The role of E-cadherin expression and E-cadherin gene promoter hypermethylation in gastric carcinoma

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Background
Gastric cancer is the third fatal cancers worldwide after lung cancer and liver cancer. Various studies have been launched to detect the markers helping predict the prognosis of different forms of this disease, thus, modifying the treatment regimen accordingly. The aim of this study was to define the role of the E-cadherin protein in such an important issue.
Patients and methods
Sixty-four patients with gastric carcinoma were included in this study. For each case, representative sections from the tumor tissue and the adjacent noncancerous tissue were assessed for E-cadherin protein expression by immunohistochemistry and promoter methylation by the methylation-specific PCR analysis technique. The results were correlated with *Helicobacter pylori* positivity and other clinicopathological variables.
Result
The frequencies of reduced E-cadherin expression and E-cadherin gene methylation were significantly higher in diffuse than intestinal-type gastric carcinoma. However, no significant relationship was found when being correlated to the T-stage and the N-stage of the corresponding lesions.
Conclusion
Reduced E-cadherin expression and E-cadherin methylation are common alterations in gastric cancer. These gene alternations facilitate cell invasion and metastatic spread with no significant correlation to the T-stage nor the N-stage of the tumor.

Keywords:
E-cadherin deletion, E-cadherin promoter hypermethylation, gastric adenocarcinoma

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Introduction

Gastric cancer is one of the leading lethal cancers worldwide. Among gastric tumors, carcinoma is the most important and the most common. Next in order of frequency are lymphomas and carcinoids and mesenchymal spindle cell tumors [1]. Numerous studies have established the role of *Helicobacter pylori* infection in gastric carcinogenesis [2–4]. However, the exact pathway of *Helicobacter*-induced gastric cancer is mostly unknown [5], although it was suggested that the pathophysiology of this process is via induction of P 53 mutation, disruption of E-cadherin/catenin-containing adherens junctions, and upregulation of antiapoptotic genes [6].

E-cadherin, a 120 KD transmembrane glycoprotein, is the prime mediator of cell–cell adhesion [7]. The E-cadherin gene (CDH1) is located on chromosome 16q22. The loss of E-cadherin-mediated cell adhesion correlates with the loss of the epithelial morphology and with the acquisition of metastatic potential by the neoplastic cells [8]. Inactivation of E-cadherin occurs in 51% of gastric carcinomas with a high percentage in the diffuse type [9,10]. Therefore, it is suggested that E-cadherin gene mutation plays a major role in the molecular evaluation of gastric cancer, especially the diffuse type [11].

It was found that downregulation of E-cadherin can also occur by hypermethylation of the promoter region of E-cadherin and may lead to altered tumor cell behavior [12–14].

More importantly, *H. pylori* is an independent risk factor associated with the methylation of E-cadherin in gastric mucosa from patients with dyspepsia [15].

A possible future role for E-cadherin had been postulated in screening for premalignant conditions of the esophagus. Since obtaining histological biopsy is a must in the diagnosis of gastric cancer [16], this study was designed with the aim of studying the relation between *H. pylori* and gastric carcinoma through

studying the effect on E-cadherin protein expression and E-cadherin gene promoter hypermethylation.

Patients and methods

After taking the approval of the ethical committee at our hospital. Sixty-four patients diagnosed with operable and resectable gastric carcinoma (T1–4, N0, 1, M0), presenting at the El-Demerdash Hospital, Ain Shams University Specialized Hospital, and El-Matareya Teaching Hospital were included in this prospective study.

Information about the patients regarding age, sex, and metastatic status were recorded. All patients were submitted to a diagnostic upper gastrointestinal endoscopy. Computed tomography with double contrast of the chest, abdomen, and pelvis were done for all cases. All patients were prepared for surgery. Intraoperatively, the site of the tumor, either fundus, body, or pylorus was specified. Resected surgical specimens of the tumor itself with safety margins and adjacent noncancerous tissues away from the tumor were examined at the Pathology Department, Faculty of Medicine, Ain Shams University. The resected specimens were classified according to the TNM classification and categorized according to the Lauran classification into intestinal and diffuse type. Patients were excluded from the study if they have received neoadjuvant therapy and if the histopathological type of the tumor was anything other than carcinoma, that is, lymphoma or sarcoma. Also, patients with recurrent cancers were excluded.

