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Expression of β-Catenin and E-Cadherin, their Clinical Significance and Association with Complexity Index of Colon Carcinoma

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Abstract

Cell-cell adhesion and communication relies greatly on the E-cadherin/catenin system.

Deregulation in this system may result in phenotypic change, which may create an opportunity for tumor cells to differentiate, metastasize and invade neighbouring tissue. In this study, we aimed to assess the protein expression of E-cadherin and β-catenin in the patients diagnosed with colon carcinoma correlating the levels with growth pattern of tumors using complexity index analysis as well as clinical and pathological features of the patients.

129 colon carcinoma patients were selected to evaluate the protein expression of E-cadherin and β-catenin through immunohistochemical staining. Complexity index of randomly selected patients diagnosed with colon carcinoma was calculated to examine the growth pattern of the tumor.

Expression of adhesion proteins was significantly perturbed in colon carcinoma patients as compared with normal mucosa ($p<0.05$). Similarly the growth pattern of tumor i.e., complexity index value was significantly related to differentiation of the tumor ($p=0.002$) and Duke's stages ($p=0.026$).

Our results suggest that E-cadherin and β-catenin may play an indicative role in colorectal cancer invasion and disease progression which may act as prognostic markers in colorectal carcinoma. Also complexity index and adhesive proteins distribution are two independent markers which should aid the development of novel strategies for prevention as well as individual treatment of colon carcinoma.

Keywords: Adhesive proteins; Immunohistochemical; Complexity index

Introduction

Colorectal cancer (CRC) is one of the most prevalent cancers and accounts for about 9% of the overall cancer incidence [1]. The factors involved in the development of CRC can be categorized into 3 groups; genetic, epigenetic and environmental factors [2]. These factors cause variations in the fine-tuned pathways of normal cell growth and proliferation. Genetic factors are one of the most significant factors, in which alterations in genes and signalling pathways result in failure of normal gene functions [3,4].

The signalling cascade has received great attention in the last few decades because of its complete or partial involvement in many cell processes. The Wnt/β-catenin pathway is includes many genes which inter-play and regulate cellular activities such as; cell proliferation, transformation, growth and invasion [4]. Mutations in the E-cadherin/β-catenin system, a key player in the Wnt signalling has been found in many CRCs. β-catenin CTNNB, a glycoprotein is a central component of the adherens junctions (AJs), as it has the ability of binding to E-cadherin in epithelial cells and by doing so, it stabilised the cytoskeleton of cells preventing abnormal cell growth [5-7]. AJs are vital in the regulation of growth and adhesion between epithelial cells. At cellular levels, β-catenin transmits contact inhibition signals that cause arrest of cell growth especially when growth of epithelial cell plate has been completed. Binding of Wnt cell surface receptors including Frizzled (FZ), LDL receptor-related proteins 6 (LRP6), stabilizes the transcription co-activator β-catenin which then enters the nucleus of cells and form a complex with T cell factor (TCF) and activates Wnt target genes such as c-Myc and cyclin D1 [8-10]. β-catenin is usually degraded through phosphorylation by GSK-3 which usually sits at the its upstream, while the signal from extracellular Wnt-1 oncoprotein and gene mutations in β-catenin results in elevated levels in the cytoplasmic pool of β-catenin [5].

Accumulation of cellular β-catenin has also been attributed to mutational loss in adenomatous polyposis coli (APC), which antagonizes CTNNB1, thereby promoting the transcription activity of the target gene [11,12]. Abnormal stimulation of Wnt/β-catenin pathway together with the up-regulation of T-cell factor (TCF)/lef proteins increases the transcription of genes that mediate CRC and this has been identified as the initial step in colorectal tumorigenesis [8]. This stage is usually followed by a gradual increase in the number of mutations in the immediate vicinity of β-catenin leading to invasive carcinoma. Previous reports indicate nuclear overexpression of β-catenin as well as interruption in the Wnt signal pathway due to the mutations in APC tumor suppressor gene. Also loss of expression could be due to mutations or hypermethylation of the genes [13-15].

