

ORIGINAL ARTICLES

Correlation of Lauren's histological type and expression of E-cadherin and HER-2/neu in gastric adenocarcinoma

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Abstract

Background: To evaluate whether a proven association between clinicopathological features and expression of molecular markers could provide a clue towards the relevance of these markers in gastric adenocarcinoma (GAC).

Aims: The present study aimed to find any relation between immunohistochemical expression of E-cadherin and HER-2/neu proteins, and Lauren's histological type and stages of GAC.

Materials and Methods: 100 cases of primary GAC diagnosed on biopsy or gastrectomy were included in the study. Immunohistochemical expression of E-cadherin and HER-2/neu was studied with respect to site, Lauren's histological type, and stages of GAC.

Results: A total of 11 cases showed a loss of E-cadherin expression and 17 cases overexpressed HER-2/neu. Loss of E-cadherin was seen in 26% of Lauren's diffuse type, but only in 5.7% of intestinal GAC ($P=0.014$). While 35% of the GAC present at greater curvature showed HER-2/neu overexpression, whereas 16% at antro-pyloric region and none at lesser curvature showed HER-2/neu overexpression ($P=0.04$). All of the 17 HER-2/neu overexpressing GAC were Lauren's intestinal type ($P=0.012$).

Conclusions: Loss of E-cadherin and HER-2/neu overexpression are associated with Lauren's diffuse and intestinal type respectively. Anti-HER-2/neu monoclonal antibody therapy would be helpful in approximately one-fifth of gastric adenocarcinoma patients.

Keywords: E-cadherin, Gastric adenocarcinoma, HER-2/neu, Lauren's classification

Introduction

An estimated 989,600 new cases per year in 2008 (8% of new cancer cases) and 738,000 annual deaths (10% of total deaths) globally have been attributed to gastric cancer.¹ High incidence rates are observed in Eastern Asia, South America, and Eastern Europe, whereas low-risk areas include Eastern and Northern Africa and North America.¹

Lauren's classification divides gastric adenocarcinomas (GAC) into two subtypes- intestinal and diffuse, and they show both biological and epidemiological differences.² Intestinal GAC is more likely to be sporadic and related to environmental factors including *Helicobacter pylori* infection, cigarette smoking, and diet.³ Diffuse GAC represent about 30-40% of gastric cancers and tend to affect mainly young females when compared to intestinal GAC.³ Hereditary

diffuse gastric carcinoma (HDGC), a subset of diffuse GAC, is associated with germline mutations in the tumor suppressor gene CDH1, which encodes for E-cadherin.³ Histopathology findings indicate that intestinal GAC are well differentiated and exhibit intestinal architecture such as tubular glandular structures.⁴ Diffuse GAC are poorly differentiated, often grow as single cells or in small groups of cells and are likely to infiltrate into the stomach wall. When identification of a gastric tumor as either intestinal or diffuse is not possible, the histological subtype of the tumor is referred as a mixed type GAC.²

The knowledge of molecular abnormalities in GAC gives an insight into the evolution of tumor. An association between clinicopathological features and expression of molecular markers could provide a clue towards the relevance of these markers in GAC.

E-cadherin is a transmembrane protein with a cell-cell adhesion and invasion suppression function. Therefore, loss of E-cadherin expression has been associated with metastasis. The method of blocking E-cadherin down regulation in tumors is one of the most important future approaches in gene therapy, which prevents metastasizing potential of almost any epithelial tumor.

A number of recent studies have reported the over-expression of HER-2/neu in a subset of 7-34% of patients with GAC.⁵ HER-2/neu protein over-expression and gene amplification are much more heterogeneous in GAC compared to breast cancer.⁵ The exact numbers are variable, but proximal GAC with intestinal phenotype is generally found to have a higher prevalence of HER-2/neu positivity (range 8-34%) than distal diffuse GAC (range 1-7%).⁵ The results from the first successful phase III clinical trial (ToGA study- Trastuzumab with chemotherapy in HER-2/neu positive advanced gastric cancer) of anti-HER-2/neu therapy in combination with chemotherapy in advanced HER-2/neu positive GAC suggest that the addition of trastuzumab to chemotherapy was associated with a significant improvement in overall survival compared to chemotherapy alone.⁶

Data on the co-expression of HER-2/neu and E-cadherin in GAC is not available. Most of the data on relation between GAC and HER-2/neu or E-cadherin expression is available for the western population. A similar data on the Indian population is required, keeping in mind the future prospects of therapy with monoclonal antibodies and gene therapy.