All the specimens were submitted to histopathological, immunohistochemical, and molecular studies for CDH1 gene methylation.

In the histopathological examination, all tumor tissue sections were examined to determine the (a) histological tumor type; intestinal or diffuse, (b) histological grade, (c) the degree of tumor extension to determine the pathologic T-stage of the primary tumor, and then (d) the lymph nodes were examined to assess the presence of lymph node metastasis (pathologic N-stage). Then, adjacent noncancerous tissues were examined to assess the (a) presence of *H. pylori* by examination of Giemsa-stained slides and (b) the presence of intestinal metaplasia or dysplasia.

Following that, immunohistochemical studies started on the neoplastic and nonneoplastic gastric tissues to detect the E-cadherin antigen. After that, molecular studies started to determine the methylation status of the DNA sequence of the CDH1 gene.

Results

In this study, we assessed the role of E-cadherin in the pathogenesis of gastric adenocarcinoma and hence, its role in determining the prognosis of such cases thus modifying the treatment decision and adopting new means of screening accordingly. Sixty-four patients with gastric carcinoma were enrolled in the study including 42 men and 22 women with a male to female ratio of 1.9 : 1. Their age ranged from 27 to 79 years with a mean of 53.2±14.1 years. Adjacent nonmalignant tissue for each case was taken as the control.

The demographic data of the studied group is shown in Table 1.

Surgical findings

On examining the resected specimens of different parts of the stomach grossly, it was found that gastric carcinoma occurred as 60% (38 cases) in the pylorus and antrum, 15% (10 cases) in the body, and 25% (16 cases) in the fundus (Table 2).

Histological findings

Forty-two out of the 64 gastric cancer cases (65.5%) were of the intestinal type (Fig. 1) and 22 (34.4%) were of the diffuse type (Fig. 2). The tumors of the intestinal type were of histological grade 1 (12 cases), grade 2 (28 cases), and grade 3 (two cases). Pathological T staging was as follows: pT2=24 cases, pT3=28 cases, and pT4=12 cases. Forty-four (68.75%) cases of tumor tissues showed lymph node metastasis.

The histological features of the gastric carcinomas are enlisted in Table 3.

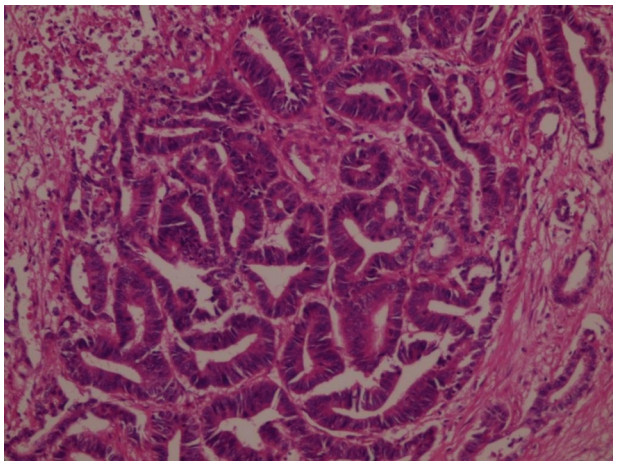
Table 1 Demographic data of the 64 studied patients with gastric carcinoma

Variables	*N* (%)
Sex	
Male	42 (65.6)
Female	22 (34.4)
Age	
≤50	24 (37.5)
>50	40 (62.5)
Mean±SD	53±14