Reduced levels of E-cadherin and it associated β-catenin has been reported in many human cancers [16,17]. In a paper that was published by Saldanha et al., 2004, they showed that, at low levels of E-cadherin in the cells, E-cadherin sequester β-catenin at the cell membrane and this in turn lead to the increase expression of MYC and cyclin D1 which alters the rate of tumor proliferation [18]. Fundamental studies have shown that, E-Cadherin plays an important role in creating tight intracellular association suppressor system of cancers cells. This glycoprotein plays a vital role in Ca^{2+}-dependent cell adhesion [19,20]. In the cytoplasm of cells, they have the ability of forming a molecular complex with proteins of neighbouring cells and by doing so; they stabilize and establish cellular junctions. Claudin and occludin are good examples of proteins which are involved in the adhesion of epithelial cells [21].

Our knowledge of both the intercellular and extracellular structure of E-cadherin has significantly been improved by results of many publications and other research work on the cadherin molecule. In line with this, the crystal structure shows cooperation between individual E-cadherin molecules to form a linear cell cadherin zipper [22]. In order to elucidate the structure of E-cadherin, Alberte et al., showed that, β-catenin and plakoglobin binds to β-catenin leading to the formation of cadherin-catenin complex (CCC) which is highly regulated by tyrosine phosphorylation [22]. Communication between E-cadherin and catenin is essential in anchoring to the cytoskeleton [19,23]. This has been suggested to be one of the main mechanisms behind the invasive suppressor system in colorectal carcinoma [24,25].

CRC usually show cellular differentiation at the invasive front with loss in epithelial phenotype which is the brain behind the metastatic potentials of originally differentiated cells. This is usually considered to be an indicator target for gene expression that coactivates other genes that are involved in tumor growth [24-26]. Growth pattern of tumor is an important prognostic marker in colorectal cancer and CRC tumors show two different kinds of growth patterns, expansive growth with smooth invasive front and infiltrative growth with high coarse and irregular border [27]. Irregular growth pattern and different shapes of cells is a typical form of infiltrative pattern of CRC tumor and is usually characterised with worse prognosis [28,29]. Multiple scoring systems have been presented to describe a tumor growth in different carcinomas [30]. In 2008, a computer based analysing technique was introduced by Franzen and Hahn-Strömberg, which quantitatively graded a tumor from 1-5 according to the outline of its invasive front. This classification is based upon the fractal dimensions and number of tumor cells/ clusters at invasive front. A tumor with smooth and regular invasive front has 1 complexity index while a 5 complexity score represents as tumor with highly coarse and irregular invasive front [27]. The proteins which are important in intercellular adhesions are equally significant in maintaining the morphology of tumor and affecting its invasion and metastasis [31,32]. Being an important part of tight junctions, β-catenin and E-cadherin has a vital role in maintaining the morphology of the tumor [33].

Aim of our study was to analyze the expression activity of β-catenin and E-cadherin and then try to correlate them with complexity of colon carcinoma, 5-years survival and clinic-pathological features of the patients like age, gender, tumor penetration, lymph node metastasis, systemic metastasis, differentiation and localisation of tumor and Duke's stages. The expression pattern of our genes of interest was evaluated using immunohistochemistry while determination of the tumor complexity at the invasive front was done using computer imaging analysis.

Materials and Methods

Patients and tumor

129 formalin-fixed paraffin embedded (FFPE) tissues blocks of colon carcinoma patients were obtained for this project from the clinic of laboratory medicine, section for pathology, Örebro university hospital, Sweden. All the patients underwent surgery as a primary treatment and were assigned serial numbers. Selected patients, male and female were between the age of 40 and 104 years. The samples were selected at random to avoid bias and to have a good representative of the whole population. Clinical data for the studied samples was extracted. This information includes age, sex, and year of diagnosis, localisation and differentiation of tumor, TNM stages (tumor wall penetration, lymph node metastasis and systemic metastasis) and Duke's stages of carcinomas. Ethical review board, Örebro University Hospital, Sweden approved this study.

