Abstract. Background: First-degree relatives of gastric cancer patients have an increased risk of developing such neoplasia, and several alterations in gastric mucosa of these subjects have been described. On the other hand, both gastric cell hyperproliferation and abnormalities of adhesion molecules have been involved in gastric carcinogenesis. We studied gastric mucosa alterations in first-degree relatives of gastric cancer patients. Patients and Methods: This prospective, case-controlled study enrolled 39 first-degree relatives of gastric cancer patients and 39 matched controls. Biopsy specimens obtained at endoscopy were used to assess epithelial cell proliferation plus E-cadherin and β-catenin expression by immunohistochemical methods. H. pylori infection was assessed by histology and a rapid urease test. Results: Gastric epithelial cell proliferation values were not significantly different between the patient and control groups. H. pylori infection significantly increased cell proliferation values both in patients and in controls, without a significant difference between the two groups. Moreover, cell proliferation values were significantly higher in cases harboring intestinal metaplasia than in those without it. Alterations of the adhesion molecules were described exclusively in those patients harboring intestinal metaplasia. In detail, a reduction of both E-cadherin and β-catenin expression was observed in 8 (67%) out of 12 first-degree relatives and in 6 (67%) out of 9 controls with intestinal metaplasia (p=0.4). These alterations were similarly distributed between H. pylori infected and uninfected cases. Conclusion: Our data showed that a family history of gastric cancer itself is not associated with gastric cell hyperproliferation. However, both cell hyperproliferation and alterations of adhesion molecules have been detected in those patients with intestinal metaplasia.

Despite a progressive reduction of incidence in recent decades, gastric cancer remains one of the most frequent tumors worldwide (1), and it represents the second cause of cancer-related deaths in the world (2). Gastric cancer most probably results from an interaction between environmental factors and a genetic predisposition (3, 4). The importance of a family history as a risk factor for gastric cancer development has been pointed out in several studies (5-10). In detail, epidemiological observations have shown that subjects with a family history of gastric cancer have a 2.6-3.5-fold increased risk of developing such neoplasia (8, 9), and the attributable risk was calculated to be 8% (10). Moreover, in a single study, it has been shown that gastric epithelial cell proliferation was significantly increased in subjects with a family history of gastric cancer as compared to controls (11). Besides these observations, Helicobacter pylori infection has been recognised as a definite type I carcinogen (12), and it has been shown to significantly increase the risk of gastric cancer development (13-16). Indeed, several changes in gastric mucosa – such as epithelial cell hyperproliferation, free oxygen radical formation, ascorbic acid reduction, increased superoxide-dismutase activity and genetic alterations – have been described in infected patients (17-20). In addition, a possible synergistic effect between H. pylori infection and a family history of gastric carcinoma in the carcinogenic process has been suggested (21). Indeed, it has been shown that H. pylori infection causes pangastritis (22), atrophic gastritis with hypochlorhydria (23) and intestinal metaplasia (24, 25) – all being well recognized precancerous conditions – more frequently in these subjects than in controls. E-cadherin and related molecules, such as α-, β- and γ-catenins, are a family of transmembrane proteins which play a pivotal role in
epithelial intercellular adhesion, exerting an invasion-suppressor function (26). Abnormalities in these adhesion molecules have been involved in gastric carcinogenesis (27). Interestingly, their down-regulation seems to correlate with both tumor invasiveness and a poor patient survival (28-30).

In order to further assess alterations in the gastric mucosa of first-degree relatives of gastric cancer patients, we designed this prospective, case-controlled study, focusing on gastric epithelial cell proliferation, E-cadherin and β-catenin expression, and a possible relationship with H. pylori infection. In a consecutive first-degree relatives of gastric cancer patients, H. pylori infection was considered present when both histology (Giemsa staining) and rapid urease testing were positive. For the purposes of the study, only patients with a normal appearing gastric mucosa at endoscopy were selected, whereas those with either an active or a past history of gastric ulcer were excluded. Both patients and controls were excluded if they had been taking proton pump inhibitors, H2-receptor antagonists, or antibiotics in the four weeks preceding the study. Those frequently taking NSAIDs (more than 1 tablet/week) as well as alcohol abusers were also not included. All patients gave their informed consent to participate.

