E-cadherin Immunohistochemical Expression in Gastric Carcinoma

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ABSTRACT

Introduction: Carcinoma of the stomach is a disease with a grave prognosis. The revelation of the genetic and molecular basis of gastric cancer has helped the development of targeted therapies which has the potential to improve survival. Decreased expression of Epithelial cadherin (E-cadherin) in gastric carcinoma is one such genetic alteration which could help in targeted therapy and prognostication. The objective of the present study was to identify E- cadherin immunohistochemical expression in gastric carcinoma and its correlation with histopathological features.

Material and Methods: Gastric biopsies and surgical specimens from a tertiary care center in South India were included and assessed by light microscopy and immunohistochemistry (IHC).

Results: Aberrant E-cadherin staining was seen in 42.8% of gastric adenocarcinoma. Aberrant staining was found with disproportionately high frequency in diffuse-type (95.5%) when compared to intestinal-type (18.6%) and mixed type (20%) with very high statistical significance with P <0.001. All (100%) poorly differentiated tumors had aberrant E- cadherin staining while only 21.2% of moderately differentiated and 12.5% of well-differentiated tumors had aberrant E-cadherin staining and this was statistically highly significant with P <0.001. In this study, there was no significant association between E-cadherin with age, gender, nature of the specimen, site of the tumor, size of the tumor, lymph node status and tumor invasion.

Conclusion: This study emphasizes the importance of identification of aberrant E-cadherin expression in gastric carcinomas which in turn helps in selecting patients for novel therapies which might improve the prognosis of this grave disease.

Keywords: Epithelial-cadherin, Gastric Adenocarcinoma, Immunohistochemistry.

INTRODUCTION

Gastric cancer is the third most common cause of cancer-related deaths worldwide. Over 90% of gastric malignancies are gastric adenocarcinomas.[1,2] Gastric carcinogenesis is a multifactorial process.[3,4] Helicobacter pylori infection, intestinal metaplasia, dysplasia, and atrophic gastritis are related to gastric adenocarcinoma.[4] The most important prognostic factors include stage followed by histologic type.[5] Patients in the same stage and histologic type may have varied prognosis, therefore additional parameters have to be identified in order to better classify the biological subsets of this disease.

Surgical resection of all gross and microscopic disease is the only proven potential curative treatment for gastric cancer, but disease recurrence is high. A better understanding of the molecular basis of cancer has led to the development of molecular targeted therapies that interferes with signaling cascade involved in cell differentiation, proliferation, and survival. Cadherins are a family of cell surface glycoproteins made up of 723-747 amino acids that act as intercellular adhesion molecules by calcium dependant homophilic binding and play an essential role in the complex process of invasion and metastasis of which the initial step is the escape of cancer cells from primary tumor involving disruption of normal cell-cell adhesion.[6,7] E-cadherin gene is located on the long arm of chromosome 16 (q22.1) and produces E-cadherin transmembrane protein. It is considered as a tumor suppressor, invasion or metastatic suppressor gene as it suppresses proliferation, invasion, motility, and differentiation. E- Cadherin is a 120 k D transmembrane glycoprotein, which is expressed on the surface of epithelial cells, at the level of the intercellular junction and is important for establishing cell polarity, maintaining epithelial integrity and cellular differentiation.[8,9] Decreased expression of E-cadherin leads to dissociation and dissemination of adenocarcinoma cells that lead to invasiveness and metastasis. Detection of loss of E-cadherin is useful for prognostication and selection of patients for targeted chemotherapy with demethylating agents. The objective of this study was to assess E-cadherin expression using immunohistochemistry in gastric carcinoma and to evaluate their correlation with histological subtypes and histological grading. We proposed to conduct this study as there is currently limited data available on immunohistochemical expression of E-cadherin in gastric carcinoma in the Indian population.

MATERIAL AND METHODS

The present study "E-cadherin Immunohistochemical Expression in Gastric Carcinoma" is a descriptive study conducted in the department of Pathology in a tertiary care hospital in South India with the cooperation of Departments of General Surgery, Medical Gastroenterology, and

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Surgical Gastroenterology during a period of two years. A total of 70 cases including total /subtotal gastrectomy specimens and gastric biopsies confirmed as gastric adenocarcinoma by histopathological examination were included in the study. Approval for the study was obtained from the Institutional Ethics Committee.

