Original Article

Evaluation of Immunohistochemical Expression of Beta-catenin and E-cadherin as Epithelial–Mesenchymal Transition Markers in Colorectal Carcinoma – An Analytical Retrospective Study in a Tertiary Care Center

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Abstract

Context: Colorectal carcinoma is a major health concern globally, with varying prognostic outcomes influenced by molecular markers. E-cadherin and beta-catenin are proteins involved in cellular adhesion and signaling pathways, and their aberrant expression has been implicated in tumor progression and metastasis. **Aims:** This study aims to evaluate the association between abnormal immunohistochemical expression of E-cadherin and beta-catenin with various clinicopathological parameters in colorectal carcinomas. **Setting and Design:** A retrospective cross-sectional 3-year analytical study. **Materials and Methods:** A total of 52 colorectal carcinoma tissue samples were analyzed using immunohistochemistry to assess the expression levels of E-cadherin and beta-catenin. Clinicopathological parameters including age, gender, tumor location, tumor differentiation, depth of invasion, perineural invasion, lymphovascular invasion, and nodal involvement were assessed and correlated with protein expression patterns. **Statistical Analysis:** SPSS version 24 was used for calculating *P* values using the Chi-squared test. **Results:** Aberrant expression of E-cadherin and beta-catenin was observed in a significant proportion of the tumors. Poorly differentiated tumors showed a marked loss of E-cadherin and abnormal beta-catenin localization. In addition, increased lymphovascular and nodal involvement were significantly associated with these aberrant expression patterns. **Conclusion:** The findings suggest that abnormal expression of E-cadherin and beta-catenin is linked to poor tumor differentiation and higher rates of lymphovascular and nodal involvement. These markers may serve as potential biomarkers for assessing prognosis in colorectal carcinoma patients.

Keywords: *Beta-catenin, colorectal carcinoma, E-cadherin, immunohistochemistry, prognosis, tumor differentiation*

Introduction

Of all the fundamental changes that happen in the cell physiology of a malignant cell, the ability to invade and metastasize is the most distinct hallmark. This ability occurs with the help of the process called epithelial–mesenchymal transition (EMT), where the malignant cells lose epithelial differentiation and gain mesenchyme-like capabilities which help the cells to detach, migrate, and gain access to blood or lymphatic vessels and disseminate in the body.[1] Colorectal cancer (CRC) is the third most frequent malignancy around the world and its 5-year survival rate reduces from 89% to 90% in localized cancers to 10%–15% in metastatic cancers.[2] Consequently, the occurrence of distant metastases is the most dreaded event during the disease course.[3] Therefore, it is vital to divulge any biological mechanism underlying metastases of CRC early.

There is mounting evidence that suggests EMT plays a very critical role in the progression of CRC. In EMT, epithelial cells lose cell-cell adhesion systems, and polarity and acquire mesenchymal phenotype which enables the cells to disseminate into surrounding and far-off sites through lymphatics or blood vessels. Of the several markers that act as EMT indicators, loss of E-cadherin expression (structural adhesion protein) is considered a hallmark of EMT. Adenomatosis polyposis coli (APC)-WNT-beta-catenin-E-cadherin pathway regulates this entire process. Beta-catenin is a molecule that is normally bound to membrane-associated E-cadherin

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and it determines the function and proper positioning of E-cadherin molecule.[4] Signaling through WNT receptor or mutations in APC gene results in the displacement of membrane-bound form of beta-catenin and increase in its concentration within the cytoplasm and nucleus and results in loss of membrane expression of E-cadherin, activation of various proto-oncogenes such as C-myc, Cyclin D1, and genes associated with invasiveness such as fibronectin and CD44.[5]

Accordingly, the present study aims at evaluating the clinical significance of immunohistochemical expression and localization of beta-catenin and E-cadherin in tumor cells of the invasive front of colorectal carcinomas in different stages and recognizing if these markers can be used as predictive markers for lymph node metastasis and poor prognosis.

Materials and Methods

The present study is a retrospective analytical study performed in a tertiary care teaching hospital in the southeastern belt of India. Institutional ethical clearance was taken as per the departmental protocol.

Inclusion criteria

All the resection specimens of colorectal adenocarcinoma received during three years (January 2021 to December 2023) were included in the study.

Exclusion criteria

Cases with a family history of colorectal adenocarcinoma, small colonoscopic biopsies, reexploration surgery specimens, resection specimens of patients who underwent neoadjuvant therapy before surgery, and gastrointestinal tumors other than colorectal adenocarcinomas were excluded from the study.

