

E- CADHERIN EXPRESSION: A SIGNATURE FOR INVASION IN GASTRIC CANCER

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ABSTRACT:

Background

Cancer cell detachment is the first step in the process of invasion and metastasis. E-cadherin, a calcium mediated intercellular adhesion molecule, plays a crucial role in cell to cell adhesion between epithelial cells. Studies have shown a correlation between decreased E-cadherin expression and cancer cell detachment. Gastric cancer with down regulation of E-cadherin results in dissociation and dissemination of tumour cells, leading to distant metastasis and poor outcome.

Aims & Objectives

The aim of our study was to analyze the expression and staining pattern of E-cadherin and to correlate it with tumour differentiation, invasion, histological variants, lymph node status and distant metastasis in gastric cancer.

Materials and Methods

Twenty cases of antral gastrectomy specimen with lymph node dissection for gastric carcinoma were retrospectively studied. E-cadherin immunostain was performed using anti E-cadherin antibody and pattern of expression was analysed and correlated with other prognostic parameters.

Results

The study included equal number of cases of intestinal and diffuse type of gastric carcinoma. Age group ranged from 4th –8th decade with maximum cases in the 5th decade.

Among the 10 intestinal type two were well differentiated, four moderately differentiated and another four were poorly differentiated type of gastric carcinoma. In normal areas, the epithelial cells, showed strong membrane staining. None of the tumours showed normal type of E-cadherin expression. All tumour cases studied showed abnormal E-cadherin expression with varying patterns. Out of 20, nine showed faint/absent E-cadherin, seven showed reduced cytoplasmic staining and four showed membrane and cytoplasmic staining. A majority of diffuse carcinoma showed absence of E-cadherin (70%) as compared to intestinal type (20%). Four cases were below the age of 40 and they uniformly showed faint/absent E-cadherin staining. Three out of four females in the study showed faint/absent E-cadherin expression. Cases with pathological stage pT3/pT4 showed faint/absent E-cadherin. All cases with distant metastasis showed faint/absent E-cadherin expression.

Conclusion

Decreased E-cadherin expression correlates with differentiation and distant metastasis. An evaluation of clinico-histological features and immunohistochemical study of E-cadherin may help in predicting the prognosis and metastatic potential in gastric cancer. This can further be utilised as a tool for predicting recurrence.

Key words: E-cadherin, gastric cancer, tumour differentiation

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INTRODUCTION:

Gastric carcinoma is the second commonest cause of cancer-related deaths in the world and is the leading cancer in southern India as reported by cancer registries in India.^(1,2) In spite of in depth studies and therapeutic advances, its prognosis and outcome has not shown much improvement. Prognostic markers may play a vital role in designing the therapeutic approach, especially with regard to aggressive chemotherapy or surgery.

Cancer cell detachment is the first step in the complex process that leads to the invasiveness and metastasis. The cadherins are a major class of adhesion molecules that play an important role in the homotypic cell-cell adhesion. E-cadherin, a member of the cadherin family, is a 123 –kD cell surface glycoprotein, calcium dependent intercellular adhesion molecule which is expressed in all epithelial cells. Decrease expression of E-cadherin leads

to dissociation and dissemination of adenocarcinoma cells. It is therefore also called the invasion suppressor gene.⁽³⁾ The present study was designed to evaluate the immunohistochemical expression pattern and significance of E-cadherin in gastric cancer.

MATERIALS AND METHODS

The paraffin blocks of twenty cases of gastric carcinoma who underwent antral gastrectomy with lymph node dissection were obtained from archival files in Department of Pathology, (Table 1). The age group ranged between 4th - 8th decade with increase incidence in the 5th decade. Sixteen were males and four females. None of the patients had undergone any other modality of treatment before surgery. Blocks selected for the study included representative areas from the tumour as well as adjacent non-cancerous tissue. The tumours were classified according to the Lauren's classification into intestinal type and diffuse type. The clinical presentation and histological features were studied.

