Evaluation of β-Catenin Expression in Relation to Staging and Grading of Colorectal Carcinoma – A Study Conducted in Jubilee Mission Medical College and Research Institute, Thrissur

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ABSTRACT

BACKGROUND

Colorectal carcinoma is the most frequently encountered malignancy worldwide. The main cause of mortality associated with colorectal malignancy is tumour invasion and metastasis. The major genomic alteration that has been found in colorectal carcinoma is mutation in the adenomatous polyposis gene. Mutated APC causes unrestricted action of the Wnt signalling pathway which results in accumulation of the β - catenin protein in the nucleus responsible for cell proliferation, differentiation and enhanced survival of colorectal epithelial cells. Role of β - catenin expression as a prognostic marker needs to be studied. It will help in aiding the possibility of the future of anti β - catenin targeted chemotherapy for the treatment of colorectal cancers.

METHODS

A total of 85 samples from histopathologically proven cases of adenocarcinoma colon were taken. Histomorphological features and their immunohistochemical expression of β - catenin were studied. Data thus obtained was analysed using SPSS version 25. Histopathological diagnosis is considered as the gold standard.

RESULTS

Among the 85 samples of adenocarcinoma 9 case were stage T1 tumours, 24 were stage T2. 31 cases were stage T3, and 21 colorectal cancers were stage T4. Statistically significant correlation was obtained between tumour stage and β - catenin expression. (P value = < 0.001) With regard to tumour grade 24 out of 85 cases were well differentiated and 45 were moderately differentiated, 16 cases were poorly differentiated. Correlation of tumour grading and β - catenin expression also were statistically significant (P value = < 0.001). 45 out of 85 cases were having no nodal metastasis. 22 cases showed metastasis in 1 to 3 lymph nodes. 18 cases were N2. No statistically significant correlation was obtained between lymph node status and β - catenin expression. (P value = 0.003)

CONCLUSIONS

In this study the membranous, cytoplasmic and nuclear scores of β - catenin shows the linear progression with the advancing stages of colorectal carcinoma. So β - catenin can be used as a prognostic marker in malignant colorectal neoplasm.

KEYWORDS

Immunohistochemistry, β – Catenin, Colorectal Carcinoma

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BACKGROUND

Colorectal cancer is one of the most common forms of gastrointestinal malignancies and the third leading cause of cancer related death in the world. Colorectal cancer account for approximately 9.4 % of total worldwide cancer cases. It is the third most commonly diagnosed cancer in men and the second in women with over 1.2 million cases worldwide. It is usually associated with poor prognosis because it is often at an advanced stage when diagnosed. The overall mortality of colorectal carcinoma is approximately 40 % with the treatment, 30 % - 50 % of patients with stage II to stage III tumours relapse within 5 years following treatment. Colorectal carcinoma (CRC) rises from benign neoplasms, like tubular adenomas and serrated polyps. The slow polyp - cancer progression sequence seen in the general population offers an opportunity to detect and remove the polyps before they undergo malignant transformation.[1] So it is the most preventable cancer. The two main pathways are APC (Adenomatous Polyposis coli) β-catenin pathway and Microsatellite instability pathway.

B - Catenin is a cell adhesion molecule. It is a member of the signal transduction pathway. Mutation of the β - catenin gene, result in activation of the Wnt signaling molecules. B - Catenin accumulation in the nucleus leads to transcriptional activation. In cases of mutation in APC gene and its subsequent dysfunction, over activity of these transcription factors is noted, which results in cellular proliferation. This indicates that the APC protein has a tumour suppressive effect.[2]