Inclusion criteria: Total or subtotal gastrectomy specimens, gastric biopsies from patients with gastric adenocarcinoma who have not received any previous treatment were included in the study.

Exclusion criteria: Cases, where there is extensive tumor necrosis without sufficient viable tumor cells for an accurate evaluation of the immunohistochemical results were excluded.

The detailed clinical history including age, gender, and results of relevant investigations done was collected or abstracted from the patient's case files. For prospective cases, total, subtotal gastrectomies and small biopsy specimens were received in the Pathology Department in 10% formalin. In every case, the standard protocol for surgical grossing of resected specimens was followed. After a detailed specimen description, multiple sections were taken from the tumor, surgical margins, omentum, mesentery, and all the lymph nodes. For retrospective cases, the histopathology reports, slides and paraffin blocks were retrieved from the archives. After conventional processing, paraffin sections of 5µm thickness were stained by hematoxylin and eosin (H and E) for histopathological study. In addition, 4µm sections were cut from a paraffin block of tumor tissue and taken on a glass slide coated with adhesive aminopropyltriethoxysilane (APES) for IHC to detect E-cadherin loss of expression. Sections were cut from the paraffin blocks in a similar manner. The H and E stained slides were studied for the tumor histology and classified into intestinal and diffuse according to Lauren's classification. p TNM staging was done in gastrectomy specimens. The technique of IHC included antigen retrieval in tris EDTA buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibody against E-cadherin protein (PATH INSITU) and linking with rabbit anti-mouse secondary antibody, enzyme linking with streptavidin-horseradish peroxidase, developing chromogen with diaminobenzidine (DAB) and counterstaining with hematoxylin. Positive and negative controls were run with each batch of slides.

E-cadherin staining: In concordance with previously published criteria, cancer cells which immunostained as strongly as normal epithelial cells were defined as positive. E- cadherin was graded according to the proportion of positive tumor cells in gastrectomy specimens. In gastric biopsies, if the majority of tumor cells within the biopsy material have taken up the stain, it was considered positive. Heterogeneous or absent staining was considered aberrant expression (Table 1).[6,7,10]

The relationship between various parameters such as age, gender, anatomic site of the tumor, histologic type and grade with loss of expression of E-cadherin was studied in all specimens. In addition, in gastrectomy specimens, the pathologic stage, lymph node status were also studied.

STATISTICAL ANALYSIS

Data was analyzed using computer software, Statistical Package for Social Sciences (SPSS) version 16. Data are expressed in their frequency and percentages. To elucidate the associations and comparisons between different parameters, the Chi-square test was used as the nonparametric test. For all statistical evaluations, a two-tailed probability of value < 0.05 was considered significant.

RESULTS

A total of 70 cases which were confirmed as gastric adenocarcinoma were included for the study. Among the total 70 specimens, 50 (71.4%) were gastric biopsies, 17 (24.3%) were total gastrectomies and 3 (4.3%) were subtotal gastrectomies. The age group of patients in the study ranged from 30- 80 yrs. The maximum incidence was at the 50-59 yr age group (34.3%). Around 57.2% occurred in the age group 40-59yrs. Of 70 cases studied, males accounted for the majority - 54 (77.1%). Antrum was the commonest site (40%) followed by antropyloric (21.4%), body (17.2%), OGJ (14.3%) and Cardia & Fundus (7.1%). Among the 20 gastrectomy specimens, 15 (75%) had tumor size <5cm and 5 (25%) had a tumor size of >5cm. Among 20 gastrectomy specimens, 11 (55%) had lymph node involvement. Among the 20 gastrectomy specimens, 40% exhibited T3 and 30% exhibited T4 stage. T1 and T2 were 15% each. Among 70 cases, 43 (61.4%) were intestinal type, 22 (31.4%) were diffuse type and 5 (7.2%) were mixed type. Among 70 cases, 33 cases (47.1%) were moderately differentiated, 21 cases (30%) were poorly differentiated and 16 cases (22.9%) were well differentiated.