Table 1: Distribution of cases included for the study

Histological type of Carcinoma	No. of cases
Intestinal	10 Well diff (2) Mod diff (4) Poorly diff (4)
Diffuse Type	10

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E-cadherin immunostain was performed using anti E-cadherin antibody. E-cadherin expression was examined by immunostaining using the avidin-biotin complex immunoperoxidase method. Four- μ m thick tissue sections on slides were deparaffinized in xylene and rehydrated serially with alcohol and water. Subsequently, the sections were heated in pressure cooker for 10 minutes to retrieve antigens. Endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol for 10 minutes. After washing with phosphate-buffered saline, the slides were then incubated with diluted (1:100) mouse monoclonal E-cadherin antibody (Clone: 36; Isotype: IgG1, BioGenex) in a moist chamber at 37°C for 1 hour. The reaction was washed with phosphate-buffered saline and probed with universal secondary antibody at 37°C for 1 hour. The results were visualized with diaminobenzidine as the chromogen. The slides were finally counterstained with Harris hematoxylin, dehydrated and mounted. For negative control, the primary antibody step was omitted. Slides with normal gastric mucosa was used as positive control. Furthermore, positive E-cadherin staining in the adjacent non-involved gastric mucosa also served as an internal positive control.

E-cadherin staining was examined under a light microscope and classified, according to the pattern of staining. The pattern of staining was described as normal type i.e. strong and membranous. An abnormal type was heterogenous i.e. membrane and cytoplasmic staining pattern, reduced cytoplasmic staining (reduced staining found only in the cytoplasm) or faint or absent (homogenous faint/absent staining). Pattern of expression was studied by two pathologists and correlated with clinical features and evidence of lymphatic/distant spread. Quantitative data was analysed by Chi square test and p value was generated for each subgroup.

RESULTS

In the mucosa of the normal gastric tissue and the non cancerous area, E-cadherin showed strong membrane staining (Figure1). E-cadherin expression in all the twenty cases of gastric cancer cases showed aberrant staining pattern (Table 2). Out of 20, nine (45%) showed faint / absent E-cadherin, 7 (35%) showed reduced cytoplasmic staining (Figure 2), and 4(20%) showed membrane and cytoplasmic staining. Diffuse type of gastric carcinoma showed faint/absent E-cadherin expression (Figure 3) as compared to the intestinal type and this was statistically significant ($p=0.03$).

Among the different cases, 5% showed involvement upto the lamina propria and submucosa (pT1), 20% showed extension into the muscularis propria and subserosa (pT2). A majority i.e. 65% showed involvement of the serosa and visceral peritoneum (pT3). About 10% showed invasion into adjacent structures (pT4). Faint/absent E-cadherin staining was noted in 7 of 15 tumours with serosal extension and 2 of 5 tumours limited to the subserosa (Table 3). However, there was no statistically significant difference between the tumours confined to subserosa and those that had invaded beyond the serosa (p value = 0.95)

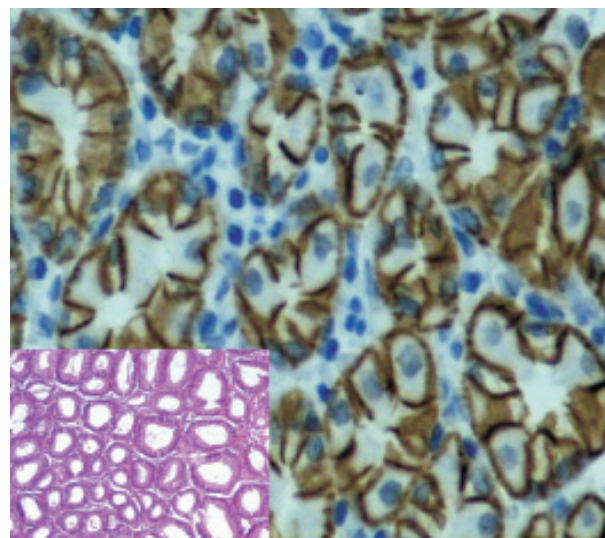


Figure 1- Normal gastric mucosa with strong membranous staining (E cadherin immunostain X100); inset show morphology of the same (H& E stain X 40)