The adenoma – carcinoma sequence of colorectal tumour genesis requires accumulation of genetic alterations. Inactivation of the adenomatous polyposis coli (APC) suppressor gene is the first event in this sequence, which results in colorectal adenoma formation.[3] It has been reported that APC mutations are associated with an accumulation of intracellular β - catenin protein, which leads to loss of control of normal β - catenin signaling.[4] The APC gene maps at position 21 q in chromosome 5 and encodes a protein with multiple functional domains that interact with proliferation and apoptosis regulators.[5] In case of APC mutation, the destruction complex fails toubiquitinise β - catenin, which leads to its accumulation of β - catenin intracellularly, potentiating its nuclear translocation, where it acts as a transcriptional activator to target genes like c – myc and cyclin D1, which subsequently play an integral role in the development of colorectal neoplasm.[6]

Studies have been undertaken to investigate and compare the expression pattern of catenin in normal colorectal tissue and in various colorectal neoplasm ; benign, premalignant and malignant. Because detection and treatment at an early stage can dramatically improve survival, there has been great interest in developing diagnostic and prognostic factors that can help to detect asymptomatic colorectal carcinoma, as well as its precursor, colorectal adenoma, and to predict the outcome of this cancer accurately. Normal colorectal tissue does not display nuclear or cytoplasmic immunoreactivity with β - catenin, and an exclusively a diffuse membranous positivity.[6]

Dysplasia is associated with decrease in membranous staining and subsequent increase in nuclear staining of the β - catenin. Studies have been undertaken for the possibility of the future of anti – β - catenin targeted chemotherapy for the treatment of colorectal cancers.

Objective

To evaluate beta catenin expression in colorectal carcinomas and to correlate its score with stage and grade of carcinoma

METHODS

The cross sectional study was conducted from November 2018 to May 2020 in the department of pathology in a private tertiary care centre in central Kerala, India. All patients diagnosed as carcinoma colon / rectum were taken. Written informed consent obtained from each patient. Resected specimen was fixed in 10 % formal saline overnight and all the gross findings recorded. Tissue bits were taken from the representative areas and processed. Sections cut at 5 microns size and stained using hematoxylin and eosin. For immunohistochemical study paraffin blocks subjected to immunohistochemical staining. Scoring of β - catenin done according to staining pattern. The study was approved by the institute ethical committee of Jubilee mission medical college and research institute, Thrissur.

Sampling Procedure

The study was conducted in 85 patients diagnosed to have an adenocarcinoma colon / rectum. Informed consent had been obtained from all patients whose specimens were collected and all the tests were done only after approval obtained from the institutional ethics committee. The resected specimens were fixed in 10 % formol saline overnight. Detailed gross examination was done on the next day. All the gross findings were recorded, including size of the specimen, gross appearance, and exact site of the tumour. Bits were taken from the representative areas and processed. Sections were cut at 5 microns size and stained using hematoxylin and eosin. All cases were staged according to American joint committee on cancer (AJCC) – TNM classification system.

For immunohistochemical study sections were cut at 3 microns size incubated at 60 degree Celsius for 1 hour. Antigen retrieval was done in EDTA buffer, then washed with TBS (Tris buffered saline). Endogenous peroxidase activity was blocked by the treatment with peroxidase block for 10 minutes and again washed in TBS. Non - specific protein binding in tissues is prevented by incubating with power block for 10 minutes at room temperature. Then the tissues were incubated with primary antibody β - catenin (EP35) along with, positive and negative controls for 40 minutes. Secondary antibody was then added followed by chromogen, diaminobenzidine and washed in distilled water and counter stained with Hematoxylin. Normal colonic epithelial cells served as internal positive controls with

membrane staining, and negative controls were obtained by excluding the primary antibody addition.