Surgical specimen staining pattern	Biopsy specimen staining pattern	E-cadherin expression
Membranous staining in <10% of tumor cells	Perceptible membranous staining in very few tumor cells	Negative
Perceptible membranous staining in 10-90% of tumor cells	Perceptible membranous staining in some of the tumor cells	Heterogenous
Strong membranous staining in > 90% of tumor cells.	Strong membranous staining in the majority of tumor cells.	Positive

Table-1: E-cadherin scoring criteria for gastric carcinoma[10]

Grade	Type			Total
	Intestinal Type	Diffuse Type	Mixed Type	
Poorly Differentiated	0	21	0	21
		100.00%		100.00%
Moderately Differentiated	27	1	5	33
	81.80%	3.00%	15.20%	100.00%
Well Differentiated	16	0	0	16
	100.00%			100.00%
Total	43	22	5	70
Chi Square: 69.529; $P < 0.001$				

Table-2: Type and grade of tumor

E –cadherin	Frequency	Percent
Aberrant	30	42.8
Normal	40	57.2
Total	70	100

Table-3: Frequency of aberrant and normal E-cadherin expression

Type	E –cadherin		Total
	Aberrant	Normal	
Intestinal Type	8	35	43
	18.60%	81.40%	100.00%
Diffuse Type	21	1	22
	95.50%	4.50%	100.00%
Mixed Type	1	4	5
	20.00%	80.00%	100.00%
Total	30	40	70
Chi Square: 36.246; $P < 0.001$			

Table 4: E-cadherin and type of tumor

Grade	E –cadherin		Total
	Aberrant	Normal	
Poorly Differentiated	21	0	21
	100.00%		100.00%
Moderately Differentiated	7	26	33
	21.20%	78.80%	100.00%
Well Differentiated	2	14	16
	12.50%	87.50%	100.00%
Total	30	40	70
Chi Square: 40.334; $P < 0.001$			

Table-5: E-cadherin and grade of tumor

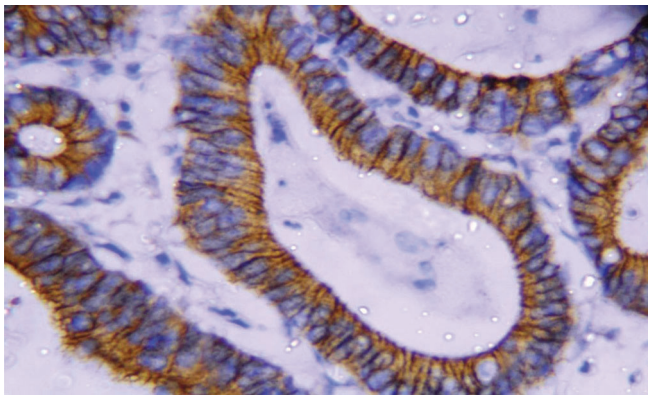

Figure-1: Photomicrograph of well-differentiated intestinal type of gastric adenocarcinoma showing normal E-cadherin expression (IHC, 400x)

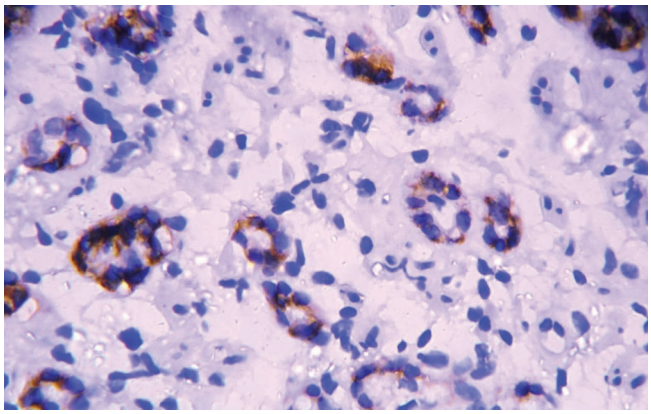

Figure-2: Photomicrograph of moderately-differentiated intestinal type of gastric adenocarcinoma showing heterogenous E-cadherin expression (IHC, 400x)

Study	Aberrant E-cadherin expression (%)					
	Type			Grade (Differentiation)		
	Intestinal	Diffuse	Mixed	Well	Moderate	Poor
Joo Y E et al[6]	32.1	80	41.7	26.7	33.3	79.3
Lazar D et al[10]	31.6	82.4	66.7	-	30	61.5
Anbiaee R et al[9]	23	41.7	-	24	-	47
Present study	18.6	95.45	20	12.5	21.21	100

Table-6: Comparison of aberrant E-cadherin expression with respect to histologic type and grade in various studies

Type and grade of tumor

Among the 21 poorly differentiated cases, all (100%) were of diffuse-type while among the 16 well-differentiated cases, all (100%) were of intestinal type. Among the 33 moderately differentiated cases, 27 (81.8%) were of the intestinal type, 5 (15.2%) were of mixed type and 1 (3%) was of diffuse type. All these findings were statistically highly significant (P <0.001) (Table 2).