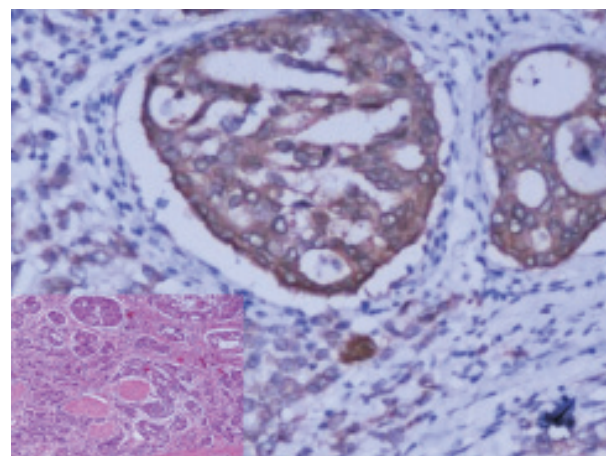


Figure 2: Reduced cytoplasmic staining of E-cadherin in moderately differentiated intestinal type gastric carcinoma (E-cadherin immunostain X 100); inset show morphology of the same (H& E X 40)

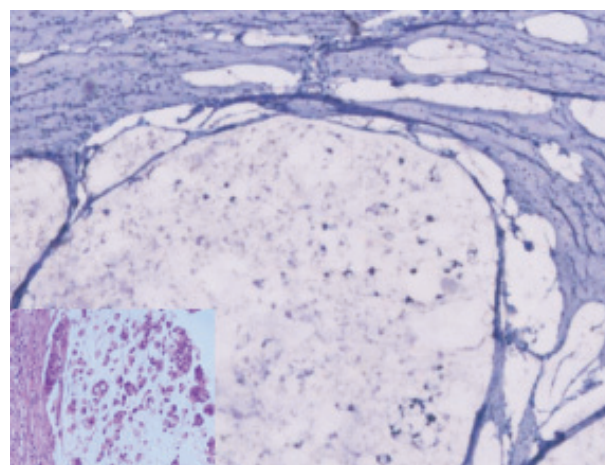


Figure 3: Total loss of E-cadherin expression in diffuse signet ring type gastric carcinoma (E-cadherin immunostain X100) ; inset show morphology of the same (H& E stain X 40)

Among the 20 cases, 25% of the cases showed no lymphnode involvement (pN0) and another 25% showed involvement of 1-6 nodes (pN1). About 40% showed 7-15 lymphnode metastases (pN2), while 10% showed greater than 15 node (pN3) involvement. Out of 5 node negative cases, 3 cases showed absent E-cadherin as compared to 6 out of 15 in node positive cases (Table 4). There was no statistically significant relation between E-cadherin expression and the nodal status (p value = 0.45).

Distant metastasis was seen in four out of twenty cases. Two cases metastasized to the peritoneum, one case showed metastatic nodules in the peritoneum and pericardium and one case showed liver involvement. All cases with distant metastasis showed faint/absent E-cadherin expression in the primary tumour. All cases below 40 years (4 cases) uniformly showed reduced cytoplasmic of E-cadherin. Three out of four females showed reduced cytoplasmic and or faint /absent of E-cadherin expression.

Table 2: E-cadherin expression among the types of gastric carcinoma

Type of staining	Total no of cases	Distribution among types of carcinoma		p-value value
Membrane & cytoplasmic staining	4	Intestinal 4	Diffuse 0	0.03 (significant)
Reduced cytoplasmic	7	Intestinal 4	Diffuse 3	
Faint or loss of staining	9	Intestinal 2	Diffuse 7	

Table 3- E-cadherin expression compared with depth of invasion

Level of invasion	Membrane cytoplasmic staining	Reduced cytoplasmic	Faint/absent staining	p-value (Not significant)
pT1 & pT2(5)	1	2	2	0.95
pT3 & pT4(15)	3	5	7	

Table 4 –E-cadherin expression in node positive and node negative cases

Node involvement	Membrane & cytoplasmic staining	Reduced cytoplasmic	Faint /absent staining	p-value (Not significant)
pN0	0	2	3	0.42
pN1, pN2, pN3	4	5	6	