Reagent

- B - Catenin EP 35 rabbit monoclonal antibody, with 1 % bovine serum albumin (BSA), and 0.05 % sodium azide (NaN3) at 1 : 20 dilution. (path insitu)

B - Catenin Scoring System

B - Catenin Immunostaining were scored according to Jass et al[7] scoring system

- B - Catenin scoring system based on the distribution of β-catenin within the cell membrane, the cytoplasm and nuclei.
- The β - catenin activation score was calculated as the sum of nuclear score, cytoplasmic score and the membrane score.
- Total score then collapsed in to grade I, grade II and grade III

For nuclear scoring three categories are given
0-no expression
+ 1 - weak expression
+ 2 - positive expression
For cytoplasmic scoring
0 - no expression
+ 1 - weak expression
+ 2 - positive expression
For membrane scoring
0 - positive membrane expression
+ 1 - negative membrane expression
Total score collapsed in to three grades.
GRADE I = 0 - 1
GRADE II = 2 - 3
GRADE III = 4 - 5

Total score of 0 reflecting normal colonic mucosa and score of 5 for tumours with strong nuclear staining (n = 2), strong and diffuse cytoplasmic staining (n = 2), loss of cell membrane staining (n = 1).

B - Catenin expression was correlated to tumour stage, grade and lymph node status. All categorical variables were expressed as frequency and percentages. Chi square test was used to obtain the association between β - catenin expression with age, sex, tumour site, stage, grade and lymph node status.

Data Analysis

All categorical variables were expressed as frequency and percentages. Chi square test was used to obtain the association between beta catenin expression with age, sex, tumour site, stage, grade, and lymph node status. Data analysis was done using SPSS software version 25.

RESULTS

63.5 % of colorectal carcinomas were above 60 years of age, 65.9 % cases were males and 34.1 % were females, Majority of tumours were located in caecum and ascending colon (48.2 %) followed by upper and mid rectal tumours (21.2 %), sigmoid colon (12.9 %), lower rectum (7.1 %), descending colon (5.9 %) and transverse colon (4.7 %), No statistically significant correlation was found between beta catenin expression and the age, sex and the site of tumour, 38.8 % of tumours were having stage T3, and least were stage T1 tumours (10.6 %), Among the stage T4 tumours 71.4 % showed beta catenin score of III, 52.9 % of cases were moderately differentiated carcinomas and 18.8 % were poorly differentiated tumours, 62.5 % of poorly differentiated tumours were having beta catenin score of III, 52.9 % tumours were having no lymph node metastasis, while 25.9 % of cases showed metastasis to 2 - 3 lymph nodes (N1), 44.4 % of N2 tumours showed beta catenin score of III, P value obtained for tumour stage and beta catenin association was statistically significant. (P value = 0.000), P value obtained for tumour grade and beta catenin expression association was also statistically significant. (P value = 0.000).