In E-cadherin IHC staining, among the 70 cases, 40 cases (57.2%) showed positive staining, 15 cases (21.4%)

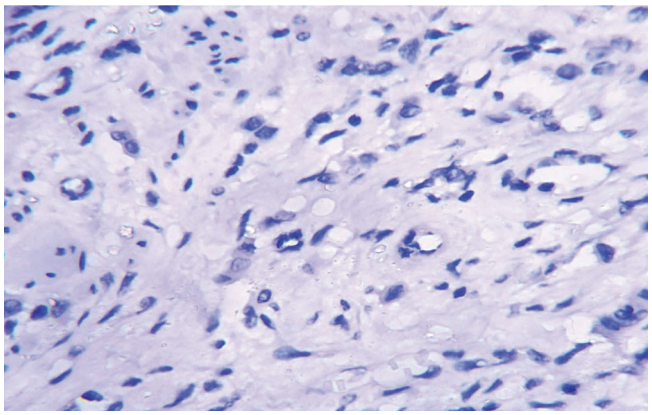

Figure-3: Photomicrograph of poorly-differentiated diffuse type of gastric adenocarcinoma with negative E-cadherin expression (IHC, 400x)

showed heterogeneous staining and 15 cases (21.4%) showed negative staining (Figures 1,2,3). The intensity of staining was found to be more or less the same in both homogenous and heterogeneous membrane staining. So in our study categorization based on the intensity of staining was considered irrelevant. Heterogenous and negative staining is considered aberrant and positive staining is considered as normal. 30 cases (42.8%) showed aberrant E-cadherin staining (Table 3).

E-cadherin and type of tumor

Among the 43 intestinal types, 8 (18.6%) had aberrant E-cadherin staining and 35(81.4%) had normal staining. Among the 22 diffuse types, 21 (95.5%) had aberrant staining and 1 (4.5%) had normal staining. Among the 5 mixed types, 1 (20%) had aberrant E-cadherin staining and 4 (80%) had normal staining. Among the 30 cases with aberrant E-cadherin staining, 21 (70%) were of the diffuse type, 8 (26.7%) were of the intestinal type and 1 (3.3%) was of mixed type. Among the 40 cases with normal E- cadherin staining, 35 (87.5%) were of the intestinal type, 4 (10%) were of mixed type and 1 (2.5%) was of diffuse type. All these findings were statistically very highly significant (P<0.001) (Table 4).

E-cadherin and grade of tumor

Among the 21 poorly differentiated cases, all had aberrant E- cadherin staining. Among the 33 moderately differentiated cases, 7 (21.2%) had aberrant E- cadherin staining and 26 (78.8%) had normal staining. Among the 16 well-differentiated cases, 2 (12.5%) had aberrant E-cadherin staining and 14 (87.5%) had normal staining. Among the 30 cases with aberrant E-cadherin staining, 21(70%) were poorly differentiated, 7 (23.3%) moderately differentiated and 2 (6.7%) were well differentiated. Among the 40 cases with normal E-cadherin staining, 26 (65%) were moderately differentiated, 14 (35%) were well-differentiated and none were poorly differentiated. All these findings were statistically very highly significant (P <0.001) (Table 5).

DISCUSSION

Carcinoma of the stomach is a disease with a grave prognosis. Understanding the genetic and molecular basis of gastric cancer has helped the development of targeted therapies which has the potential to improve survival. Decreased expression of E-cadherin in gastric carcinoma is one such genetic alteration which could help in targeted therapy and prognostication. Decreased expression of E-cadherin, a major class of cell adhesion molecule leads to dissociation and dissemination of adenocarcinoma cells that lead to invasion and metastasis. Therefore the detection of loss of E-cadherin is useful for the prognostication and selection of patients for targeted chemotherapy with demethylating agents.