Table 2 Percentage of gastric carcinoma

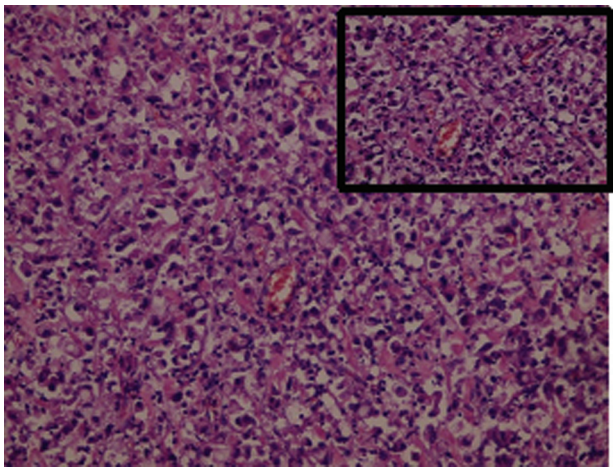
	N (%)
Pylorus and antrum	38 (60)
Body	10 (15)
Fundus	16 (25)

Figure 1



A case of gastric adenocarcinoma, intestinal type (hematoxylin and eosin, ×200).

Figure 2

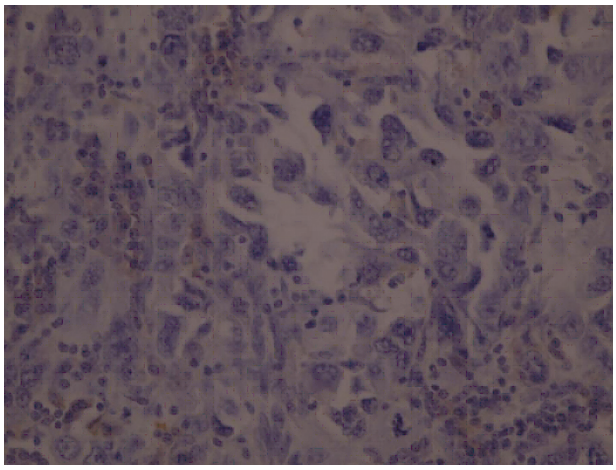


A case of gastric adenocarcinoma, diffuse type (hematoxylin and eosin, ×200, inset ×400).

Table 3 Histological features of 64 gastric cancer cases

Variables	*n* (%) (N=64)
Histological type	
Diffuse	22 (34.5)
Intestinal	42 (65.5)
Grade	
Grade 1	12 (18.75)
Grade 2	28 (43.75)
Grade 3	2 (3.12)
Pathological T-stage	
T2	24 (37.5)
T3	28 (43.7)
T4	12 (18.8)
Pathological N-stage	
N0	20 (31.3)
N1	44 (68.7)

Figure 3



A case of gastric carcinoma, diffuse type, showing reduced E-cadherin expression (no membranous staining). Immunohistochemistry (×400).

All noncancerous tissue samples showed chronic gastritis. *H. pylori* organisms were detected in 22 of the 64 tissue specimens (34.5%), intestinal metaplasia was found in 20 (31.25%) cases, and dysplasia was noted in 16 (25%) cases.

E-cadherin protein immunohistochemistry and gene methylation

Reduced E-cadherin expression was noted in 28 of the 64 gastric cancer cases (43.8%) (Figs 3, 4). The remaining 36 (56.2%) cases showed preserved E-cadherin expression (Figs 5, 6). On the other hand, E-cadherin gene methylation was detected in 22 (34.4%) cases (Fig. 7). No significant relationship was obtained between E-cadherin protein expression and promoter methylation (Table 4).