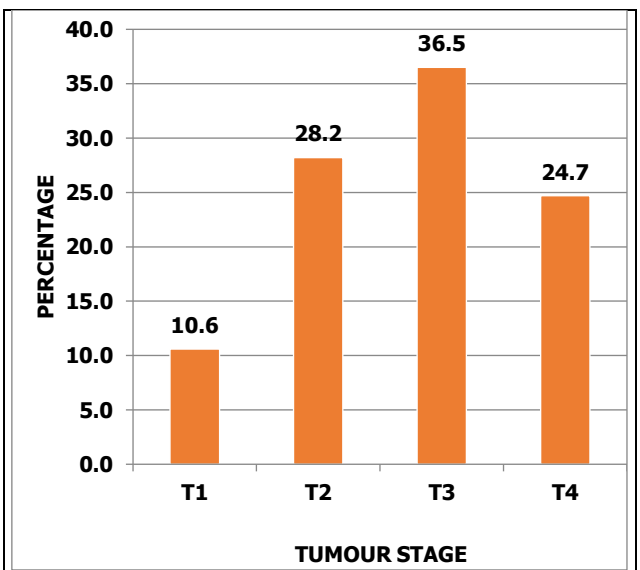

Figure 1 (a). Tumour Stage

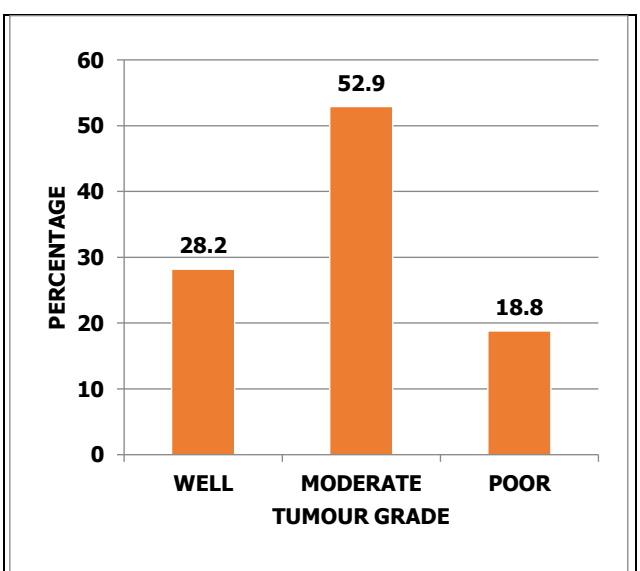

Figure 1 (b). Tumour Grade

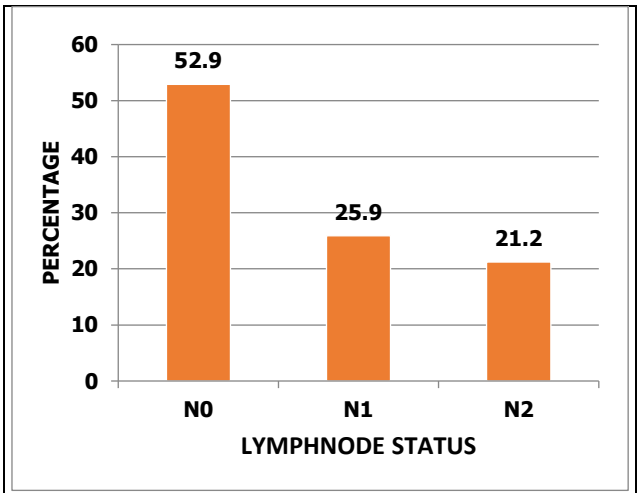

Figure 1 (c) - Lymph Node Status

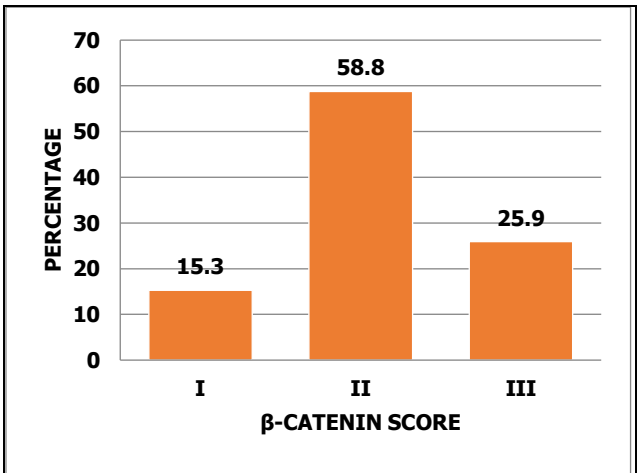

Figure 1 (d). β - Catenin Score

Stage	Frequency i	ii	iii	Total	P Value
t1	4 (44.4 %)	5 (55.6 %)	0 (0.0 %)	9 (100 %)	0.000
t2	6 (25.0 %)	18 (75.0 %)	0 (0.0 %)	24 (100 %)	
t3	3 (9.7 %)	23 (69.7 %)	6 (18.2 %)	31 (100 %)	
t4	0 (0.0 %)	6 (28.6 %)	15 (71.4 %)	31 (100 %)	
Total	**13 (15.3 %)**	**50 (58.8 %)**	**22 (25.9 %)**	**85 (100 %)**	

