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Expression of E-Cadherin and B -Catenin in Gastric Carcinoma and its Correlation with the Clinicopathological Features

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Abstract: ***Background: The E-cadherin-catenin complex plays a crucial role in epithelial cell-cell adhesion and in the maintenance of tissue architecture. Abnormal expression or function of this complex results in loss of intercellular adhesion, with possible consequent cell transformation and tumour progression. Methods: Immunohistochemical staining for E-cadherin and β-catenin was performed on paraffin sections of 80 gastric carcinomas at a tertiary hospital. Results: Since E-cadherin and b-catenin complex are transmembranous molecules, normal membrane staining is observed in gastric mucosa. Abnormal expression of E-cadherin and β-catenin was demonstrated in gastric carcinoma. Of the 80 gastric carcinoma cases studied 40 were Intestinal type and 39 were Diffuse type with 01 Mixed type. According to cancer grade, 27 were Well Differentiated, 14 were Moderately Differentiated and 39 were Poorly Differentiated. Abnormal staining for E-cadherin and β-catenin was seen in 50% and 47.5% respectively in 40 cases of Intestinal type, and in 87.18% and 76.9% respectively in 39 cases of Diffuse type. 1 case of Mixed type carcinoma showed abnormal staining for both markers. Abnormal staining for both markers were seen more in Poorly differentiated than in Well and Moderately differentiated carcinomas. Conclusion: The present study shows that E-cadherin and β-catenin are implied in the initiation and progression of gastric carcinomas.***

1. Introduction

E-cadherin (CDH1) is a 120 kDa trans-membrane glycoprotein that mediates calcium dependent cell-to-cell adhesion. CDH1 gene, located on chromosome 16q22, is expressed in all epithelial cells. The cytoplasmic domain of E-cadherin complexes with cytoplasmic proteins-Catenins[1,2]. The catenin, consists of three proteins: (α, β and γ). β -catenin complexes with E-cadherin, while α-catenin links this complex to the actin cytoskeleton[3,4]. Perturbation of E-cadherin mediated cell adhesion leads to tumor progression and metastasis[5, 6]. Germline mutations in the CDH1 gene predisposes to sporadic diffuse-type gastric cancers and the subsequent inactivation due to methylation, mutation or Loss of Heterozygosity (LOH) of second allele of E-cadherin leads to Hereditary Diffuse Gastric Cancer (HDGC).[7,8,9] It is postulated that the under-expression of E-Cadherin accounts for invasive potential of epithelial tumors and appears to be a late event. Thus, the E-cadherin gene is also called an "invasion suppressor gene".

β-Catenin is a 92 kDa protein, localized at chromosome 3p21, binds directly and tightly to E-cadherin, affecting the strength of cell-cell adhesion[1o]. It is also involved in the Wingless/Wnt signaling pathway,involved in cell division[11]. In the absence of a mitotic signal, β-Catenin is bound in a complex formed by APC gene product, GSK-3β and adapter protein Axin. This complex degrades free cytoplasmic β-Catenin by ubiquitin-proteosome system. In the presence of a mitotic signal, Wnt family of glycoproteins bound to frizzled receptors activates disheveled (DSH) protein. DSH deactivates the β-Catenin degradation complex, leads to stabilization of cytoplasmic β-Catenin, which translocates to nucleus and binds to transcription factors Tcf, leading to activation of gene expression. Uncontrolled activation of this signaling pathway leads to uncontrolled proliferation of target cells and contributes to development of malignancy[12].

Role of Cadherin-Catenin Complex in Oncogenesis

Aberrant expression of E-cadherin and/or catenin is seen in a proportion of carcinoma in situ and invasive carcinomas. Loss of expression of E-cadherin in poorly differentiated carcinomas and relatively strong expression in well differentiated carcinomas suggests its role in development and progression of malignant tumor[13]. Low expression in highly metastasizing carcinomas indicates its role in the development of metastasis.

Abnormal activation of Wnt signaling pathway leads to uncontrolled cell proliferation[11]. Alterations in β-catenin gene leading to loss of cell-cell adhesion have been observed in gastric cancer cell lines derived from signet ring cell carcinoma of stomach and these show diffuse growth pattern[14].