Immunohistochemistry

Sectioning of samples: For each patient, a tumor area and normal mucosa area was chosen. Blocks were incubated on ice for 20 minutes and then sectioned in 4μm sections by using (LEICA RM 2155) microtome. Sections were mounted over glass slides (Superfrost® plus-Thermoscientific) and dipped briefly into hot water at 50°C. These samples were then heated for protein retrieval 62.3°C (Nuve, EN400, Lab Klimat AB) for one hour. Before staining, all the slides mounted with tissue sections were preheated in high PH buffer (PH 9.5) for one hour. This step was completed by using PT link, Dako, according to the manufacturer's instructions.

Staining: Immunohistochemical staining was performed using Dako Techmate and DAB Envision (Dakopatts, Dako, Denmark) according to manufacturer's protocol. Slides were incubated with primary antibodies for 30 minutes. The primary antibodies used were monoclonal anti-E-cadherin (mouse IgG 2a, BD Biosciences, San Jose, USA) and anti-beta-catenin (mouse IgG 1, BD Biosciences, San Jose, USA), (Table 1). After staining sectioned slides were exposed to ethanol in ascending concentrations and xylene before mounting.

Target protein	Nature of antibodies	Subtype	Dilution	Manufacturer
CTNNB1	purified mouse anti β-catenin monoclonal antibodies	IgG 1	1:1000	BD Biosciences, San Jose, USA
CDH1	monoclonal mouse anti E-cadherin	IgG 2a	1:800	BD Biosciences, San Jose, USA

Table 1: Beta- catenin and E-cadherin antibodies with their information used in this study.

Evaluation: Slides were mounted for light microscope (Olympus BX45 manual microscope, Germany) evaluation of immunoreactivity by a pathologist (VH-S). Staining intensity of β-catenin and E-cadherin was semi-quantitatively evaluated into four categories where 0= <10% of cells stained, 1=10-50% of cells stained, 2=50-80% of cells stained and 3= >80% of cells stained. Both tumors as well as normal mucosa sections were assessed for beta-catenin and E-cadherin staining. Staining distribution was confined to cell membrane, nucleus and cytoplasm (Table 2).

Expressing proteins	0n (%)	1n (%)	2n (%)	3n (%)	P value
β-catenin tumor membrane	13 (10.1)	17 (13.2)	56 (43.4)	43(33.3)	0.000
β-catenin normal membrane	0 0)	33 (25.6)	3 (2.3%)	96(72.1)	
β-catenin tumor nucleus	58 (45.0)	29 (22.5)	21 (16.3)	21 (16.3)	0.550
β-catenin normal nucleus	128 (99.2)	0 (0)	0 (0)	1 (0.8)	
β-catenin tumor cytoplasm	2 (1.6)	26 (20.2)	22 (17.1)	79 (61.2)	0.000
β-catenin normal cytoplasm	0 (0)	33 (25.6)	2 (1.6)	94 (72.9)	
E-cadherin tumor membrane	4 (3.1)	11 (8.5)	37 (28.7)	77 (59.7)	0.000
E-cadherin normal membrane	0 (0)	33 (25.6)	0 (0)	96 (74.4)	
E-cadherin tumor nucleus	123 (95.3)	4 (3.1)	2 (1.6)	0 (0)	0.001
E-cadherin normal nucleus	127 (98.4)	0 (0)	1 (0.8)	1 (0.8)	
E-cadherin tumor cytoplasm	2 (1.6)	33 (25.6)	20 (15.5)	74 (57.4)	0.000
E-cadherin normal cytoplasm	0 (0)	33 (25.6)	0 (0)	96 (74.4)	
0= no expression, 1= low expression, 2=medium expression, 3= high expression					

Table 2: β-catenin and E-cadherin expression in tumor vs. normal samples.