Clinical features, demographic facts, and other relevant data were retrieved from the departmental archives.

Sections were readied following routine 10% buffered formalin fixation, grossing, automatic tissue processing (Leica TP1020), embedding, and manual microtomy. Grossly tumor location was considered as left when it was present distal to splenic flexure. Hematoxylin and eosin-stained sections were used to identify the histological variant, differentiation, depth of invasion, perineural invasion, lymphovascular invasion, and nodal status. Immunohistochemistry for beta-catenin and E-cadherin were performed in representative sections from all cases, manually using manufacturers' protocol. Antigen retrieval was performed using a pressurized decloaker. Immunohistochemical expression was analyzed and scored by two independent pathologists who were blinded to the clinical data. Only representative sections from the invasive front of the tumor tissue were analyzed. For the markers beta-catenin and E-cadherin, both localization (membranous, cytoplasmic, and/or nuclear expression) and intensity of expression (+++ strong, ++ moderate, +weak, and 0 negative) were analyzed. Tumors were considered immunopositive when at least 10% of tumor cells were immunoreactive.[6] Data analysis, tabulation, and statistical processing were performed using Microsoft Excel 2020 and Statistical Package for Social Sciences (SPSS version 24, IBM, Armonk, New York, USA). Chi-squared test was used for calculating the value of significance. $P \leq 0.05$ was considered significant.

Results

After considering all the inclusion and exclusion criteria, a total of 52 cases were included in the present analysis.

Patient demographics and tumor histopathological features

The mean age of the population in the present study was 54.5 years (age ranged between 34 and 77 years). The male-to-female incidence ratio was 0.53 with a female preponderance. The most common location for the tumor was left, i.e., distal to the splenic flexure of the colon (30 cases). Of the 52 cases included most cases showed well-to-moderate differentiation (42 cases). Lymphovascular invasion was seen in 28 cases and perineural invasion was seen in 25 cases. The most common stage was T3 (22 cases) and N0 (27 cases) status.

E-cadherin expression in tumor cells

As indicated in Table 1, E-cadherin showed variability in both localization and intensity of staining. Of the 52 cases included in the present study, 16 cases showed a complete absence of E-cadherin expression. Of the remaining 36 cases, 30 cases showed normal membranous expression and 6 cases showed nuclear and/or cytoplasmic expression. When E-cadherin expression was correlated with clinicopathological features, absence of E-cadherin expression was seen to be significantly associated with poorly differentiated adenocarcinomas ($P = 0.0001$), presence of lymphovascular invasion ($P \leq 0.0001$), and higher N status ($P \leq 0.0001$).

Beta-catenin expression in tumor cells

As indicated in Table 2, beta-catenin was expressed in all the 52 cases included in the study. However, localization and intensity varied from case to case. Intensity of staining was less than adjacent normal tissue in all the cases. Nuclear and/or cytoplasmic expression of beta-catenin was seen in 31 of the 52 cases and was found to be significantly associated with female gender, poorly differentiated tumors ($P = 0.02$), presence of lymphovascular invasion ($P = 0.006$), and higher nodal ($P = 0.0002$) status.

When examining the expression of beta-catenin and E-cadherin individually in each case, it was observed that in all instances where E-cadherin was either completely

Table 1: Clinicopathological parameters with respect to E-cadherin expression

Clinicopathological parameters	*n*	E-cadherin expression in neoplastic cells			*P*
		Membranous	Nuclear and/or cytoplasmic	Absent	
Age (years)					
≤50	22	10	2	10	0.14
>50	30	20	4	6	
Gender					
Males	18	12	3	3	0.24
Females	34	18	3	13	
Tumor laterality					
Left	30	18	4	8	0.72
Right	22	12	2	8	
Tumor differentiation					
Well	24	19	3	2	0.0001
Moderate	18	10	3	5	
Poor	10	1	0	9	
Mucinous					
Absent	36	24	4	8	0.10
Present	16	6	2	8	
Perineural invasion					
Absent	27	18	2	7	0.36
Present	25	12	4	9	
Lymphovascular invasion					
Absent	24	22	0	2	<0.0001
Present	28	8	6	14	
Depth of invasion					
Tumor invades submucosa (T1)	2	2	0	0	0.18
Tumor invades muscularispropria (T2)	19	11	4	4	
Tumor invades sub serosa or nonperitonealized pericolic tissue (T3)	22	14	0	8	
Tumor invades other organs or perforates visceral peritoneum (T4)	9	3	2	4	
Nodal involvement					
No nodal involvement (N0)	27	24	0	3	<0.0001
1–3 regional lymph node involvement (N1)	23	6	6	11	
4 or more regional nodal involvement (N2)	2	0	0	2	

absent or localized in the cytoplasm and/or nucleus, beta-catenin was found to localize in the cytoplasm and/or nucleus. Figures 1-3 are photomicrographs of some of the cases included in the study.