Materials and methods

The study was conducted in the Department of pathology of a tertiary care center. Hundred cases of primary GAC diagnosed on both gastric biopsies and proximal and radical gastrectomies were included. Patients diagnosed with adenocarcinoma of gastroesophageal junction, metastatic gastric tumors or primary GAC, and those who had received prior chemotherapy were excluded from the study. After overnight fixation in neutral buffered formalin, grossing of the surgical specimen was done. Biopsy tissue bits from the proximal and distal margins of the gastrectomy specimen, full thickness tumor sections, and lymph nodes were submitted for further analysis. Sections from tumor were studied for Lauren's histological type and depth, and extent of invasion. Number of lymph nodes involved and pathological stage of tumor was given for cases where gastrectomy was performed. Immunohistochemistry

(IHC) using ready-to-use monoclonal mouse anti-human E-cadherin NCH-38 (Dako N1620) and 1:300 dilution of polyclonal rabbit anti-human HER-2/neu oncoprotein (Dako A04585) was performed. Normal human mammary gland tissue was used as a positive control for E-cadherin and a known case of HER-2/neu positive (3+) breast carcinoma was used as a positive control for HER-2/neu. Controls were maintained for every batch of IHC. Membranous and/or cytoplasmic positivity for E-cadherin in <10% of malignant cells was interpreted as loss of E-cadherin expression by tumor cells and vice versa. Complete/lateral/basolateral membranous positivity of HER-2/neu in >10% of malignant cells was taken as HER-2/neu positive GAC, irrespective of staining intensity. Twenty two subjects who underwent gastrectomy were followed up for symptoms and signs of recurrence. Statistical analysis was done using SPSS10.0 software and $P < 0.05$ was considered as statistically significant.

Results

Peak incidence of GAC was noted in the sixth decade of life in females and seventh decade in male patients. The male: female ratio was 3:1. Majority of the tumors (69%) were located in the antro-pyloric region of the stomach with 17% on greater curvature and 14% on lesser curvature of the stomach. Most (83%) of the tumors were of the ulcerating type on gross examination. Using Lauren's classification for GAC, 70 cases were of intestinal type, 27 were of diffuse type, and 3 cases of mixed type. Pathological staging was done for 22 cases where surgical specimens of gastrectomy were available. Seven cases (32%) had stage II disease, 9 cases (41%) had stage III disease and 6 cases (27%) had stage IV disease.

Immunohistochemistry: Normal gastric mucosal glands in all cases were membranous positive for E-cadherin and negative for HER-2/neu. Loss of E-cadherin was seen in 11 cases and overexpression of HER-2/neu in 17 cases of GAC (Fig. 1 and 2). Concomitant loss of E-cadherin and overexpression of HER-2/neu was seen in 2 cases. Nearly 35% (6/17) of the GAC present at the greater curvature showed HER-2/neu overexpression as compared to only 16% (11/69) at the antro-pyloric region and none (0/14) at the lesser curvature. HER-2/neu overexpression was found significantly more common in tumors located on the greater curvature (Pearson coefficient 6.303, $P=0.04$). All of the 17 GAC (100%) that showed HER-2/neu overexpression were of Lauren's intestinal type, thereby indicating a significant association between the two (Pearson coefficient 8.7,

Fig. 1: Loss of E-Cadherin immunostaining in Lauren's diffuse type gastric adenocarcinoma (red arrows) with positively staining normal gastric mucosal glands (HEx400)

