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Original Article

E-Cadherin Expression in Patient with Gastric Carcinoma in Relation to Histological Prognostic Parameters

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Abstract

Background: E-cadherin (epithelial-cadherin), encoded by the CDH1 gene, is a transmembrane glycoprotein playing a crucial role in maintaining cell-cell adhesion. E-cadherin has been reported to be a tumor suppressor and to be down regulated in gastric cancer.

Objective: The aim of this study is to find out the expression of E-cadherin in patients with gastric carcinoma and it's relation to histological prognostic parameters. This study also aimed to investigate the morphological parameters of prognostic value: histologic type and tumor grading, depth of invasion, lympho-vascular invasion, perineural invasion and lymph node metastasis.

Methods: This descriptive cross sectional study was carried out from March, 2015 to June, 2017 at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The study materials were gastric tissue taken from 42 patients who underwent total or partial gastrectomy operation in Surgery Department of BSMMU and few private hospitals and diagnosed as gastric carcinoma in Pathology department of BSMMU.

Result: Among 42 cases, 26 were male and 16 were female with a male/female ratio of 1.6:1. Thirty two samples were total gastrectomy specimen and twelve samples were partial/subtotal gastrectomy specimen. Ages of the patients ranged from 20 to 76 years and the highest number(33.3%) of cases belonged to 50-59 years age group. This study showed E-cadherin expression did not show association with lymphovascular invasion, perineural invasion and depth of invasion. The rate of E-Cadherin positivity shows association with histological type, histological grading and lymph node metastasis.

Conclusion: Although alterations in E-cadherin and its expression may serve as promising biomarkers or therapeutic targets in gastric cancer but in this study E-cadherin expression was significantly more frequent in comparison with the histological type, histological grading and lymph node metastasis.

Keywords: E-cadherin, Gastric Carcinoma, Histological parameter

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Introduction

Gastric carcinoma is a malignancy that arises from the gastric mucosa, and though its incidence and mortality rates have fallen dramatically over the past 70 years, stomach cancer is the fourth most prevalent cancer and the second most common cause of cancer death worldwide.[1,2]

Gastric cancer is a multifactorial disease. Epidemiological studies point to a role for H. pylori, although its importance is disputed. H. pylori seem to be principally associated with carcinoma of the body of the stomach and the distal stomach rather than the proximal stomach. As Helicobacter is associated with gastritis, gastric atrophy and intestinal metaplasia, the association with malignancy is perhaps not surprising. Several other risk factors have been identified as being important in the etiology of gastric cancer. Patients with pernicious anemia and gastric atrophy are at increased risk, as are those with gastric polyps. Patients who have had peptic ulcer surgery, particularly those who have had drainage procedures such as Billroth II or Polya gastrectomy, gastroenterostomy or pyloroplasty are at approximately four times the average risk. Carcinoma is associated with cigarette smoking and dust ingestion from a variety of industrial processes. Diet appears to be important, as illustrated by the often quoted example of the

change in the incidence of gastric cancer in Japanese families living in the USA. Excessive salt intake, deficiency of antioxidants and exposure to N-nitroso compounds are also implicated. Genetic factors are also important but imperfectly elucidated.[3]

The most useful classification of gastric cancer is the Lauren classification. In this system there are principally two forms of gastric cancer: intestinal gastric cancer and diffuse gastric cancer. In intestinal gastric cancer, the tumor resembles carcinoma found elsewhere in the tubular gastrointestinal tract and forms polypoid tumors or ulcers. In contrast, diffuse gastric cancer infiltrates deeply into the stomach without forming obvious mass lesions but spreading widely in the gastric wall.[3]

Cadherin is a superfamily of calcium-mediated membrane glycoproteins, forming one of the four classes of adhesion molecules. Some common cadherins expressed by epithelial cells are E-cadherin, N-cadherin, and P-cadherin.[4] The cadherins are responsible for the homotypic cell-cell adhesion. In carcinogenesis, the tumour cell has to dissociate from one another before it can invade or metastasize. Therefore, these adhesion molecules are thought to play an important role this has a much worse prognosis. E-cadherin is expressed in all epithelial cell types. Under-expression of the E-cadherin is found in gastric, hepatocellular, esophageal, breast, prostatic, bladder and gynaecological carcinomas and correlates with infiltrative and metastatic ability.[4] It has been postulated that the under-expression of E-cadherin in these tumours may account for their invasive potential and appear to be a late event.