Dysfunction of E-cadherin and β-catenin can also be due to its failure to localize to the membrane or bind to cytoskeleton despite occurring in large numbers[15.16]. This occurs due to alterations in their phosphorylation status due to overexpression of EGFR, c-erbB2 and c-met, leading to development of cancers[17,18,19,]. Abnormal expression of E-cadherin-catenin complex correlates with grade of differentiation, invasiveness, metastasis and tumor stage[20]. Aberrant expression also correlates well with disease relapse, disease free survival and overall survival and has been shown to be an independent prognostic marker for shorter survival[21].

Loss of the E-cadherin locus on chromosome 16 (16q22) occurs in gastric (24%), hepatocellular (50%), lobular breast (50–100%) and oesophageal (66%) carcinomas as suggested by several reports[22,23]. Several studies have reported

germline mutations in the E-cadherin gene in families with an inherited diffuse type of gastric cancer. Frequent somatic mutations of β-catenin gene have been found in small colorectal adenomas and intestinal type gastric cancer[24]. Genetic alterations in β-catenin abolishes cell-cell adhesiveness, as seen in some signet ring cell carcinoma of stomach and these show diffuse growth pattern.

2. Materials and Methods

A prospective study was carried, over a period of 2 years (Nov 2015 to Oct 2017) at Osmania General Hospital, Hyderabad.

A total of 80 cases of surgically excised / biopsy specimens of Gastric carcinomas, were taken up for the study. H&E stained sections of these cases were reported according to Laurens classification as: Intestinal type- 40, Diffuse type- 39 and Mixed type – 1.

Representative areas of gastric carcinoma were marked on the slides and the blocks. Using a hollow needle, tissue cores with regions of interest are removed to prepare a tissue microarray for IHC staining. 6 cores of 5mm each were arranged on each slide. The kits for E-cadherin and β-catenin Immunohistochemical staining were obtained from Biogenex Company. Staining was done according to manufacturer's protocol. Normal gastric mucosa included within the tissue sections acted as positive controls. The fibroblasts and lymphocytes in these samples were used as negative controls. Two micro sections,4-5µm thick, from each tissue microarray paraffin blockwere taken on poly-L-lysine coated slides for immunostaining.

Scoring and Evaluation

Scoring of E-cadherin immunohistochemical staining was done according to the system of Jawhari [25].

0-No staining.
1-Cytoplasmic staining without membranous staining.
2-Cytoplasmic and membranous staining in the same case.
3-Normal membranous immunoexpression.

- Abnormal patterns- scores 0, 1 and 2.
- Normal pattern- score of 3.

Scoring of β-catenin immunohistochemical staining was done according to the system of Sergio et al[26]:

0-No or weak dot like membranous staining.
1-Membranous staining in <25% of tumor cells.
2-Membranous staining in 25-75% of tumor cells.
3-Membranous staining in >75% of tumor cells.

- Abnormal patterns- scores 0,1 and 2.
- Normal pattern - score of 3.

Statistical Analysis

Correlation between clinicopathological factors, E-cadherin and β-catenin expression was evaluated using Chi square test. p-values <0.05 were considered statistically significant.

3. Results

Total cases in the present study were 80. Of these 56 males and 24 females,ages ranged from 18 to 80 years. Overall mean age was 54 years, with 56 years for males and 50 years for females. Majority of cases were seen in the 6th and 7th decades, with most seen in 6th decade for males and 5th and 6th decade for females. Majority of the cases involved the pyloric antrum- 48 cases (60%) followed by body-20 cases (25%). Case distribution according to Laurens' classification was Intestinal type- 40 cases, Diffuse type- 39 cases and one case of Mixed type. Distribution according to histological grades were Well differentiated-27 cases, Moderately differentiated-14 cases and Poorly differentiated- 39 cases.

Table 1: Co-expression of E-cadherin and β-catenin with p-value:

	E-cadherin aberrant expression	E-cadherin normal expression	p-value
β-cateninaberrant expression	42	8	0.000145
β-cateninnormal expression	13	17	Significant