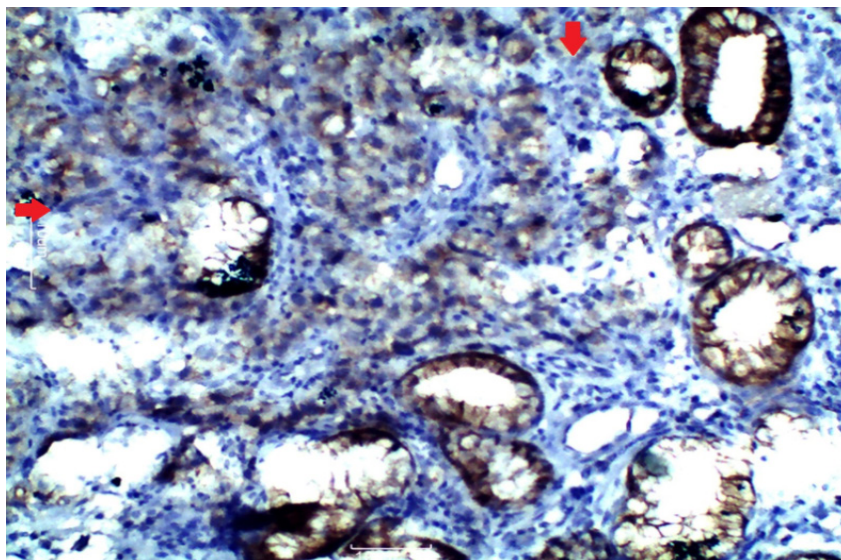
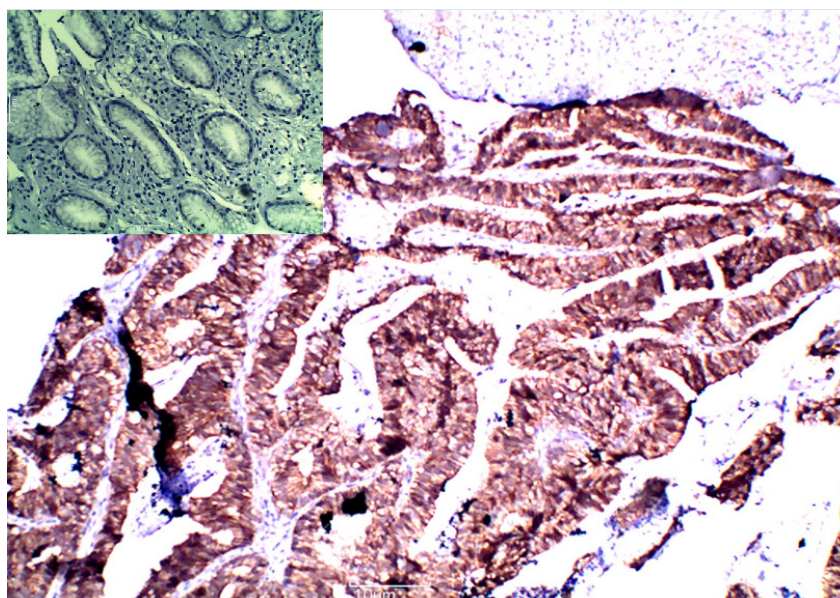


Fig. 2: Positive HER-2/neu immunostaining (3+) in Lauren's intestinal type gastric adenocarcinoma (HEX400). Inset: Normal gastric mucosal glands are negative for HER-2/neu immunostaining (HEX400)



P=0.012) (Table 1).

Statistically significant association was seen between Lauren's diffuse type and loss of E-cadherin. Of the 11 cases that showed loss of E-cadherin expression, 7 (63.63%) cases were of diffuse type (Pearson coefficient 8.513, P=0.014) (Table 1). Depth of invasion, lymph node involvement, and stage was studied in the 22 cases

of gastrectomy. No statistically significant relation was established between these parameters and immunostaining (P >0.05). Co-expression of E-cadherin and HER-2/neu was not significant.

Twenty two patients who underwent gastrectomy were followed up for symptoms and signs of recurrence for a duration ranging from 4 months to 2 years. However, 14

Table 1: Relation between Lauren's type of GAC and immunostaining with E-cadherin and HER-2/neu (P=0.014 and 0.012 respectively)

Lauren's type	Total	E-Cadherin expression (No. of cases)		HER-2/neu expression (No. of cases)	
		Absent	Present	Present	Absent
Intestinal	70	4	66	17	53
Diffuse	27	7	20	0	27
Mixed	3	0	3	0	3
Total	100	11	89	17	83

cases were lost to follow-up during the period. Recurrence of gastric adenocarcinoma at the site of surgery was noted in four cases. All the cases of recurrence had expressed HER-2/neu. None of the cases had loss of E-cadherin expression. Two of the cases were poorly differentiated and two were moderately differentiated. Since the number of cases showing recurrence was only four, no statistical significance was calculated for them.