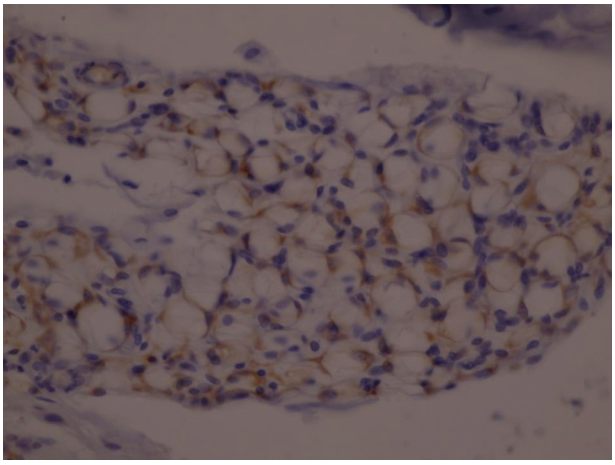
In the noncancerous tissues

Preserved membranous expression of E-cadherin antigen was noted in all the 64 (100%) tissue specimens including those with *H. pylori*, intestinal metaplasia, or dysplasia (Fig. 8). However, E-cadherin gene methylation was detected in 36 (50.6%) specimens. E-cadherin was methylated in 12 out of 22 *H. pylori* positive cases (54.54%). Half of the tissue specimens with intestinal metaplasia (50%) and 10 of the dysplastic lesions (62.5%) had methylated E-cadherin (Table 5).

Relationship between E-cadherin protein expression and clinicopathological variables

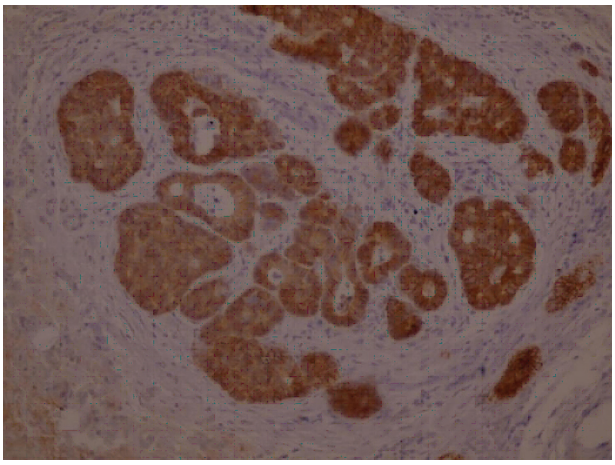
Reduced E-cadherin expression was significantly more frequent in the diffuse-type gastric carcinomas than the intestinal type (P=0.02). No significant relationship was detected between E-cadherin expression and other variables (Table 6).

Figure 4



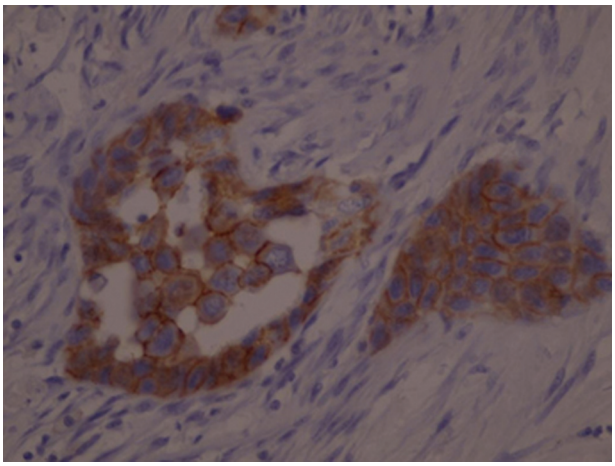
A case of gastric carcinoma, diffuse type showing reduced E-cadherin expression (membranous staining in <60% of tumor cells). Immunohistochemistry (×400).

Figure 5



A case of gastric carcinoma, intestinal type, showing preserved E-cadherin expression (membranous staining in >60% of tumor cells). Immunohistochemistry (×100).

Figure 6

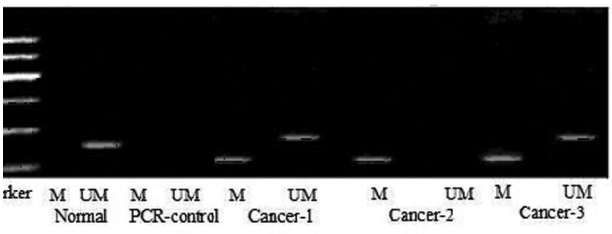


Previous case, higher power (×400).

