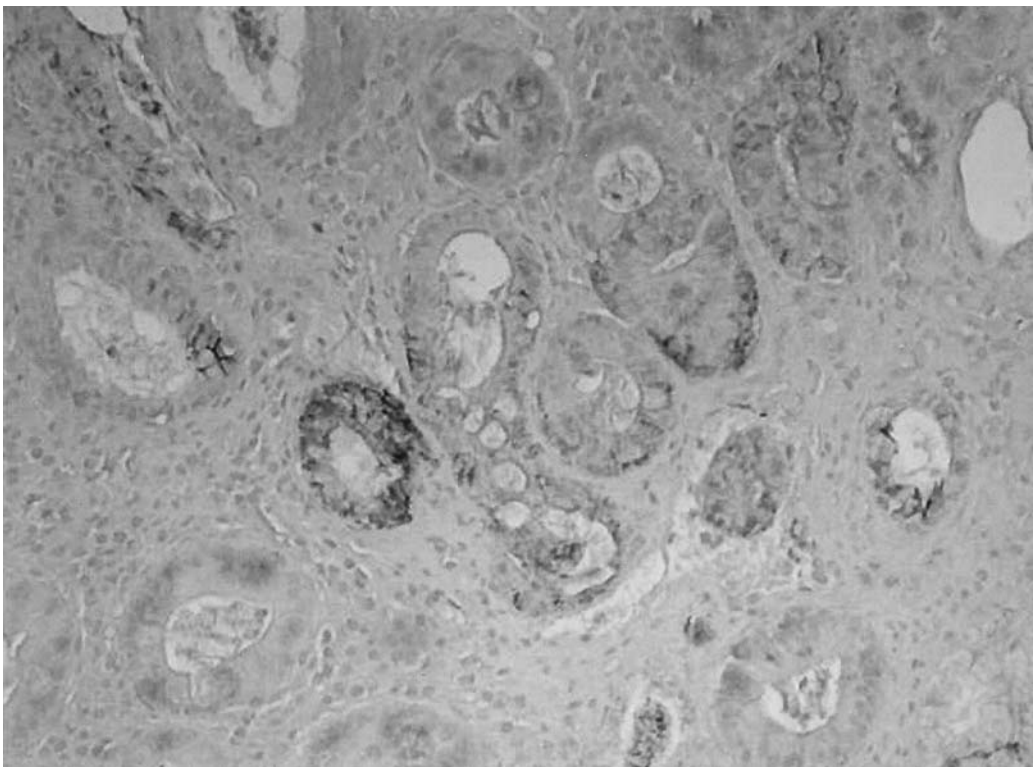

Figure 1. *Reduction of E-cadherin expression on metaplastic glands as compared with an adjacent normal gland. (Original magnification 20x).*