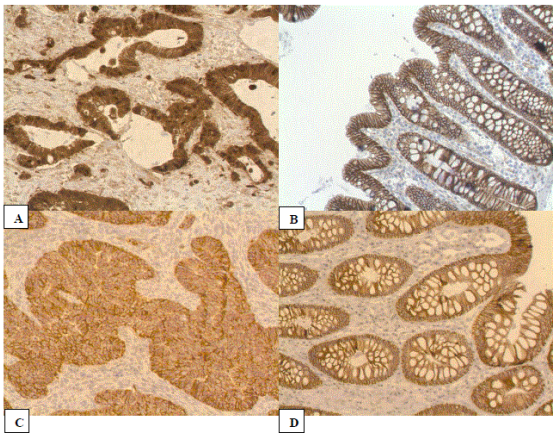

Figure 1: Growth patterns of colon carcinoma. Figure 1A representing the tumor with low complexity index (CI=1) while Figure 1B indicates a tumor with high complexity index (CI=5). Slides stained with cytokeratin.

Computer-based image analysis: Image analysis was performed as described by a previous study by Franzén and Hahn-Strömberg [27]. Briefly, Photographic images were taken at the invasive boarder which has been stained with anti-cytokeratin CAM 5.2 and the images were launched into a computer. During processing, certain changes are impacted on the images such as; colouring the immunohistochemically stained sections and tumor boarders to black while the background is white (Figure 1).

The threshold images i.e., black and white were used for calculating the numbers of cells in the tumor clusters while the dark outlined invasive front of the tumors was used in calculating the fractal dimensions [27]. These two features were then used in tree-based recursion partitioning technique to estimate which assign arbitrary numbers to the tumors based on the complexity at the invasive fronts. The numbers usually range between 1 and 5 (with 1 indicating smooth boarders while 5 indicate highly irregular or complex boarders). For every slide 5-15 pictures were taken from each tumor section and the mean value of their fractal dimensions and numbers of cancer cells in the clusters were used for estimating the tumor complexity.

Statistical analysis: SPSS 16.0 was used to perform the statistical analysis. Fisher's exact and chi square tests were used to analyse the expression of β-catenin and E-cadherin and association of expression of adhesion proteins with clinic-pathological features of the patients. Survival analysis was examined by using Kaplan Meier's test. A P-value less than 0.05 ($P<0.05$) was considered to be statistically significant.

Results

Patients data

Among 129 patients samples, 69(53.49%) were male and 60(46.51%) female. Age of the patients were divided into 2 groups, group 1 comprises of patients with age <60 years while >60 years comprised of group 2. There were 72(55.81%) patients in group 1 and 57(44.19%) in group 2. Regarding TNM staging system, Tumor wall penetration data was separated into two categories, Ta = (T1 + T2) and Tb = (T3 + T4) includes 24(18.60%) and 105(81.40%) patients respectively. In lymph node metastasis, 68(52.71%), 38(29.46%) and 21(16.28%) patients were at N0, N1 and N2 stage respectively.

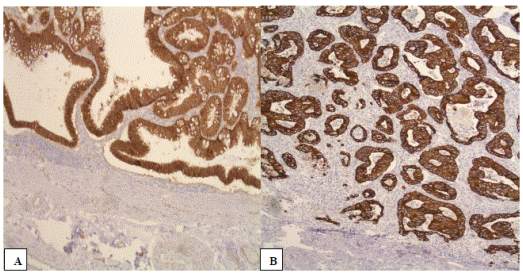

Figure 2: Comparison between β-catenin staining pattern at IF of CRC showing difference in nuclear staining pattern between (A) Tumor and (B) normal colon tissue for β-catenin and C and D indicate the membrane expression of E-cadherin in tumor and normal colon samples respectively. IHC slides are stained with monoclonal antibodies (β-catenin and E-cadherin).

The number of samples were 119(92.25%) at M0 and 5(3.88%) at M1. Samples were also divided according to differentiation of the tumor where 21(16.28%) were low, 80(62.02%) were medium and 26(20.16%) were highly differentiated tumors. Similarly 75(58.14%)

tumors were on the right flexure and 47(36.43%) were on left flexure of the colon. According to Duke's tumor stages, 14(10.85%), 37(28.68%), 37(28.68%) and 8(6.20%) tumors were at A, B, C and D stages respectively.