To consolidate the results, a total of 11 cases showed a loss of E-cadherin expression and 17 cases overexpressed HER-2/neu. Lauren's diffuse type gastric adenocarcinoma is associated with loss of E-cadherin expression and intestinal type gastric adenocarcinoma is associated with overexpression of HER-2/neu. Gastric tumors located on greater curvature show overexpression of HER-2/neu.

Discussion

The knowledge of molecular abnormalities in gastric cancer may provide a footprint for their genetic and molecular backgrounds. Study of molecular markers provides scope for better understanding of disease. It also enables the customization of targeted therapies economically and appropriately to reduce the morbidity and mortality in the Indian setting. In this study, an attempt was made to establish any relation between immunohistochemical expression of E-cadherin or HER-2/neu and histopathological features like site, Lauren's type or stage of the tumor.

In our study, 26% (7/27) patients with diffuse GAC exhibited loss of E-cadherin expression. Berx *et al.* found 13 of 26 (50%) diffuse GAC to have reduced E-cadherin expression.⁷ Correlation of E-cadherin expression with

staging and lymph node invasion was studied in 22 post-gastrectomy cases and no significant association noted. This was in agreement with Shimoyama *et al.* who found that down-regulation of E-cadherin expression was not significantly associated with the process of metastasis to regional lymph nodes, although the disruption of cell-cell contact is considered to be a prerequisite for metastasis.⁸ However, a meta-analysis by Xing *et al.* revealed that E-cadherin expression is decreased in primary tumors than in the normal gastric tissues and is associated with tumor stage, depth of invasion, and metastasis.⁹ This difference between various studies could be explained by the Bukholm *et al.* study where re-appearance of E-cadherin in metastatic cells was demonstrated in invasive lobular breast carcinomas.¹⁰ This possibly highlights that re-establishment of cellular contact may prevent apoptosis. However, the size of the study group was small and hence no conclusions can be drawn in this aspect from the present study.

A statistically significant correlation was found between HER-2/neu over-expression and GAC located on greater curvature (P=0.04) in our study. In previous studies, it has been correlated with proximally located GAC.¹¹ This difference in the correlation between HER-2/neu expression and site of GAC noted in the present study and previous studies could be explained by the fact that the previous studies included cases of adenocarcinomas occurring at the gastroesophageal junction, while the current study excluded them. The tumors of the gastroesophageal junction are believed to be a separate entity, probably originating from the distal esophagus. All GAC expressing HER-2/neu had Lauren's intestinal morphology (P=0.012). Conversely, 24% cases with intestinal type morphology

revealed HER-2/neu overexpression. This is in agreement with previous studies where up to 34% of intestinal GAC showed an overexpression of HER-2/neu receptor protein.¹² Studies by Lakshmi *et al.* and Rajagopal *et al.* have reported that 37.8% and 32.7% of intestinal GAC showed HER-2/neu overexpression respectively.^{13, 14} In the present study, stage of the tumor did not correlate with HER-2/neu over-expression. More research needs to be done in this regard, as very few studies have found HER-2/neu expression is associated with a higher stage.¹⁵ ¹⁶ This is crucial as stage of the tumor is an independent prognostic factor in GAC.

Only two cases showed a concomitant overexpression of HER-2/neu and loss of E-cadherin. The expression of these two immunohistochemical markers is not related. In fact, as both these markers are differently expressed in relation to tumor grade and histological type, absence of correlation between the two markers is justified.

Conclusion

Lauren's diffuse type gastric adenocarcinoma is associated with loss of E-cadherin expression and intestinal type gastric adenocarcinoma is associated with overexpression of HER-2/neu. Gastric tumors located on greater curvature show overexpression of HER-2/neu. Approximately one-fifth of patients with gastric adenocarcinoma would benefit from anti-HER-2/neu monoclonal antibody therapy in the Indian scenario. A therapy that blocks E-cadherin down-regulation in tumors would serve as an important future approach in gene therapy in one-tenth of gastric adenocarcinoma patients.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Citation

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