E-cadherin (epithelial-cadherin), encoded by the CDH1 gene, is a transmembrane glycoprotein playing a crucial role in maintaining cell-cell adhesion. Besides genetic mutations in CDH1 gene to induce hereditary diffuse gastric cancer (HDGC), epigenetic factors such as DNA hypermethylation also contribute to the reduction of E-cadherin in gastric carcinogenesis. As E-cadherin is vitally involved in signaling pathways modulating cell proliferation, survival, invasion, and migration, dysregulation of E-cadherin leads to dysfunction of gastric epithelial cells and contributes to gastric cancer development.[5] According to recent studies, the role of E-cadherin in carcinogenesis does not limit only to invasion and metastasis, this molecule being apparently involved in modulating of intracellular signaling, and thus promoting tumor growth.[6]

The expression of E-cadherin has been studied by immuno histochemical method. Decreased expression has been observed in gastric cancer ranging from 17% to 92% depending on the method and the definition used.[7,8] Direct correlation between E-cadherin and the grade of tumour differentiation has also been observed in all these studies. In addition, it was shown that patients with E-cadherin positive tumors had significantly better 3- and 5-year survival rates than patients with E-cadherin negative tumors. The decreased expression of E-cadherin was mainly observed in diffuse type and less in intestinal type of gastric cancer. One possible mechanism for the decreased expression is mutation in the E-cadherin gene and/or loss of heterozygosity in 16q22.1.[9] Mutations of the E-cadherin gene have been reported in 50% of diffuse carcinomas of the stomach.[9] Recently, the report of germline mutations in kindred with early onset diffuse gastric carcinoma became the first description of a molecular basis for familial gastric cancer of the diffuse type.[10] The other possible mechanism of decreased cadherin expression is by CpG methylation of the cadherin gene.[11]

Materials and Methods

This was a cross sectional observational study carried out from March, 2015 to June, 2017 at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The studied materials were represented by gastric tissue with consecutive convenience sampling. The tissue were taken from 42 patients who underwent total or partial gastrectomy operation in Surgery Department of BSMMU and few private hospitals and were diagnosed with gastric carcinoma in Pathology department of BSMMU. Inclusion criteria was gastric tissue were taken from partial or total gastrectomy sample diagnosed as gastric carcinoma in pathology laboratory and exclusion criteria were gastric carcinoma treated with chemotherapy and other cancers like lymphoma, carcinoid tumor, undifferentiated carcinoma, GIST etc. In the laboratory, tissue processing, paraffin embedding, sectioning of the paraffin blocks, H & E staining were done according to the standard protocol followed at BSMMU. Cases were classified and interpreted according to Lauren's classification.[12] The expressions of E-cadherin were semi-quantitatively analyzed in the neoplastic cells under microscope. Performing an adaptation of the methodology proposed by Scholten et al., (2006), two parameters were evaluated: Staining intensity and positivity in tumor cells. Regarding the staining intensity, cases were analyzed under light microscopy (×400 magnification), using the following 4-point scoring system: 0 =(absent), 1= (weak), 2= (moderate), 3= (strong). The positivity in tumor cells varied from 0 = 0%, 1= 1-25%, 2= 26-50%, 3= 51-75%, 4=>75% The degree of membranous staining, the staining score, was calculated as the sum of staining intensity and positivity in tumor cells (range 0-7). Total staining score of 5 or more considered as positive. Similarly 4 or less was considered as negative. Immunohistochemistry, E-cadherin expressions of the submitted blocks were performed using Daco Cytomation at immunohistochemistry laboratory, department of pathology, BSMMU.