Among these specimens, 83 were stained with cytokeratin-8 for morphometrical processing of images and after assessing complexity index, 16(19.28%) tumors were with low complexity index, 44(53.01%) with medium complexity index and 23(27.71%) were with high complexity index.

β-catenin and E-Cadherin expression

Immunohistochemical staining was performed for β-catenin and E-Cadherin. In normal and tumor cells, nucleus, cytoplasmic and membranous staining was evaluated. Results indicate significance differences in staining ratios among tumor and normal samples in β-catenin and E-cadherin (Table 2). An even expression of β-catenin and E- cadherin was observed in normal epithelium cells while a varying distribution of these proteins was experienced in tumor cells (Figure 2). The aberrations in expression were significant in both studied proteins P<0.05) Table 2.

Association of β-catenin staining with Clinicopathological parameters

We compared the β-catenin nucleus staining properties with the clinical and pathological parameters of the colon carcinoma patients (Table 3).

Parameters		0n (%)	1n (%)	2n (%)	3n (%)	P value
Gender	Male	27(20.9)	19(14.7)	10(7.8)	13(10.1)	0.309
	Female	31(24.0)	10(7.8)	11(8.5)	8(6.2)	
Age	<60	33(25.6)	18(14.0)	8(6.2)	13(10.1)	0.344
	≥60 years	25(19.4)	11(8.5)	13(10.1)	8(6.2)	
Tumor penetration	Ta	0(0)	3(2.3)	8(6.2)	13(10.1)	0.614
	Tb	4(3.1)	8(6.2)	29(22.5)	64(49.6)	
Lymph node metastasis (N)	N0	1(0.8)	6(4.7)	21(16.5)	40(31.5)	0.748
	N1	2(1.6)	3(2.4)	8(6.3)	25(19.7)	
	N2	1(0.8)	2(1.6)	7(5.5)	11(8.7)	
Systemic metastasis (M)	M0	4(3.2)	11(8.9)	35(28.2)	69(55.6)	0.382
	M1	0(0)	0(0)	0(0)	5(4.0)	
Localisation	Right colon	3(2.5)	7(5.7)	18(14.8)	47(38.5)	0.888
	Left colon	1(0.8)	4(3.3)	14(11.5)	28(23.0)	
Differentiation	Low	0(0)	4(3.1)	8(6.3)	9(7.1)	0.320
	Medium	4(3.1)	5(3.9)	23(18.1)	48(37.8)	
	High	0(0)	2(1.6)	6(4.7)	18(14.2)	
Duke's stages	A	0(0)	1(1.0)	5(5.2)	8(8.3)	0.916
	B	1(1.0)	3(3.1)	12(12.5)	21(21.9)	
	C	3(3.1)	3(3.1)	12(12.5)	19(19.8)	
	D	0(0)	2(2.1)	2(2.1)	4(4.2)	

Table 3: Correlation between β-catenin nuclear expression and Clinicopathological parameters of the patients diagnosed with colon carcinoma.

There was no significant association between β-catenin and other parameters. The correlation between β-catenin nucleus expression with clinic-pathological parameters was as gender (p=0.309), age (p=0.344), tumor wall penetration (p=0.688), lymph node metastasis (p=0.722), systemic metastasis (p=0.854), localisation of tumor (p=0.61), differentiation (p=0.722) and Duke's stages (p=0.820).

Association of E-cadherin staining with clinicopathological parameters

Here the correlation of the percentage of cell membranes stained with E-cadherin was scored with the different clinic-pathological characteristics of the different colon carcinoma patients.

A significant association was observed between E-cadherin membrane expression and age of the patients (p=0.044). The relationship between E-cadherin membrane expression and other

parameters was as gender (p=0.420), TNM (p=0.614, p=0.748 and p=0.382 respectively), differentiation (p=0.320), localization (p=0.888) and Duke's stages (0.916) (Table 4).