Table 4 Relationship between E-cadherin protein expression and gene methylation in the 64 gastric carcinoma cases

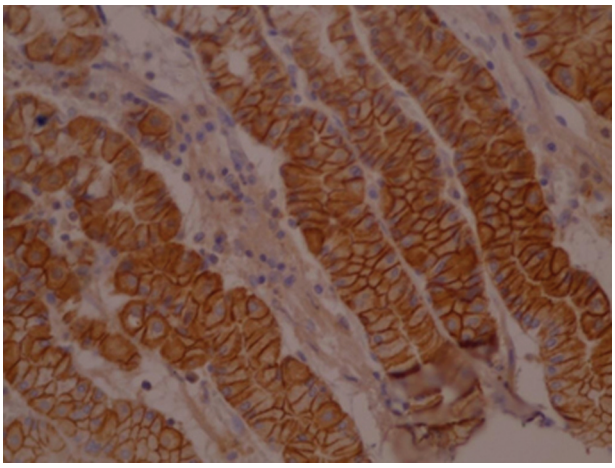
Methylation status	Total number [*n* (%)]	E-cadherin protein expression		*P* value
		Preserved	Reduced	
Unmethylated	42 (34.4)	26	16	0.289
Methylated	22 (65.6)	10	12	

Figure 7



Methylation-specific PCR showing first lane (column): a hundred base pair ladder (molecular weight marker), second and third lanes: normal gastric mucosa sample showing the unmethylated (UM) band only at 180 base pair (bp), fourth and fifth lanes: PCR control containing no DNA, sixth to 11th lanes: cases of GC showing the methylated (M: 120 bp) and unmethylated (UM: 180 bp) bands.

Figure 8



Noncancerous gastric tissue showing preserved E-cadherin expression, (membranous staining in <60% of cells). Immunohistochemistry (×200).

Table 5 Gene methylation in 64 noncancerous tissue samples

Methylation status	Total [*n* (%)]	*H*elicobacter *pylori* positive	Intestinal metaplasia	Dysplasia
Unmethylated	28 (49.1)	10 (45.46)	10 (50)	6 (37.5)
Methylated	36 (50.9)	12 (54.54)	10 (50)	10 (62.5)
Total	64	22	20	16

Table 6 Relationship between E-cadherin protein expression and clinicopathological variables

Variables	E-cadherin		χ^2	*P*
	Preserved	Reduced		
Sex				
Male	22	20	0.01	0.81
Female	12	10		NS
Age				
≤50	14	10	0.07	0.794
>50	22	18		NS
Helicobacter pylori in the adjacent tissue				
Negative	20	22	Fisher	0.067
Positive	16	6		NS
Histologic type				
Diffuse	8	14	5.39	0.020*
Intestinal	28	14		S
Histologic grade				
Grade 1	10	2	2.31	0.129
Grades 2 and 3	17	12		NS
Pathologic T-stage				
T2 and 3	28	24	0.65	0.419
T4	8	4		NS
Nodal status				
N0	10	10	0.46	0.497
N1	26	18		NS

S, significant.

Table 7 Relationship between E-cadherin gene methylation and clinicopathological variables

Variables	E-cadherin		χ^2	*P*
	Unmethylated	Methylated		
Sex				
Male	28	14	Fisher	0.41
Female	12	10		NS
Age				
≤50	10	14	Fisher	0.003
>50	32	8		S
Helicobacter pylori in the adjacent tissue				
Negative	32	10	6.04	0.014
Positive	10	12		S
Histologic type				
Diffuse	10	32	6.04	0.014
Intestinal	12	10		S
Histologic grade				
Grade 1	10	2	0.002	0.965
Grades 2 and 3	24	5		NS
Pathologic T-stage				
T2 and 3	36	16	0.002	0.965
T4	6	6		NS
Nodal status				
N0	12	8	fisher	0.5772
N1	30	14		NS

S, significant.