Table 2: Comparison of E- Cadherin and β - Catenin in different variables

Comparison of E - Cadherin and β -Catenin in different variables		E - Cadherin Expression			β - Catenin Expression		
		Aberrent	Normal	p value	Aberrent	Normal	**p value**
Sex	Male(n-56)	37	19	0.429795 (p>0.05)	35	21	1 (p>0.05)
	Female(n-24)	18	6	Not Significant	15	9	Not Significant
Age (Mean)	n-80	56.72	52.93	-	55.82	53.10	-
Location	Cardia	4	1	0.072 (p>0.05) Not Significant	3	2	0.9415 (p>0.05) Not Significant
	Fundus	7	0		5	2	
	Body	17	3		13	7	
	P. Antrum	27	21		29	19	
Grade	Well.Diff	15	21	0.004449 (p>0.05) Significant	12	15	0.024866 (p<0.05) Significant
	Mod.Diff	7	7		8	6	
	Poor.Diff	34	5		30	9	
Laurens	Intestinal	20	20	0.000382 (p>0.05) Significant	19	21	0.007059 (p<0.05) Significant
	Diffuse	34	5		30	9	
	Mixed	1	-		1	-	

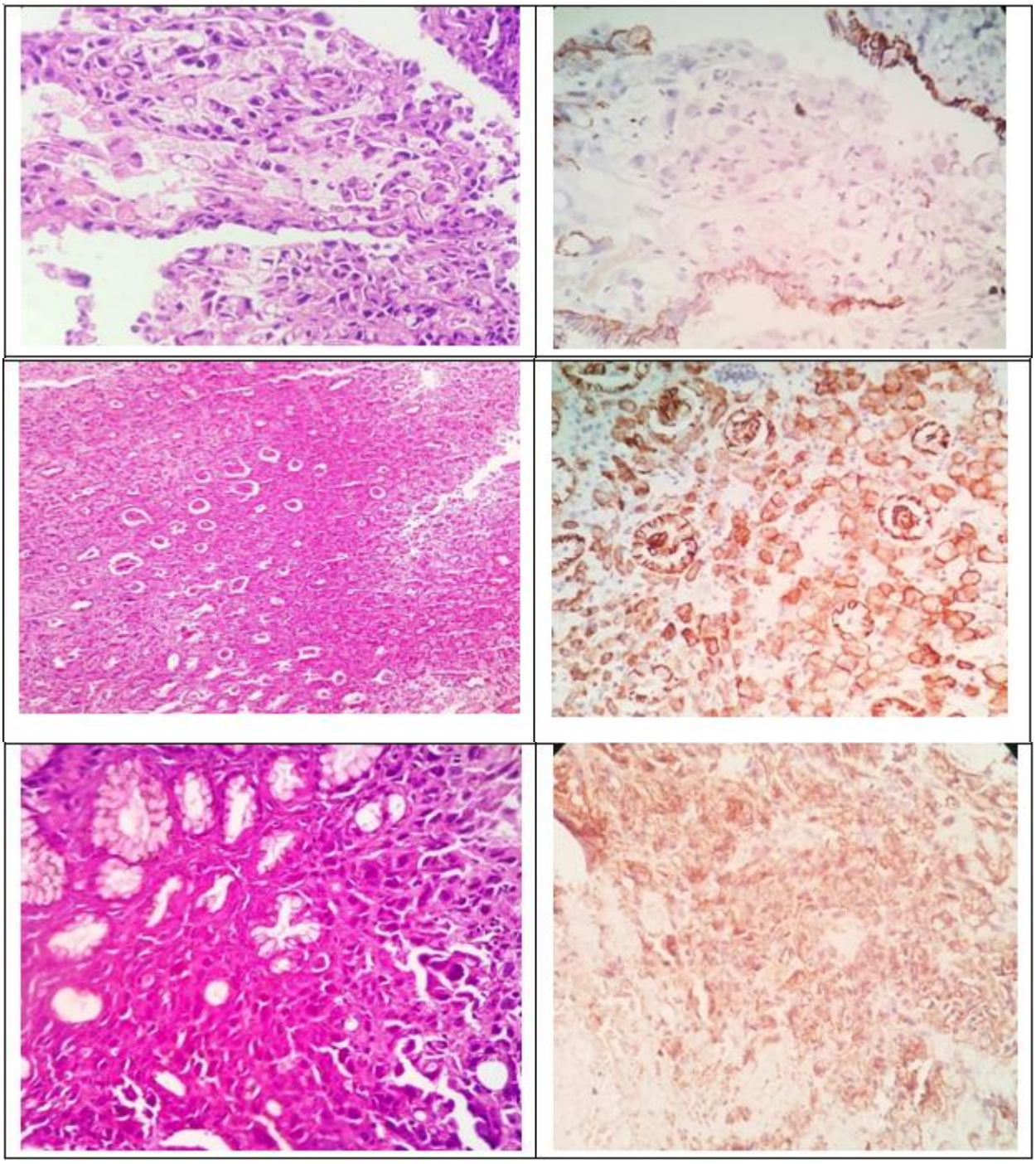

Figure 1&2: A case of diffuse gastric carcinoma showing signet ring cells on H&E and loss of E-cadherin immunoexpression. 3&4 A case of diffuse gastric carcinoma showing diffuse pattern and cytoplasmic staining of E-cadherin. 5&6 A case of diffuse gastric carcinoma showing diffuse pattern and cytoplasmic staining of B-Catenin.