Patients and Methods

Patients. Consecutive patients complaining of dyspeptic symptoms and with a family history of non-cardia gastric carcinoma, referred to a single Endoscopy Unit for upper endoscopy, were taken into account. In detail, only first-degree (i.e. a parent or sibling) relatives of patients with gastric cancer were enrolled in the study. During the study period, matched patients without a family history of gastric cancer were selected as controls. Both patients and controls were included if they had been taking proton pump inhibitors, H2-receptor antagonists, or antibiotics in the four weeks preceding the study. Those frequently taking NSAIDs (more than 1 tablet/week) as well as alcohol abusers were also not included. All patients gave their informed consent to participate.

Endoscopic procedure. After overnight fasting, all patients underwent endoscopy with biopsies (2 samples from the antrum and 2 samples from the corpus) for histology and to look for H. pylori infection. A rapid urease test (1 sample from the antrum) was also carried out. At histology, intestinal metaplasia was recorded as present or absent. Two further biopsy specimens from the antrum were collected in order to assess epithelial cell proliferation plus E-cadherin and β-catenin expression by immunohistochemistry. H. pylori infection was considered present when both histology (Giemsa staining) and rapid urease testing were positive. For the purposes of the study, only patients with a normal appearing gastric mucosa at endoscopy were selected, whereas those with either an active or a past history of gastric ulcer and those with gastric erosions were not enrolled.

Immunohistochemistry. For E-cadherin, β-catenin and cell proliferation assessment, immunohistochemistry was carried out by the avidin-biotin-peroxidase method. The sections were deparaffinized in xylene and rehydrated through a graded alcohol series to distilled water. Antigen retrieval was performed by immersing the slides in 10 mM citrate buffer (pH 6.0) and heating them in a microwave for 3 cycles, 5 minutes each, at 750 Watts. Endogenous peroxidase activity and non-specific binding were blocked by incubation with 3% hydrogen peroxide and nonimmune serum, respectively. Sections were then incubated with monoclonal antibodies against E-cadherin (Clone 36, 1:2500 dilution, Transduction Laboratories, Lexington, KY, USA), β-catenin (Clone 14, 1:500 dilution; Transduction Laboratories) and monoclonal antibodies against Ki-67 (Clone MIB-1, 1:100 dilution, YLEM, Italy) for 1 hour at room temperature. Immunoreactivity was revealed with the chromogen DAB test and the sections were counterstained with Mayer’s haematoxylin solution for 7 minutes. Negative control sections were prepared by substituting primary antibody with buffered saline.

A semi-quantitative approach was used for scoring both E-cadherin and β-catenin expression, according to the method previously described by Ma et al. (31). Briefly, the staining pattern of the intestinal metaplastic areas was compared with that of the adjacent normal gastric mucosa. Expression of adhesion molecules in metaplastic areas was considered ‘normal’ when both the intensity and the frequency of the cell membrane stains were equivalent to those found on the bordering non-metaplastic gastric mucosa, ‘reduced’ when the staining was less than the adjacent mucosa, and ‘negative’ in the absence of staining. A quantitative approach was used for scoring the Ki67 expression. The number of cells was determined by counting the positively-stained nuclei on 10-20 randomly selected fields at 400x magnification.

All slides were reviewed by two observers who were unaware of both clinical and endoscopic data. All sections on which the two observers disagreed were re-evaluated and final agreement was achieved.

Statistical analysis. Data between patient subgroups were compared by using the Student’s t-test for unpaired data, and the Fisher’s exact test with Yate’s correction for small numbers, as appropriate. A p value less than 0.05 was considered statistically significant.