Results

A total number of forty two diagnosed cases of gastric adenocarcinoma were taken for this study. Thirty samples were total gastrectomy specimen and twelve samples were partial/subtotal gastrectomy specimen. Ages of the patients

ranged from 20 to 76 years. The subjects were grouped on the basis of decades and the highest number of cases 14(33.33%) belonged to 50-59 years age group (Figure-1). Among 42 cases, 26 were male and 16 were female with a male/female ratio of 1.6:1 (Table-1). The association between expression of E-cadherin and histological prognostic parameters are summarized in 2nd table. This study showed E-cadherin expression did not show association with lymphovascular invasion, perineural invasion and depth of invasion. The rate of E-Cadherin positivity shows association with histological type, histological grading and lymph node metastasis (Table 2)

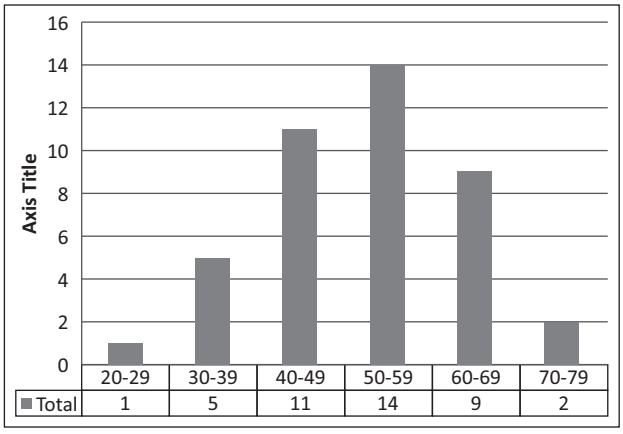

Figure 1: Age distribution of gastric carcinoma cases (n=42)

Table 1: E-Cadherin positivity according to sex distribution of gastric carcinoma cases

Attributes		No of cases	E-Cadherin immunoreactivity		p value
			Positive	Negative	
Sex	Male	26 (61%)	18 (42%)	8 (19%)	1.00
	Female	16 (38%)	8 (19%)	8 (19%)	

Table 2: Correlation of E-cadherin expression with histological prognostic parameters

Histological parameters		No of cases	E-Cadherin immunoreactivity		p value
			Positive	Negative	
Histo-logical types	Intestinal type	21 (50%)	20 (48%)	1 (2%)	0.0001
	Diffuse type	21 (50%)	6 (14%)	15 (36%)	
Grading	Well differentiated	9 (21%)	8 (19%)	1 (2%)	0.003
	Moderately differentiated	7 (17%)	7 (17%)	0	
	Poorly differentiated	26 (62%)	11 (26%)	15 (36%)	
Depth of invasion	Serosal invasion	18 (43%)	9 (21%)	9 (21%)	0.558
	Subsersosal invasion	9 (21%)	6 (14%)	3 (7%)	
	Muscularis-propria invasion	12 (29%)	9 (21%)	3 (7%)	
	Uptosub-mucosa invasion	3 (7%)	2 (5%)	1 (2%)	
Lympho-vascular invasion	Present	25 (60%)	16 (38%)	9 (21%)	0.735
	Absent	17 (40%)	10 (24%)	7 (17%)	
Perineural invasion	Present	27 (64%)	19 (45%)	8 (19%)	0.130
	Absent	15 (36%)	7 (17%)	8 (19%)	
Lymph node metastasis	Present	27 (64%)	17 (40%)	10 (24%)	0.0447
	Absent	15 (36%)	10 (24%)	5 (12%)	

Discussion

In this study, there were 42 cases of gastric carcinoma. Among these, 21 cases were intestinal type and 21 cases were diffuse type. The intestinal type of gastric carcinoma showed positive E-cadherin expression in 20 (48%) cases and diffused type of gastric carcinoma showed positivity in 6 (14%). This association is statistically significant (P<0.001).

Among 42 cases, 9 (21%) were well differentiated tumors, 7 (17%) were moderately differentiated and 26 (62%) were poorly differentiated morphologically. In poorly differentiated carcinoma, 15 (58%) cases were E-cadherin negative; whereas all the cases of moderately differentiated carcinoma of intestinal type showed positive E-cadherin expression and 1 (2%) out of 9 cases of well differentiated

carcinoma was E-cadherin negative. This indicates association of poorly differentiated gastric carcinoma with loss of E-cadherin is statically significant, ($p<0.05$).