Table 1. Association of β-Catenin Score with Tumour Stage

Grade	Frequency I	Ii	Iii	Total	P Value
Well	11 (45.8 %)	12 (50 %)	1 (4.2 %)	24 (100 %)	0.000
Moderate	1 (2.2 %)	33 (73.3 %)	11 (24.4 %)	45 (100 %)	
Poor	1 (6.3 %)	5 (31.3 %)	10 (62.5 %)	16 (100 %)	
Total	**13 (15.3 %)**	**5 (58.8 %)**	**22 (25.9 %)**	**85 (100 %)**	

Table 2. Association of Beta Catenin Score with Tumour Grade

Grade	Frequency I	Ii	Iii	Total	P Value
Well	11 (45.8 %)	12 (50 %)	1 (4.2 %)	24 (100 %)	0.000
Moderate	1 (2.2 %)	33 (73.3 %)	11 (24.4 %)	45 (100 %)	
Poor	1 (6.3 %)	5 (31.3 %)	10 (62.5 %)	16 (100 %)	
Total	**13 (15.3 %)**	**5 (58.8 %)**	**22 (25.9 %)**	**85 (100 %)**	

Table 3. Association of β-Catenin Score with Tumour Grade

Lymphnode Status	FREQUENCY I	Ii	Iii	Total	P Value
N0	9 (20.0 %)	32 (71.1 %)	4 (8.9 %)	45 (100 %)	0.003
N1	1 (4.5 %)	11 (50.0 %)	10 (45.5 %)	22 (100 %)	
N2	3 (16.7 %)	7 (38.9 %)	8 (44.4 %)	18 (100 %)	
Total	**13 (15.3 %)**	**50 (58.8 %)**	**22 (25.9 %)**	**85 (100 %)**	

Table 4. Association of β-Catenin Score with Lymph Node Status

Figure 5 (a) β-Catenin Immunohistochemistry Shows Absent Membrane Staining Weak Nuclear Staining and Strong Cytoplasmic Staining. Overall Score 4 (1+1+2)

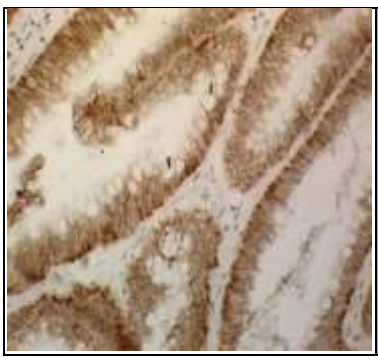

Figure 5 (b). β-Catenin Immunohistochemistry Shows Absent Membrane Staining Weak Cytoplasmic and Absent Nuclear Staining Overall Score 2 (1+1+0)

DISCUSSION

Colorectal cancers are the leading cause of cancer related mortality in the world. Early diagnosis of the colorectal cancers, surgical treatment, and response to adjuvant therapy contributed to better outcome in patients. Expression of β - catenin immunohistochemistry in benign and malignant lesions of colon and rectum were done in several studies, Wong SC et al and Iwamoto et al.[6,8] Here total 85 cases of colorectal adenocarcinoma were taken and classified according to stage, grade and lymph node status and relationship with β - catenin expression were studied.

54 % of our cases were above 60 years of age group, and not a single case was below 20 years. No statistically significant relationship were obtained between patients age and β - catenin expression (p value = 0.123). The study done by Mojarad et al showed patients presented with colorectal carcinoma in younger age group having intense β - catenin nuclear expression compared with older age group individuals and interestingly this difference was statistically significant (P = 0.010). This finding was not in line with our study. We did not find any correlation between β - catenin expression and patient's age. Studies done by Bhattacharya et al[9] and Mohamed et al[10] also could not find out any association between age and β - catenin expression.

Majority of cases in our study (65.9 %) were males. 53.6 % of male colorectal cancers and 69 % of female colorectal cancers showed grade II β - catenin score .No statistically significant relationship obtained between sex and β - catenin score (p value = 0.010) In study done by Wanitsuwan et al majority of colorectal cancers were in males and they showed with strongest nuclear expression of β - catenin compared to female patients. The study conducted by Kazem et al[11] also showed higher β - catenin in males. (p = 0.004). Study done by Bhattacharya et al,[9] Mohamed et al[10]

were in line with our finding and no statistically significant relationship was there between gender and β - catenin expression.