4. Discussion

The present study was done in the Upgraded Department of Pathology, Osmania general hospital, Hyderabad. Staining patterns of E-cadherin and β-catenin were evaluated in gastric carcinomas.

In the present study, ages of the patients ranged from 18 to 80 years. The mean was 53.89 and median was 57.5. Male to female ratio was 2.3:1 with 56 males and 24 females. The ages in various studies ranged from 18-94 years[27,30].In our study, mean age in patients with aberrant expression of E-cadherin was 56.72 and with expression β-catenin was 55.8. There was no statistical significance between the age, mean, median and the aberrant expression of E-cadherin and β-catenin.

In the present study, four cases below the age of 30 years, showed E-cadherin and β-catenin aberrant expression. In the cases aged 31 to 40 years, 5 of 6 in males and 3 of 4 in females showed aberrant expression. Of the 6 cases between 71 to 80 years, 2 of 6 showed aberrant E-cadherin expression and 1 of 6 showed aberrant β-cateninexpression. Thus aberrant expression is seen more commonly in the extremes of ages in the present study.

The present study, in concordance with other studies, compared the relation between location of the tumor in the stomach to aberrant expression of E-cadherin and β-catenin and showed no statistical significance between the variables.Majority of cases in the present study involved the lower 1/3rd part of the stomach. Other studies also showed majority of the cases involvement of the same. Percentage of cases showing aberrant expression of E-cadherin and β-catenin were more frequent in the upper 2/3rd part of the stomach.

In the present study, of the 40 cases of intestinal type gastric carcinomas, half showed aberrant E-cadherin expression and about half of the cases showed aberrant β-catenin expression. In the diffuse type of gastric carcinomas, 34 cases out of 39 (87.2%) showed aberrant expression of E-cadherin and 30 out of 39 (76.9%) showed aberrant expression of β-catenin. Mixed type of gastric carcinoma in the present study, diagnosed as Mixed Adeno-neuroendocrine Carcinoma (MANEC), showed aberrant expression of both E-cadherin and β-catenin.

In the study by Yong-Ning-Zhou et al [27], expression of E-cadherin and β-catenin in gastric carcinomas was compared to the clinicopathological features and patient survival. Of the 163 cases of gastric carcinoma studied, 108 were intestinal type, 40 were diffuse type and 15 were of mixed type. Aberrant E-cadherin and β-cateninexpression were seen majorly in diffuse type of gastric carcinomas and is in concordance with the present study. This study showed a significant correlation between aberrant β-cateninexpression and lymph nodal metastasis. It also concluded of a positive relation between survival and retention of membranous expression of β-catenin. Thus aberrant β-catenin expression can be used as a useful prognostic marker.

Study by Elena Fricke[28] et al have shown mutation in the gene encoding E-cadherin in 16/24 cases (66%) of diffuse gastric carcinomas and E-cadherin was detectable in all the tumor samples, but the localization and intensity of expression of E-cadherin differed. A possible reason for the abnormal localization of E-cadherin is because it redistributes to cytoplasm due to loss of cell-cell contact.

Table 5: Comparison of aberrant expression of E-cadherin and β Catenin with histological types of gastric carcinoma in present study and other studies