Parameters		0 n (%)	1 n (%)	2 n (%)	3 n (%)	P value
Gender	Male	1(0.8)	4(3.1)	21(16.3)	43(33.3)	0.420
	Female	3(2.3)	7(5.4)	16(12.4)	34(26.4)	
Age	<60 years	1(0.8)	6(4.7)	15(11.6)	50(38.8)	0.044
	≥60 years	3(2.3)	5(3.9)	22(17.1)	27(20.9)	
Tumor penetration (T)	Ta	0(0)	3(2.3)	8(6.2)	13(10.1)	0.614
	Tb	4(3.1)	8(6.2)	29(22.5)	64(49.6)	
Lymph node metastasis (N)	N0	1(0.8)	6(4.7)	21(16.5)	40(31.5)	0.748
	N1	2(1.6)	3(2.4)	8(6.3)	25(19.7)	
	N2	1(0.8)	2(1.6)	7(5.5)	11(8.7)	
Systemic metastasis (M)	M0	4(3.2)	11(8.9)	35(28.2)	69(55.6)	0.382
	M1	0(0)	0(0)	0(0)	5(4.0)	
Localisation	Right colon	3(2.5)	7(5.7)	18(14.8)	47(38.5)	0.888
	Left colon	1(0.8)	4(3.3)	14(11.5)	28(23.0)	
Differentiation	Low	0(0)	4(3.1)	8(6.3)	9(7.1)	0.320
	Medium	4(3.1)	5(3.9)	23(18.1)	48(37.8)	
	High	0(0)	2(1.6)	6(4.7)	18(14.2)	
Duke's stages	A	0(0)	1(1.0)	5(5.2)	8(8.3)	0.916
	B	1(1.0)	3(3.1)	12(12.5)	21(21.9)	
	C	3(3.1)	3(3.1)	12(12.5)	19(19.8)	
	D	0(0)	2(2.1)	2(2.1)	4(4.2)	

Table 4: Correlation between E-cadherin membrane expression and clinic-pathological parameters of the patients diagnosed with colon carcinoma.

Complexity index: The growth pattern of tumors and complexity index was calculated and correlated with β-catenin, E-cadherin expressions as well as clinic-pathological parameters of the patients diagnosed with colon carcinoma to detect any possible affiliation between them. There was significant relationship of complexity index with differentiation of tumor (p=0.002) and Duke's stages (p=0.026). The association of complexity index of tumor with other parameters was as, β-catenin nuclear expression (p=985), E-cadherin membrane expression (p=0.507), gender (p=0.105), age (p=0.611), tumor penetration (p=0.654), lymph node metastasis (p=0.800), systemic metastasis (p=0.418) and localization (p=0.154) (Table 5).

		Complexity index			
Parameters		**Low CI n (%)**	**Medium CI n (%)**	**High CI n (%)**	**P value**
Gender	Male	4(4.8)	24(28.9)	13(15.7)	0.105
	Female	12(14.5)	20(24.1)	10(12.0)	
Age	<60 years	6(7.2)	23(27.7)	12(14.5)	0.611
	≥60 years	10(12.0)	21(25.3)	11(13.3)	
Tumor penetration (T)	Ta	3(3.6)	5(6.0)	4(4.8)	0.654