Results

A total of 78 patients were enrolled in the study. Thirty-nine consecutive first-degree relatives of gastric cancer patients...
were evaluated. H. pylori infection was detected in 19 (48.7%) of these patients. At histology, chronic active gastritis was observed in all the infected patients, whilst it was absent in the remaining 20 uninfected patients. Intestinal metaplasia was present in 14 out of 39 patients (35.8%), being significantly more frequent in patients with H. pylori infection than in uninfected patients (10/19 vs 4/20; p=0.037). For each patient, a control subject, matched for gender, age (±2 years), endoscopic finding, histological picture and presence of H. pylori infection was selected (ratio 1:1). As expected, there were no statistically significant differences between the two groups in terms of demographic and clinical characteristics (Table I).

**Gastric cell proliferation.** Gastric epithelial cell proliferation was assessed in all patients and controls. As shown in Table II, gastric epithelial cell proliferation values were not significantly different between the patient and control groups. Similarly, when the data of patients and controls were analyzed separately according to H. pylori infection, no significant difference in the proliferation index emerged between matched sub-groups. On the contrary, taking into account all subjects (patients and controls), infected cases showed significantly higher gastric epithelial cell proliferation values than those without infection (29.6±9.2 vs 16.6±6.4; p<0.0001). Moreover, H. pylori infection similarly increased cell proliferation values as compared to uninfected subjects, both in patients (27.9±8.9 vs 14.9±4.9; p<0.0001) and in controls (31.1±9.3 vs 18.2±7.4; p<0.0001) groups. Finally, our data showed that epithelial cell proliferation values were significantly higher in cases (patients and controls) harboring intestinal metaplasia than in those without it (30.4±9.9 vs 19.9±9.3; p=0.0001).

**E-cadherin expression.** Twenty-three first-degree relatives of gastric cancer patients and 23 controls were selected for evaluation of E-cadherin expression. The two groups were matched for age (56.5±11.5 vs 56.1±11.6 years; p=0.9), sex (M/F: 6/17 vs 9/14; p=0.09), presence of H. pylori infection (12/23 vs 11/23; p=0.2), and prevalence of intestinal metaplasia (12/23 vs 9/23; p=0.2).

A normal membranous staining for E-cadherin, primarily distributed in the apical-lateral membrane, was observed in all patients with either normal gastric mucosa or chronic active gastritis. On the contrary, a reduction in adhesion molecule expression was detected in patients harboring intestinal metaplasia. In detail, a reduction of E-cadherin expression in the metaplastic areas as compared to the adjacent glands was observed in 8 (67%) out of 12 first-degree relatives and in 6 (67%) out of 9 controls (p=0.4). No case of completely negative staining on metaplastic areas was observed in our series. Sub-grouping patients with intestinal metaplasia according to the presence of H. pylori infection, we found that E-cadherin expression in the metaplastic areas was similarly reduced in the infected cases (patients and controls) and those uninfected (9/14 vs 5/7; p=0.4). Moreover, no difference emerged between subjects with and without infection, both in patients (6/9 vs 2/3) and controls (3/5 vs 3/4) with intestinal metaplasia.

**β-catenin expression.** β-catenin expression was evaluated in the same 23 patients and 23 matched controls studied for E-cadherin. The β-catenin immunostaining was shown to be homogeneously distributed on the surface of epithelial cells in all patients with either normal gastric mucosa or chronic active gastritis, and no case of completely negative staining was observed in our series. On the contrary, a reduction of β-catenin expression was detected in gastric metaplasia. In detail, a reduction of β-catenin expression in the metaplastic areas was similarly observed in both first-degree relatives and controls (8/12 vs 6/9; p=0.4). Moreover, the reduction of β-catenin immunostaining in metaplastic areas was similarly observed in infected cases (patients and controls) and those uninfected (9/14 vs 5/7; p=0.4). Finally, no difference emerged between subjects with and without infection, both in patients (6/9 vs 2/3) and controls (3/5 vs 3/4) with intestinal metaplasia.