Previous studies have shown variable expression of E-cadherin ranging from 20% up to 90% of tumors.[13] found positive E-cadherin immunoreactivity in 65% of gastric carcinomas, mainly intestinal-type tumors (69.38%) compared with only 45% of the diffuse type tumors. They have also noted the negative staining in the remaining 30.61% of intestinal type carcinomas (all poorly differentiated tumors) and 54.54% of the diffuse type carcinomas. Shimoyama, and Hirohashi also reported variable decrease (between 17% and 92%) of E-cadherin expression in gastric carcinomas, mainly for poorly differentiated intestinal-type tumors and diffuse type carcinomas.[7] Zhou et al., also demonstrated a significant association between expression of E-cadherin-catenin complex and tumor differentiation.[14] Schizas et al., showed that 27% of the cases of gastric carcinoma showed negative E-cadherin expression in IHC.[15] Similarly, Czyzewska et al., (2010) found positive immunoreactivity in 65% of gastric carcinoma cases, mainly in intestinal type tumors (69.38%) compared with only 45% of the diffuse type tumors. They have also noted negative staining in the remaining 30.61% of intestinal type carcinoma (all poorly differentiated tumors) and 54.54% of the diffuse type carcinoma.[16] So, present study result is consistent with previous study. This study also found significant association between lymph node metastasis with loss of E-cadherin expression. Out of 42 cases, 27 showed regional lymph node metastasis and 15 showed no regional lymph node metastasis. Out of 27 regional lymph node positive cases, 17(40%) were E-cadherin positive and 11(24%) were negative. Most E-Cadherin positive cases show lymph node metastasis (P value<0.05). Wu et al., showed that expression of E-cadherin is negative in 10 (71.4%) of the 14 patients with lymph node metastasis.[17] The difference between two groups are statically significant. This result is similar to the study conducted by Stanculescu et al., which shows E-cadherin expression is preserved in 67.5% of intestinal type carcinoma associated with lymph node metastasis, with a variable immunopositivity index. The result is consistent with previous study.[13]

Stanculescu et al. showed that among intestinal-type carcinoma with deep parietal invasion (serosal involvement) E-cadherin was expressed with low immunopositivity in moderately differentiated tumors (22.22% of cases); in poorly differentiated tumors the immunoexpression of E-cadherin was very low (22.22% of cases) or negative (66.66%).[13] Most of tumors with peritoneal dissemination showed very low E-cadherin immunopositivity or negative staining (summing 60% cases), all of them being poorly differentiated. Similar study showed by Mayer et al., 1993; Chan, 2006 the marked reduction of E-cadherin expression in advance tumors. This study is consistent with those studies.[6,8]

Lymphovascular invasion was observed in 25(60%) out of 42 cases and 17(40%) showed no lymphovascular invasion. Among the 25 cases of lymphovascular invasion, 16(38%) showed E-cadherin positive reactivityand 9(21%) showed negative E-cadherin reactivity. Out of 17 without lymphovascular invasion, 10(24%) showed positive E-cadherin expression and 7(17%) showed negative expression. Stanculescu et al., showed that vascular and lymphatic invasion were predominant between E-cadherin negative intestinal type tumors.[13] No correlation was found between E-cadherin expression and lymphatic pathway of invasion (Mayer et al.,). Present study could not establish the association between E-cadherin expression and lymphovascular invasion. This may result from relatively smaller sample size, lack of extensive sampling, probable improper preservation time or use of fixative. Larger study and/or extensive sample are needed to proper evaluation of this study.

In this study 27(64%) cases showed perineural invasion. Out of these, 8(19%) were E-cadherin negative and 19(45%) cases were E-cadherin positive. There is no statistical difference between E-cadherin expression and status of perineural invasion. This study is consistent with the findings of Shun CT et al.[18]

Conclusion

Around two third of the evaluated tumors showed E-cadherin expression in immunohistochemistry and there is a significant association with Lauren classification, histological grade and lymph node metastasis. But we did not find any association between E-cadherin expression with lymphovascular invasion, perineural invasion and depth of invasion. E-cadherin was intensely expressed more frequently in gastric carcinomas of intestinal type and well differentiated while poorly differentiated tumors and diffuse type carcinomas demonstrated a high rate of negative reactivity. Irrespective of histological type, we found an association between E-cadherin expression and adverse prognostic parameters particularly lymph node metastasis. These results suggest that the E-cadherin may be a useful marker of differentiation and prognosis in gastric cancer.

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