	Tb	13(15.7)	39(47.0)	19(22.9)	
Lymph node metastasis (N)	N0	7(8.5)	24(29.3)	10(12.2)	
	N1	5(6.1)	12(14.6)	6(7.3)	0.800
	N2	3(3.7)	8(9.8)	7(8.5)	
Systemic metastasis (M)	M0	15(19.0)	40(50.6)	21(26.6)	0.418
	M1	0(0)	3(3.8)	0(0)	
Localisation	Right colon	9(11.7)	23(29.9)	16(20.8)	0.154
	Left colon	6(7.8)	19(24.7)	4(5.2)	
Differentiation	Low	3(3.7)	1(1.2)	9(11.0)	
	Medium	10(12.2)	32(39.0)	9(11.0)	0.002
	High	3(3.7)	10(12.2)	5(6.1)	
Duke's stages	A	3(4.8)	1(1.6)	3(4.8)	
	B	4(6.5)	18(29.0)	4(6.5)	0.026
	C	6(9.7)	7(11.3)	10(16.1)	
	D	0(0)	4(6.5)	2(3.2)	
E-Cadherin membrane expression	no expression	1(1.2)	2(2.4)	1(1.2)	
	low expression	1(1.2)	4(4.8)	2(2.4)	
	medium expression	2(2.4)	10(12.0)	8(9.6)	0.507
	high expression	6(7.2)	28(33.7)	12(14.5)	
β-catenin nucleus expression	no expression	8(9.6)	22(26.8)	12(14.6)	
	low expression	2(2.4)	7(8.5)	5(6.1)	
	medium expression	2(2.4)	8(9.8)	3(3.7)	0.985
	high expression	3(3.7)	7(8.5)	3(3.7)	

Table 5: Association between complexity index of tumor, β-catenin, E-cadherin expression and clinicopathological parameters of the patients diagnosed with colon carcinoma.

Survival analysis

A 5-years survival data of the patients diagnosed with colon carcinoma was correlated with β-catenin nuclear expression, E-cadherin membranous expression and complexity index of tumors but we could not find any statistically significant association. The association of β-catenin, E-cadherin and complexity index of tumors with 5-years survival of patients was as p=0.872, p=0.738 and p=0.255 respectively (Table 6) (Figure 3).

		Live n (%)	Died n (%)	Total n (%)	P value
Complexity index	Low complexity index	8(12.5)	8(12.5)	16(25.0)	0.255
	Medium complexity index	21(32.81)	23(35.94)	44(68.75)	
	High complexity index	7(10.94)	16(25.0)	23(35.94)	
E-Cadherin membrane expression	no expression	3(2.33)	1(0.78)	4(3.10)	0.738
	low expression	6(4.65)	5(3.88)	11(8.53)	

	medium expression	14(10.85)	23(17.83)	37(28.68)	
	high expression	38(29.46)	39(30.23)	77(59.69)	
β-catenin nucleus expression	no expression	26(20.16)	32(24.81)	58(44.96)	0.872
	low expression	16(12.40)	13(10.08)	29(22.48)	
	medium expression	9(6.98)	12(9.30)	21(16.28)	
	high expression	10(7.75)	11(8.53)	21(16.28)	

Table 6: Survival analysis in colon carcinoma patients; correlation of complexity index, β-catenin and E-cadherin expression with 5-years survival of patients diagnosed with colon carcinoma.

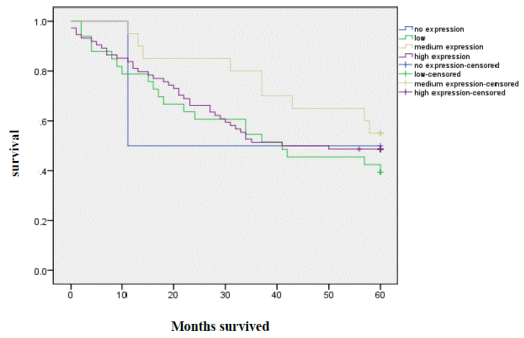

Figure 3: A Kaplan-Meier curve showing the association between E-Cadherin membrane expression and survival of the patients diagnosed with colon carcinomas.

Discussion

The E-cadherin/catenin system is a very important complex which is essential in the regulation of cell proliferation, transformation, growth, adhesion and invasion [6]. Alteration in this complex disrupts the interaction and communication between E-cadherin and beta catenin resulting in the loss of intercellular adhesiveness, a major cause of many forms of human cancers [16]. A deregulation in the E-cadherin/catenin system may be a good prognostic marker in CRC patients.