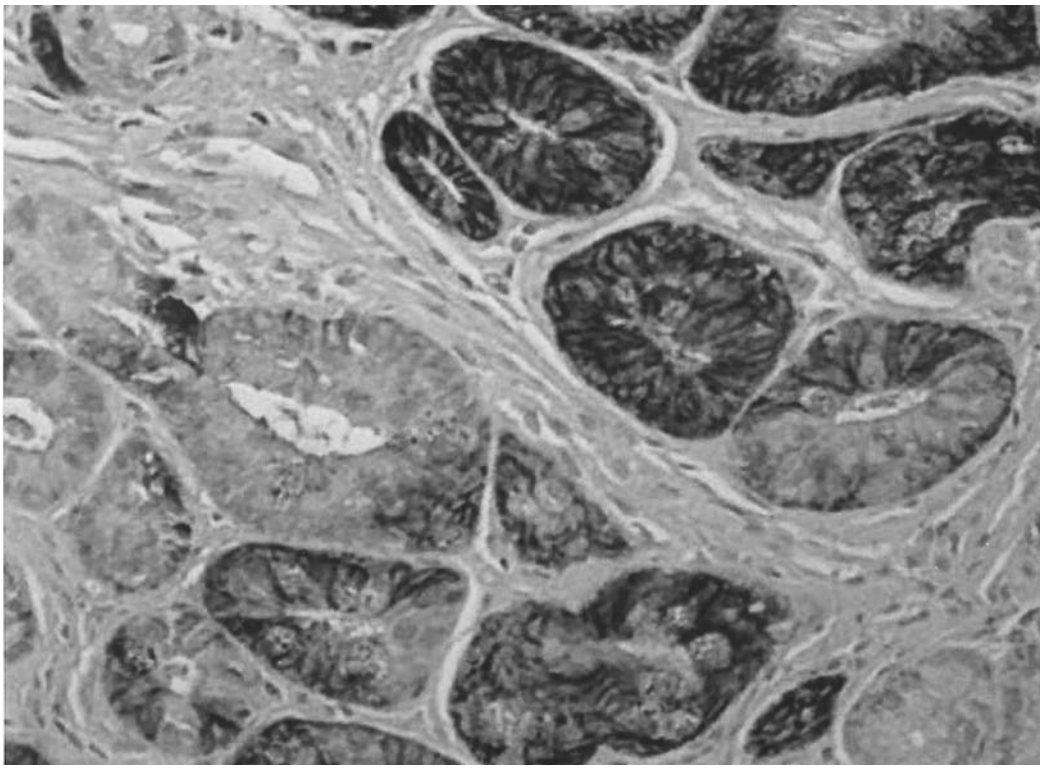

Figure 2. *Normal E-cadherin expression in gastric mucosa of patients without intestinal metaplasia. (Original magnification 20x).*

Discussion

The E-cadherin protein complex plays a critical role in the control of intercellular adhesion, which is essential for cell division, cell differentiation and architecture maintenance of normal epithelium (22, 23). Moreover, it is widely accepted that either loss or marked down-regulation of E-cadherin expression is implicated in the multi-step process of gastric carcinogenesis (9-14). Indeed, E-cadherin gene transfection in tumour cells has been shown to inhibit their invasiveness in experimental studies (24). Furthermore, some studies seem to show a relationship between E-cadherin alteration and poor long-term survival of patients with gastric cancer, presumably due to its role in favouring the development of metastases (9, 10). On the contrary, controversy still exists regarding the down-regulation of E-cadherin expression in gastric precancerous lesions, such as intestinal metaplasia. In detail, two previous studies found a reduction of E-cadherin expression in the metaplastic area of the gastric mucosa (12, 13), whilst a further two studies failed to confirm such an alteration (10, 14). However, these studies were mainly based on the gastrectomy specimens of patients with gastric cancer and, therefore, an advanced step of the carcinogenetic cascade was evaluated.

In the present study, we assessed the role of both intestinal metaplasia and *H. pylori* infection in triggering E-cadherin alterations in the gastric mucosa without coexisting dysplastic or neoplastic changes. As far as intestinal metaplasia is concerned, E-cadherin expression was found to be significantly reduced in the metaplastic areas of the majority of patients with such mucosal alteration. Subgrouping these patients according to *H. pylori* status, no difference in E-cadherin expression emerged between infected and uninfected cases. To our knowledge, this is the first endoscopic study in which such an immunohistochemical analysis was performed in patients harbouring intestinal metaplasia without concomitant neoplastic lesions in the upper gastrointestinal tract. Our findings, therefore, suggest that E-cadherin down-regulation is an early event in the gastric mucosa prior to development of gastric cancer. The role of the loss of E-cadherin expression in intestinal metaplasia is still unclear. In patients with intestinal metaplasia, both an epithelial cell hyperproliferation and a reduction of the apoptotic process have been clearly detected (25). Therefore, it would be intriguing to evaluate whether the loss of E-cadherin expression – reducing intercellular adhesion – could be involved in altering the apoptotic/proliferation balance. Furthermore, it could be speculated that the reduction of intercellular adhesion in the foci of intestinal metaplasia could facilitate the penetration of carcinogens into the gastric mucosa, thus favouring the carcinogenic process. Further studies are warranted in this field.

Our data showed that *H. pylori* infection did not affect E-cadherin expression in the metaplastic or in the normal gastric mucosa. Therefore, we failed to confirm the finding of a previous study, in which E-cadherin down-regulation was observed in 75% of *H. pylori*-positive patients as compared to 17% of uninfected patients (20). According to our data, down-regulation of E-cadherin expression seems to be linked to the presence of metaplastic changes not to *H. pylori*, although this infection is clearly implicated in the onset of intestinal metaplasia (26). A similar behaviour has been reported for epithelial cell proliferation in metaplastic mucosa which remains markedly increased even after *H. pylori* eradication (27), suggesting that some molecular modifications in intestinal metaplasia are, at least in part, irreversible.

In conclusion, our study showed that E-cadherin alteration can be identified in the early stages of intestinal metaplasia, before the development of more advanced neoplastic lesions, whilst *H. pylori* infection does not seem to play a direct role in this process.

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