Discussion

Diverse molecular pathways are involved in colorectal carcinogenesis and hence even tumors with the same TNM stage behave variedly and have different prognoses.[7] Hence, the search for different prognostic markers has always remained a theme of research.

EMT is a molecular process which is essential for "loosening up," invasion, migration, and metastasis in any malignant epithelial tumor progression. Various membrane receptor complexes play an important role in cell–cell adhesions and interactions. Most noteworthy of all is the E-cadherin, a transmembrane glycoprotein, which is normally expressed in the basolateral membranes of epithelial cells. Loss or abnormal localization of this protein has been associated with loss of epithelial differentiation, EMT, tumor progression, invasion, and metastasis because of loss of cell adhesiveness.[8-10] Beta-catenin is a very versatile protein essential for cellular homeostasis, embryonic development, organogenesis, stem cell maintenance, cell proliferation, migration, differentiation, apoptosis, and progression of various human diseases, including cancer.[11]

In the current analysis, we found loss or abnormal localization of E-cadherin in 22 cases (42.3%) and abnormal localization of beta-catenin in 31 cases (59.6%) of all cases. These results are in concordance of those obtained by Stanczak *et al*.[6] However, other studies have reported a much higher incidence of this aberrant localization (varying between 80% and 100%).[8-10] These differences in results could be because of the use of different inclusion and exclusion criteria, the use of different antibody clones from different manufacturers' and biological differences of the tumors *per se*.[3]

In the current analysis, whenever there was a decrease or aberrant localization of E-cadherin, aberrant expression of

Table 2: Clinicopathological parameters with respect to beta-catenin expression

Clinicopathological parameters	Number of cases	Beta-catenin expression in neoplastic cells		*P*
		Membranous	Nuclear and/or cytoplasmic	
Age (years)				
≤50	22	6	16	0.09
>50	30	15	15	
Gender				
Males	18	11	7	0.02
Females	34	10	24	
Tumor laterality				
Left	30	12	18	0.94
Right	22	9	13	
Tumor differentiation				
Well	24	14	10	0.006
Moderate	18	7	11	
Poor	10	0	10	
Mucinous				
Absent	36	17	19	0.13
Present	16	4	12	
Perineural invasion				
Absent	27	14	13	0.07
Present	25	7	18	
Lymphovascular invasion				
Absent	24	16	8	0.0003
Present	28	5	23	
Depth of invasion				
Tumor invades submucosa (T1)	2	2	0	0.06
Tumor invades muscularispropria (T2)	19	10	9	
Tumor invades sub serosa or nonperitonealized pericolic tissue (T3)	22	8	14	
Tumor invades other organs or perforates visceral peritoneum (T4)	9	1	8	
Nodal involvement				
No nodal involvement (N0)	27	18	9	0.0002
1–3 regional lymph node involvement (N1)	23	3	20	
4 or more regional nodal involvement (N2)	2	0	2	

beta-catenin was seen concomitantly. These findings are concordant with earlier reports.[6,12]

Absence or aberrant cytoplasmic/nuclear expression of E-cadherin was found to be highly significantly associated with poor differentiation ($P = 0.0001$), presence of lymphovascular invasion ($P \leq 0.0001$), and higher nodal status ($P \leq 0.0001$). However, in the study by Melincovici *et al*.,[8] a significant correlation occurred only between aberrant E-cadherin expression and tumor differentiation ($P = 0.018$).