**Discussion**

Although there has been a remarkable and largely unexplained fall in the incidence of gastric cancer throughout the industrialized world over recent years, the fatality rate remains extremely high, positioned as the second most common cause of cancer-related death in the world (1, 2). A family predisposition for gastric cancer development has been clearly established in several studies (5-10). Moreover, a synergistic interaction between family predisposition and H. pylori infection has recently been put forward. Indeed, some histological alterations involved in gastric carcinogenesis – such as pangastritis, atrophic gastritis and intestinal metaplasia – have been encountered more frequently in the gastric mucosa of relatives of gastric cancer patients with H. pylori infection than in matched controls (22-25). A previous study found that gastric cell proliferation was significantly increased in 19 first-degree relatives of gastric cancer patients as compared to matched controls, irrespective of H. pylori infection (11). A persistent increase in cell proliferation is known to be a predisposing factor for cancer development, since a proliferating cell is more likely to be affected by endogenous and exogenous mutagens against its DNA stability (4, 17). In the present study, however, a family history of gastric cancer did not seem to significantly affect gastric cell proliferation. Although the discrepancy between our data and those previously reported is unclear, some hypotheses may be put forward. In the
previous report (11), patients and controls were not matched for intestinal metaplasia prevalence, which was estimated to be two-fold higher in relatives of gastric cancer than in matched controls in another study by the same authors (24). It is well known that intestinal metaplasia itself is associated with a hyperproliferative status (32, 33), as further confirmed in the present study. Therefore, it might be that the previously observed hyperproliferative status in patients with a family history of gastric cancer may be simply due to a higher prevalence of intestinal metaplasia in these subjects (24). In our series, in which patients and controls were matched for the presence of intestinal metaplasia, such a difference in proliferative status did not emerge. On the other hand, in agreement with the results of several studies (19, 32-34), we found that H. pylori infection significantly increased cell proliferation in the gastric mucosa. Moreover, we observed that H. pylori presence increased cell proliferation values to a similar extent in both first-degree relatives and controls. Therefore, the gastric mucosa of first-degree relatives of gastric cancer patients seems to behave similarly to that of controls in terms of its epithelial cell proliferation during H. pylori infection.

Mutations of the CDH1 gene encoding for E-cadherin have been reported in both hereditary (27) and sporadic forms of gastric cancer (35). Altered expression of adhesion molecules, such as E-cadherin and β-catenin, appears to correlate with a poor prognosis in gastric cancer patients (28-30). Moreover, alterations of E-cadherin and β-catenin expression have been detected in precancerous conditions such as intestinal metaplasia (31, 36-39), although the data are still controversial (29, 40-42).

This is the first study assessing the expression of E-cadherin and β-catenin in gastric mucosa of first-degree relatives of gastric cancer patients. Our data show that alterations in the membranous staining for both E-cadherin and β-catenin were confined to those patients harboring intestinal metaplasia. However, such reduction appeared to be similarly distributed between first-degree relatives of gastric cancer patients and matched controls. Our findings regarding both E-cadherin reduction and β-catenin expression in patients with intestinal metaplasia are in agreement with previous studies (31, 36-39).

Regarding a possible relationship between alterations of E-cadherin expression in gastric mucosa and H. pylori infection, conflicting data are available in the literature. A previous study reported a significant association between E-cadherin expression and H. pylori infection (43), whereas another study failed to confirm such a finding (44). In the present study, we investigated for a possible relationship between either E-cadherin or β-catenin expression and H. pylori infection. Our data showed that the prevalence of alterations of either adhesion molecule did not significantly differ between infected and uninfected patients, suggesting that H. pylori infection does not seem to be directly involved in such alterations. However, it should be underlined that H. pylori is clearly involved in the onset of intestinal metaplasia, which in turn appeared to be associated with cell hyperproliferation as well as with both E-cadherin and β-catenin reduction. Therefore, the previous observation of there being different bacterium-host interactions between patients with a family history of gastric cancer versus controls, resulting in a significantly higher prevalence of intestinal metaplasia triggered by H. pylori infection in these patients, could explain, at least in part, the higher risk of gastric cancer described in epidemiological studies.

In conclusion, our data found that a family history of gastric cancer itself is not associated with gastric cell hyperproliferation. In first-degree relatives of gastric cancer patients, however, the higher prevalence of H. pylori-related intestinal metaplasia (24, 25), which we proved to be linked with both cell hyperproliferation and alteration of adhesion molecules, could be involved in the carcinogenic process together with other identified molecular alterations (45-47).

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References