The colorectal neoplasms in this study shows a predilection for proximal segment of the large intestine i.e., caecum and ascending colon. Most of the cases were right sided colon cancers (48.2 %) and least were transverse colon cancers (4.7 %). This agrees with study conducted by Bhattacharya et al. Wong et al[6] However, studies conducted by Mi et al,[12] Schlemper et al.[13] found that distal colon and rectum, to be the most common site of colorectal neoplasm. In the present study, rectum was the second most common site for colorectal neoplasm. No statistically significant link was detected between tumour location and β - catenin expression

Regarding the tumour stage majority of tumours were in stage T3. We found that 55.6 % of T1 tumours showed β - catenin score of II and no T1 stage tumours showed score of III. 75 % of T2 tumours and 67.7 % of T3 tumours showed β - catenin score II. Thus majority of T1, T2 and T3 tumours showed β - catenin score II, while among T4 tumours 71.4 % were score III. It indicates that more deeply invasive tumours showed a higher grade of β - catenin expression than superficial tumours. Similar findings have been reported previously in studies conducted by Kazem et al and Elzagheid et al.[11,14] Kazem et al[11] showed significant association between grade III β - catenin expression in colorectal carcinomas, and advanced tumour stage, [$p < 0.002$], with more deeply invasive tumours showing higher grade expression of β - catenin than superficial tumours. Similar findings have been reported by Moon et al and Wong SC et al.[6]

With respect to tumour grade 52.9 % were moderately differentiated tumours followed by well differentiated tumours (28.2 %). This corroborated with the findings of Wong et al.[6] 50 % of well differentiated tumours showed β - catenin score II. 73.3 % of moderately differentiated tumours also showed score of II. 62.5 % of poorly differentiated tumours were having a β - catenin score of III.

Grading and β - catenin expression showed a statistically significant relationship (p value - 0.000). 62.5 % of poorly differentiated tumours showed grade III β - catenin expression, and only 4.2 % of well differentiated cases were grade III. These findings are supported by other studies Sayed et al. Kazem et al[11] Willertand Jones et al.

Study done by Kazem et al[11] Kitagawa et al[15] showed Grade III expression of β - catenin with deteriorating tumour grade and showed statistically significant correlation. [$p = 0.032$]. However, Wong SC et al[6] reported lower expression of β - catenin in high grade tumours. These discrepancies could be due to the use of different β - catenin scoring system of Immunostaining.

With respect to lymph node status 52.9 % cases of colon cancers were free of lymph node metastasis. We found that most cases of negative node status (N0) (32 case, 71.1 %) had grade II β - catenin expression and 50 % of N1 node status also showed β - catenin score of II. Majority of N2 node status (44.4 %) showed β - catenin score III. No statistically significant correlation was obtained between lymph node status and β - catenin expression in our study which was in line with Baldus et al.[16] They reported no association between β - catenin expression and lymph node status.

However, Study done by Wong et al[6] showed patients with colorectal cancer having high nuclear β - catenin expression had a higher incidence of lymph node metastasis ($\chi^2 = 16.99$; $p < 0.005$) and shorter overall survival.

Though our sample size is limited, this study confirms that deeply invasive and high - grade colorectal carcinoma shows intense β - catenin expression. So this study highlights the possibility of use of immunomarker β - catenin to detect the malignant potential of colorectal lesions. So β - catenin study will help in the early management and prevention of the development of a subsequent malignancy.

CONCLUSIONS

The statistically significant correlation of β - catenin with AJCC - TNM staging may supports its use as an additional prognostic marker in colorectal adenocarcinoma. In future β - catenin marker could also be used in small biopsies to predict tumour stage and grade to plan appropriate treatment strategy.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
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