Different prognostic markers including growth pattern of colon carcinoma have been studied to analyse a tumor [34,35]. In present study, we assessed 129 samples from the patient diagnosed with colon carcinoma and analysed the growth and expression pattern of β-catenin and E-cadherin. We found that β-catenin and E-cadherin expression in tumor samples was highly perturbed and significantly different from normal samples ($p>0.05$). Similar results were presented by Hahn Strömberg V et al., which indicate the pivotal role of adhesion proteins in development of colon cancer [21]. Similarly Wang et al., showed that nuclear β-catenin expression is a good prognostic marker in CRC development [11]. In accordance with our results, some other studies demonstrate the similar findings [36-38]. Upregulation of β-catenin has been found associated with exaggerated activity of Wnt signaling pathway [39]. These epithelial-stromal transition spots might be one of the possible molecular therapeutic targets.

E-cadherin is found to be involved in the regulation of β-catenin and other molecules in cell adhesion processes [40]. By using microarrays and transcriptional intervention, Kuphal and Behrens, described how E-cadherin affects the Wnt pathway in CRC [41]. Similarly, in another study, loss of E-cadherin being indispensable in transformation from adenoma to carcinoma sequence in CRC was independent of β-catenin/ Tcf related system [42]. Lugli et al., demonstrate that nuclear β-catenin expression and loss of E- cadherin membranous expression could be adverse independent prognostic markers in colon carcinoma [43]. In our study, accordance with previous reports, E-cadherin membranous expression was significantly low and disturbed in tumors as compared with normal samples.

We also compare the expression results of β-catenin and E-cadherin with clinicopathological parameters like age, gender, tumor penetration, lymph node metastasis, systemic metastasis, differentiation and localisation of tumor and Duke's stages of the patients diagnosed with colon carcinoma but no correlation was observed except E-cadherin membrane expression was significantly associated with age factor of the colon carcinoma patients. Previous studies show similar results which indicate that there are some factors other than β-catenin and E-cadherin expression which are dependent upon clinicopathological parameters [21,44].

Tumor growth pattern was observed by measuring complexity index of randomly selected 83 tumors. Results indicate that complexity index of tumor is not associated with β-catenin and E-cadherin expression in colon carcinoma. Other researchers, in agreement with our findings, describe the similar results [21,45]. When we compare complexity index with other clinicopathological parameters of the patients, differentiation of tumor and Duke's stages were significantly associated with complexity index ($p=0.002$ and $p=0.026$ respectively). These findings indicate that complexity is an important marker when analysing a tumor. Similar outcomes were experienced by other researchers as well [29,46].

To detect any direct influence of adhesion proteins expression and complexity index on prognosis of the colon carcinomas, we assessed a 5-years survival of the patients and correlated with adhesion proteins expression and growth pattern of tumor. Results did not indicate any direct association which can be explained as the aetiology of malignant diseases and particularly, CRC is multifaceted so there could be other causes which are affecting the prognosis of tumors in colon carcinoma [47]. Meanwhile, A reason for these inconclusive finding could be because of some patients underwent surgery and got survival benefits as described by Lodge et al. [48].

Conclusion

Conclusively, our study was performed to analyse the expression patterns of β-catenin and E-cadherin and growth patterns of tumor and to examine if there is some correlation between adhesion proteins expression and complexity index along with their prognostic significance in colon carcinomas. Our results were in accordance with previous studies showing that expression of adhesion proteins is perturbed in colon carcinoma and growth pattern of tumor is not directly associated with distribution of these proteins. It seems there is a complex interplay between the adhesion proteins and there are some factors other than β-catenin and E-cadherin expression, which are involved in modifications of complexity of tumors and subsequently leading to affect the prognosis of colon carcinomas.

Conflict of Interest

The authors declare that they have no competing interests

Author's Contribution

Abrar Ahmad carried out sectioning, immune-histochemical staining, the image analysis and the statistical analysis. He also drafted the manuscript. Victoria Hahn-Strömberg conceived idea of the study, design, coordination, funding, ethical approval from EPN and helped draft the manuscript.

All authors read and approved the final manuscript.

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