Relationship between E-cadherin gene methylation and clinicopathological variables

The frequency of E-cadherin gene methylation was significantly higher in the younger age group (*P*=0.003).

Gastric carcinomas with *H. pylori* positive cases in the noncancerous tissues had a significantly higher rate of E-cadherin gene methylation than those with *H. pylori* negative cases (*P*=0.014). As in the case of E-cadherin protein expression, methylated E-cadherin was significantly more frequent in diffuse type than intestinal-type gastric carcinomas (*P*=0.014). No significant association could be obtained between E-cadherin methylation and other clinicopathological factors (Table 7).

Discussion

Gastric cancer is one of the most common malignancies worldwide. It has a high rate of mortality mainly due to metastatic spread [17]. In Egypt, it constitutes 1.6% of all cancer [18]. Gastric carcinogenesis is a multistep process with the sequence: intestinal metaplasia – dysplasia – invasive carcinoma [19]. This sequence involves a set of genetic and epigenetic events [20].

E-cadherin gene is a tumor suppressor gene expressed in the epithelial cells. Its main function is to maintain cell-to-cell adhesion [21]. E-cadherin gene alterations and the silencing of E-cadherin expression by the methylation of the E-cadherin promoter are frequent findings in gastric cancer [9,22]. It is claimed that these gene alternations facilitate cell invasion and metastatic spread [23,24].

The main risk factor for gastric cancer has been attributed to infection by *H. pylori* [25].

The aim of this study was to assess the relationship between *H. pylori* infection in gastric carcinoma and E-cadherin status. We also tested the association between E-cadherin abnormality and other clinicopathological factors.

Lastly, E-cadherin alteration was investigated in the noncancerous cases to demonstrate its frequency in intestinal metaplasia and dysplasia.

Reduced expression of E-cadherin protein was detected in 43.8% of our gastric carcinoma cases. A wide variation in the percentage of reduced E-cadherin expression in gastric carcinomas ranging from 19 to 85% has been reported (26–35). Although several methodological variables may explain these differences, we speculate that the most important cause is the discrepancy in the interpretation of E-cadherin immunostaining results and the cutoff levels

used for classification into preserved and reduced expression. Therefore, comparison of immunohistochemical results in the literature necessitates standardization, not only methodology, but also interpretation of the results.

In the current study, about one-third of gastric carcinomas showed E-cadherin gene methylation. This frequency approximates that obtained by Graziano *et al.* [36] who found E-cadherin promoter methylation in 28.6% of their 70 gastric carcinoma cases. However, other studies reported much higher rates of E-cadherin methylation in gastric carcinomas [29,30,37–39]. This difference may be attributed to the different methods used for methylation analysis or population variation.

The most important result in the current study was the significant association between *H. pylori* infection in the adjacent nonneoplastic tissue and the presence of E-cadherin promoter methylation in the cancerous tissue. This finding supports the study of Ferrasi *et al.* [39] who observed that in the intestinal gastric adenocarcinoma, methylation in the E-cadherin promoter was more frequent in the group of *H. pylori* Cag A (positive) than in those with *H. pylori* Cag A (negative). The role of *H. pylori* in the regulation of E-cadherin expression has been described in some studies, showing that after eradication of *H. pylori*, E-cadherin methylation is decreased [40,41]. Chan *et al.* [29] proposed that *H. pylori* might cause E-cadherin mutation and this mutation can be one of the initial changes in gastric carcinogenesis. It is worth mentioning that the studies of Chan *et al.* [29] and Anbiaee *et al.* [33] could not observe an association between *H. pylori* infection and E-cadherin protein expression or methylation in gastric cancer. The authors explained this lack of association by the possibility that *H. pylori* infection may disappear with the development of gastric cancer as a consequence of possible changes in the milieu of neoplastic cells which renders them unsuitable for the presence of *H. pylori*. Therefore, we preferred to examine the presence of *H. pylori* in the adjacent noncancerous tissue rather than in the neoplastic tissues to avoid the possibility of absence of *H. pylori* organisms in the cancerous tissue.