DISCUSSION

E-cadherin a major intercellular cell adhesion molecule, in addition to its property of adhesiveness plays a role in growth and development. Due to its role in carcinogenesis, it is viewed as an invasion as well as a growth suppressor gene.⁽³⁾ It has been proposed that the loss of E-cadherin-mediated cell to cell adhesion is a prerequisite for tumour cell invasion and metastasis formation.⁽⁴⁾ Down regulation of E-cadherin in gastric cancer correlates with infiltrative and metastatic ability.⁽⁵⁾ Re-establishing the functional cadherin complex, e.g. by forced expression of E-cadherin, results in a reversion of an invasive lesion to a benign epithelial phenotype in cultured tumor cells.^(4,6) Studies have demonstrated a functional relation between the invasive phenotype and decreased E-cadherin expression and also a correlation between the metastatic activity of tumor cells and the level of E-cadherin expression in vitro.⁽⁷⁾ In addition, a possible association between a decreased expression of E-cadherin and tumor progression has been shown in

vivo.⁽⁸⁾ These observations indicate that E-cadherin may play an important role in cancer invasion and metastasis by mediating cancer cell dissociation.

The present study revealed abnormal pattern of E-cadherin expression in all cases of gastric cancer, although the pattern of expression varied from case to case. We observed a higher frequency of E-cadherin negativity in diffuse type carcinoma than in intestinal type and this was statistically significant. These findings correlated with other similar studies in literature.^(9,10) Mayer et al, in a similar study showed a correlation between decrease E-cadherin expression and cellular differentiation which was also evident in our cases.⁽¹⁰⁾ These observations suggest that E-cadherin expression on tumor cells may be one of the factors responsible for glandular differentiation in gastric carcinomas. Hence, E-cadherin expression in tumor cells may play a role in the genesis of histologic types of human gastric carcinomas. Behrens et al revealed the role of E-cadherin in the establishment of an invasive phenotype in human carcinoma cell lines.⁽¹¹⁾

Our study did not show any statistically significant correlation between E-cadherin expression and depth of tumour invasion. E-cadherin-negative tumors were significantly more frequent in cases with peritoneal metastases than in those without. This finding reinforces the fact that E-cadherin-negative cancer cells may be a prerequisite for metastasis in view of loss of cell to cell adhesion property of cadherins. In this study, E-cadherin-negative tumors showed a no significant correlation with lymph node status. Unlike, Wu et al who demonstrated a positive correlation with nodal metastasis⁽¹²⁾ Gabbert et al found that patients with E-cadherin positive tumors had significantly better 3 and 5-year survival rates than patients with E-cadherin negative tumors.⁽¹³⁾

We analysed the staining pattern, intensity and the percentage of cells in positive cases. Staining was strong and membranous in normal gastric epithelium, but gradually decreased in intensity and percentage, and the pattern was changed towards cytoplasm in carcinoma. In our study, there was good correlation between E-cadherin expression and tumour differentiation. Poorly differentiated and diffuse type showed reduced E-cadherin expression. This was in concordance with study reported in literature.⁽¹⁴⁾ Tumor-node-metastasis, staging, differentiation, histologic classification, and tumor size are known to carry strong prognostic value. However, E-cadherin could serve as a prognostic marker to predict the invasive and metastatic potential and thereby help in assessing the survival status in gastric tumours.

Increased methylation within the promoter regions of genes play a key role in the inactivation of many important genes during the development of cancer. E-cadherin promoter methylation is associated with reduced E-cadherin expression. Helicobacter infection is a known associated risk factor for gastric cancer and pylori bacilli have shown to down regulate E-cadherin expression.⁽¹⁵⁾ H pylori infection has been associated with methylation of E-cadherin gene and in these cases reversal of methylation in the gastric epithelium may arrest the process and prevent future development of gastric cancer.⁽¹⁶⁾

To conclude, a combined analysis of clinico-histological features and E-cadherin expression may help to predict the prognosis and metastatic potential in gastric cancer thus providing useful information for recurrence and survival.

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