The current analysis showed that cytoplasmic and/or nuclear expression of beta-catenin was significantly associated with female gender ($P = 0.02$), poorly differentiated tumors ($P = 0.006$), presence of lymphovascular invasion ($P = 0.0003$), and higher nodal involvement ($P = 0.0002$). Our findings are in concordance with those of Youssef and Osman,[13] Lugli *et al*.,[14] Tunuguntla *et al*.,[15] and Melincovici *et al*.[8] However, Youssef and Osman[13] and Lugli *et al*.[14] also found a significant association between nuclear expression of beta-catenin and depth of invasion. The results of the present study show that aberrant expression of EMT proteins is associated with aggressive behavior of the tumor. These findings can be substantiated by considering the role of these molecules in cell-cell interactions, invasion, and migration.[4]

The literature review has shown that aberrant expression of E-cadherin and beta-catenin is a poor prognostic marker not only in colorectal carcinomas but also in carcinomas of the breast, liver, prostate, bladder, and cervix.[8] Hence, enhanced research in developing cancer therapeutic agents that can stabilize the membrane E-cadherin-beta-catenin glycoprotein complexes and inhibitors of nuclear beta-catenin can be the way forward.[11]

The current analysis supports previous literature that EMT plays an important role in colorectal carcinoma progression and highlights that localization of the

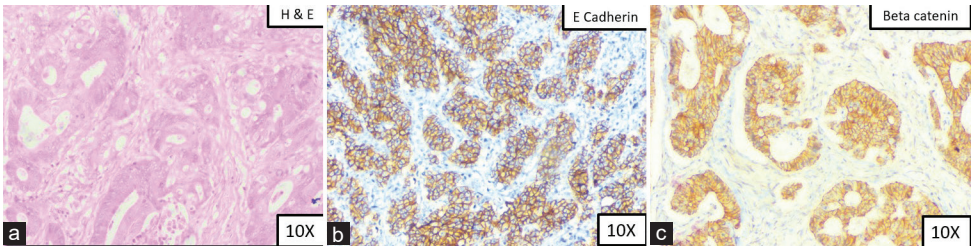

Figure 1: Photomicrograph showing (a) Well-differentiated colorectal adenocarcinoma, (b) Normal membranous expression of E-cadherin, (c) Normal membranous expression of beta-catenin

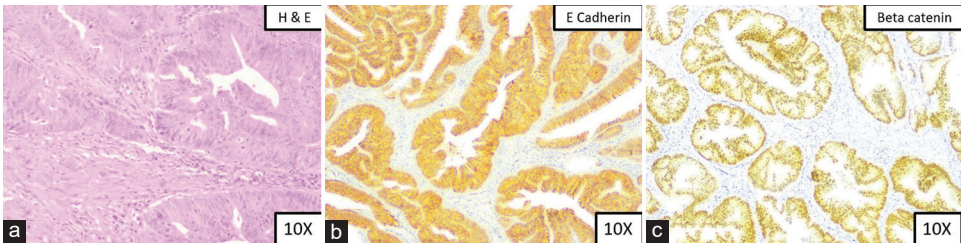

Figure 2: Photomicrograph showing (a) Well-differentiated colorectal adenocarcinoma, (b) Aberrant cytoplasmic and nuclear expression of E-cadherin, (c) Aberrant nuclear expression of beta-catenin

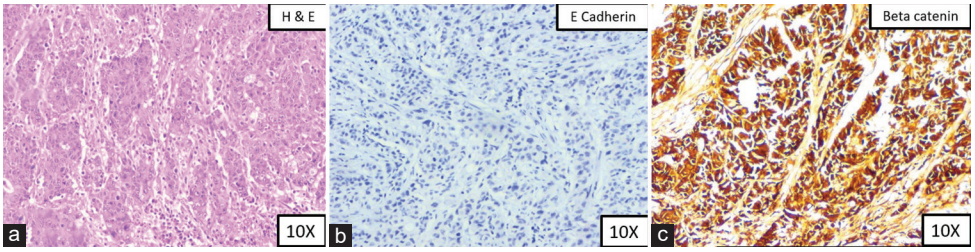

Figure 3: Photomicrograph showing (a) Poorly differentiated colorectal adenocarcinoma, (b) Aberrant absent expression of E-cadherin, (c) Aberrant cytoplasmic and nuclear expression of beta-catenin

protein expression is more important when compared to the intensity of staining, of EMT markers and these markers can be useful in predicting colorectal carcinoma progression in every case individually, irrespective of the stage at diagnosis, and hence can result in personalized therapy.

Limitations of the present study

Small sample size (52 cases), selection bias (being a retrospective and single-centered analysis), and nonavailability of survival data are some of the limitations of the present study.

Conclusion

The current analysis found that abnormal immunohistochemical expression of E-cadherin and beta-catenin is linked to poorly differentiated tumors, and increased lymphovascular and nodal involvement, suggesting that these markers may indicate a poor prognosis in colorectal carcinomas.

Ethical statement

Institutional ethical clearance was taken. Certificate number: GMC/IEC/109/2023, Dated 13.06.2024.

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Conflicts of interest

There are no conflicts of interest.

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