Study	No of cases	Aberrant expression of E-Cadherin	p value	Aberrant expression of β -Catenin	p value
Yong-Ning-Zhou et al[27]	Intestinal=108	36 (33.3%)	p<0.05 significant	34 (31.5%)	p<0.05 significant
	Diffuse=40	29 (72.5%)		28 (70%)	
	Mixed=15	10 (66.7%)		11 (73.3%)	
Yaw Ohene et al[34]	Intestinal=28	17 (60%)	p<0.05 significant	19 (67%)	p<0.05 significant
	Diffuse=7	7 (100%)		7 (100%)	
	Mixed=6	6 (100%)		6 (100%)	
In Mok Jung et al[28]	Intestinal=44	10 (23%)	p>0.05 NS	32 (73%)	p>0.05 NS
	Diffuse=67	24 (36%)		48 (72%)	
Jolanta et al[33]	Intestinal=61	21 (34.4%)	p>0.05 NS	24 (39.4%)	p>0.05 NS
	Diffuse=30	16 (53.3%)		19 (63.3%)	
Present study	Intestinal=40	20	p<0.05 significant	19	p<0.05 significant
	Diffuse=39	34		30	
	Mixed=1	1		1	

In the present study, aberrant E-cadherin expression was noted in less than 50% of well differentiated, 50% of moderately differentiated and more than 75% of poorly differentiated carcinomas. This shows that with poor differentiation and high grade of tumors, there is loss of E-cadherin expression which can be used for prognostication. Similar patterns of expression were noted with the use of β-catenin. The values of aberrant expression for E-cadherin and β-catenin were statistically significant. These values were in concordance with other similar studies.

With rise in number of cases of gastric carcinoma in developing countries, there has been a need for diagnosing gastric carcinomas early and for better prognostication. Study by Runjanetetal[29] emphasis on the potential applications of nuclear and para-nuclear E-cadherin immunostaining in cases of gastric carcinoma. E-cadherin accumulates in the cytosol due to failure of integration of E-cadherin in to cell adhesion complex. This accumulated E-cadherin in the cytosol is transported into the nucleus and can be demonstrated through immunostaining.

In the study by Philip et al[30],para-nuclear immunostaining of E-cadherin was compared to the histological types of gastric carcinomas. Of the 173 cases studied, 18% of intestinal type, 30% of diffuse type and 30% of mixed type gastric carcinomas showed prominent, punctate to vesicular, para-nuclear E-cadherin immunostaining. A greater proportion of diffuse and mixed type of gastric carcinomas displayed para-nuclear immunostaining for E-cadherin. Accumulation of E-cadherin has been attributed to the defect in transport to the cell surface and its integration in to cell adhesion systems.The residual non-neoplastic tissue included in the sections were also screened for para-nuclear staining. Para-nuclear staining and supra-nuclear staining within columnar gastric epithelium was noted in 59% of the cases that showed para-nuclear staining in the neoplastic tissue. This suggests that alteration in immunoexpression of E-cadherin may be one of the early changes in gastric carcinogenesis.

In a study by Dong Kyun Woo et al[31], role of aberrant expression of β-catenin and mutations in β-catenin exon 3 were assessed in gastric carcinomas.Genetic alterations in β-catenin correlated with the nuclear accumulation of β-catenin, thus indicating its role in gastric carcinogenesis.

This study also noted that determination of membranous expression of β-catenin may be used as a prognostication marker for predicting patients' survival.

Understanding the mechanisms in silencing or mutation of genes encoding E-cadherin and β-catenin is important for targeted therapy. Hypermethylation has been proposed as a possible mechanism for silencing of tumor suppressor genes. Graziano et al[32] analysed gastric carcinomas for immunoexpression of E-cadherin and CDH1 promotor hypermethylation. Majority of cases that showed CDH1 promotor hypermethylation, also showed loss of E-cadherin expression and diffuse pattern on histology. Thus, patients with CDH1 promotor hypermethylation may represent an ideal setting for testing demethylating drugs. These drugs can also be used as chemoprevention for patients with hereditary diffuse gastric carcinomas. As CDH1 promotor hypermethylation is not the sole mechanism for silencing of CDH1 gene, there can be failure of response to demethylating drugs.

5. Conclusion

A significant correlation was found between membranous E-cadherin and β-catenin immunoexpression and intestinal type gastric carcinomas; aberrant E-cadherin and β-catenin immunoexpression and diffuse type of gastric carcinomas.

Thus, the present study shows that E-cadherin and β-catenin are implied in the initiation and progression of gastric carcinomas, as its expression is lost in advanced stages of the disease and high grade tumors. Diffuse carcinomas are associated with absence of membranous staining of E-cadherin and β-catenin and show absent or cytoplasmic staining for E-cadherin and nuclear and/or cytoplasmic staining for β-catenin.Absence of membranous expression of E-cadherin and β-catenin is associated with invasion, metastasis and thus with poor prognosis.

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