In the present study, 70 cases of gastric adenocarcinoma were evaluated by light microscopy to determine the histologic type, histological grade, invasion, and lymph node status. Immunohistochemistry was done to find out the E-cadherin status of tumor and correlation was done between its expression and histologic type and grade of the tumor. In the present study, as per Lauren's classification, 61.4% were the intestinal type which accounted for the majority, followed by 31.4% of diffuse type and 7.2% of mixed type. This result is in concordance with literature documenting the proportion of the diffuse type of gastric carcinoma of around 30% among gastric carcinomas.[11]

In the present study among the 70 cases, 47.1% were moderately differentiated, 30% were poorly differentiated and 22.9% were well differentiated. The study by Rajagopal I *et al*[12] from South India showed a similar distribution of histological grade among cases.

On comparing grade with type, among the 21 poorly differentiated cases, all (100%) were of the diffuse type while among the 16 well-differentiated cases; all (100%) were of intestinal type. Among the 33 moderately differentiated cases, 27 (81.8%) were of the intestinal type, 5 (15.2%) were of mixed type and 1 (3%) was of diffuse type. All these findings were statistically highly significant (P <0.01). In the present study, the majority of intestinal types of gastric carcinomas were either well or moderately differentiated and diffuse types were poorly differentiated which is an established fact as per literature.

In the present study, of the 70 cases, 30 (42.8%) showed aberrant E-cadherin expression. Previous studies[6,9,10] show the prevalence of E- cadherin aberrancy as 11-52% of gastric adenocarcinoma. In contrast to another study from India by Dewan K *et al*[13], the present study shows a higher rate of E-cadherin aberrancy. This could be explained by the fact that the present study included both heterogeneous and negative E-cadherin staining as aberrant while only negative E-cadherin staining was considered abnormal in the earlier mentioned study.

In the present study as per Lauren's classification, aberrant E-cadherin expression was found with disproportionately high frequency in diffuse-type (95.5%) when compared to intestinal-type (18.6%) and mixed type (20%) with a very high statistical significance with P <0.001. These findings are in concordance with other studies showing a high rate of E-cadherin aberrancy in diffuse-type compared to the

intestinal type of gastric adenocarcinoma (Table 6).

In the present study as per CAP grading, all (100%) poorly differentiated tumors had aberrant E-cadherin expression while only 21.2% of moderately differentiated and 12.5% of well-differentiated tumors had aberrant E-cadherin expression and this was statistically very highly significant with P <0.001. These findings are in accordance with previous studies that show a higher rate of E-cadherin aberrancy in poorly differentiated tumors compared to better-differentiated tumors (Table 6). These findings correlate with poor intercellular adhesion and the invasive tendency of poorly differentiated and diffuse types of gastric adenocarcinoma due to aberrant expression of E-cadherin which is a protein with invasion suppression role.

In this study, there was no significant association between E-cadherin with age, gender, nature of the specimen, site of the tumor, size of tumor, lymph node status and tumor invasion. The present study has a few limitations. The proportion of gastrectomy specimens was less compared to gastric biopsies which could have influenced the tumor grading, typing and also IHC staining because of the relatively small representative tissue in the gastric biopsy. Another limitation is inadequate patient follow up to allow comparison of prognosis and survival rate between tumor subgroups and their IHC profiles. E- cadherin does not have a standardized staining protocol. Also, various study groups have used different protocols for E-cadherin aberrancy which could affect the comparison between various studies. Due to the relatively smaller sample size, findings of this study have to be confirmed with future large scale studies.

Identification of decreased expression of E-cadherin will help to identify patients prone to invasive and metastatic tumors. Newer methods of blocking E- cadherin downregulation is one of the future approaches in gene therapy.

CONCLUSION

Aberrant E- cadherin expression was seen in 42.8% of gastric adenocarcinoma. E-cadherin aberrancy was noted in all poorly differentiated tumors. Also, 95.45% of diffuse-type showed aberrant E-cadherin expression indicating the invasive nature of these tumors. There is no significant association of E- cadherin with age, gender, nature of the specimen, site of the tumor, size of the tumor, lymph node status and tumor invasion. Our study highlights the importance of identification of aberrant E-cadherin expression in gastric adenocarcinoma which helps in prognostication and identifying patients suitable for novel therapeutic interventions.

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