Another important finding in our study was the significant association between the histological tumor type and both E-cadherin protein expression and promoter methylation. In this context, the diffuse gastric carcinoma showed a significantly higher frequency of reduced E-cadherin expression (P=0.02) and promoter methylation than the intestinal type (P=0.01). This result agrees with other studies which suggested that inactivation of E-cadherin may induce the dissociation of tumor cells due to loss of intercellular adhesions [27,28].

The pathological T-stage of the tumor and the lymph node metastasis were not significantly related to the protein expression or the gene methylation of E-cadherin in gastric carcinoma in our study. Although these data are in concordance with several studies [27,31,33,37,39], it contradicts with others [28,32]. Again, these discrepancies between different studies may be due to differences among ethnic populations, limited number of cases, or the different methods used [28]. On the other hand, these contradictory results may point to the fact that gastric carcinogenesis is a complex process and could be induced as a consequence of genetic aberrations in several genes or pathways. This necessitates larger profiling studies which permit the examination of several genes in a single or multiple genetic pathways.

For the assessment of the relationship between the age group and E-cadherin status, we used 50 years as the cutoff age which is being increasingly used to define the young age group [34,38,39]. It was interesting to note that methylation of E-cadherin is significantly higher in the younger age group (P=0.003). This is consistent with the study of Schildberg *et al.* [34] who demonstrated that the expression of E-cadherin is significantly downregulated in the younger age group. The authors concluded that this association would normally be linked to a poorer outcome.

An analysis of the noncancerous tissue has shown that 50% of the cases with intestinal metaplasia (10/20) and 62.5% of the dysplastic lesions (10/16) had methylated E-cadherin. However, immunohistochemistry showed normal membranous staining for E-cadherin in all noncancerous tissue samples with or without gene methylation. Our findings are consistent with those of Chan *et al*. [29] who found E-cadherin methylation in 57% of cases of intestinal metaplasia, while E-cadherin immunostaining was normal in all cases. Similarly, Zheng *et al.* [42] reported E-cadherin methylation in 78% of dysplastic gastric lesions with normal membranous staining in all cases. Chan *et al.* [29] commented that immunohistochemistry may not be as sensitive as PCR in detecting the subpopulation of cells with gene methylation and hence downregulation of E-cadherin. The presence of E-cadherin promoter methylation in the nonneoplastic gastric tissue suggests that this alteration is involved

in the early steps of gastric carcinogenesis. Also, it suggests the possibility of innovative chemopreventive strategies aiming at the preservation of E-cadherin function [23].

Our study did not find a significant correlation between the reduced E-cadherin protein expression and its gene methylation. The reason for this discordance has been clarified by Kim *et al.* [38] who reported a significant association between E-cadherin methylation and reduced expression in gastric cancer. However, they found that seven tumors with E-cadherin methylation maintained E-cadherin expression and 14 tumors lacking E-cadherin methylation showed reduced or absent E-cadherin expression. This genotype-phenotype discordance in 23.3% (21/90) of their cases was explained at the molecular level as follows: the methylation may occur in E-cadherin allele carrying an inactivating somatic mutation, while the function of the remaining E-cadherin allele is preserved. In this case, E-cadherin protein expression was maintained despite positive methylation analysis. Conversely, if somatic mutations inactivate both E-cadherin alleles and/or alternative molecular mechanism knock out E-cadherin, a loss of E-cadherin expression can occur even in unmethylated tumors [38].

Conclusion

Silencing of the E-cadherin gene by promoter methylation contributes, at least partially, to the development and progression of *H. pylori*-associated gastric carcinoma. The frequency of reduced E-cadherin expression and methylation are significantly higher in diffuse-type gastric carcinoma than the intestinal type and in the younger age group, suggesting a poorer outcome. However, E-cadherin protein expression and the gene methylation are not related to the pathologic T-stage or lymph node metastasis. E-cadherin methylation may be involved in the early steps of gastric carcinogenesis, suggesting a role for treatment with demethylating drugs.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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