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A Clinicopathological Study of β-catenin in Colorectal Adenomas

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Abstract

Background and objectives: Colorectal adenomas are considered benign tumors, which originate from the mucus-secreting colonic epithelium creating polyps that protrude into the lumen of the intestine. β -catenin is a member of the catenin protein family and it has a dual function that controls the coordination of cell-cell adhesion and gene transcription. The aim of this study was to investigate the immune expression of β - catenin in colorectal adenomas and to describe β-catenin correlation with clinicopathological parameters.

Methods: This study was retrospective in nature and performed on 94 formalin-fixed tissue blocks which were obtained from the histopathology department at Rizgary Hospital and some private laboratories in Erbil city during the period from 2014- 2018, The tissue cuts were prepared in 4 µm thick and placed on special salinized slides, samples were deparaffinized and put into a buffer solution for 20 min at 95-99°C, cooled in room temperature for 20 min.

Results: The expression of β - catenin was seen in 73.4% of the samples, 41.5% of them with grade II while 31.9% exhibited grade III and 26.6% had grade I β -catenin expression. Except for the type of polyp, there was no statistically significant association between β - catenin expression and the clinicopathological. For patients diagnosed with multiple adenomas, the size of the polyp and grade of expression were remarkably different between the various polyps of the same patient.

Conclusions: No statistically significant association between β-catenin expression and clinicopathological parameters except for the type of adenoma.

Keywords: β - catenin, colorectal adenomas, colorectal polyps, clinicopathological parameters, Erbil city.

Introduction

Colorectal adenomas are benign tumors originating from the mucus-secreting colonic epithelium and forming polyps that project into the lumen of the intestine, traction on the mass may form a stalked polyp or it may be sessile without a definable stalk.[1] Colorectal adenomas are of many types and categories including: conventional adenomas (which comprise the tubular, tubulo-villous and villous adenomas), sessile serrated adenoma and traditional serrated adenoma. Conventional adenomas are the most common types comprising 65-85% of adenomas.[2]These adenomas have the potential to convert to colorectal adenocarcinoma in the future.[3-5] The prevalence of adenomas within a population, and the prevalence of people with multiple adenomas, geographically parallels the prevalence of colon cancer. The frequencies of both cancer and adenoma increase with increasing age, with the highest increase in the age group of 50 – 59 years.[6] Adenomas of colon are estimated to be present in 20-53% of the United States population older than 50 years of

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age.[7] β -catenin is a member of the catenin protein family and it has a dual-function that controls the coordination of cell-cell adhesion and gene transcription.[2] β -catenin is a sub-set of the cadherin protein complex and behaves as an intracellular signal transducer in the Wnt signaling system.[8, 9] The recognized Wnt signaling cascade which is acting through β -catenin can modify a variety of significant cellular processes as proliferation, survival, apoptosis, differentiation, cell adhesion and motility.[10] A huge body of knowledge proposes that abnormal activation of Wnt signaling after APC loss is a main reason which leads to establishment of colon adenoma.[8, 11] Loss-of-function and gain-of-function studies of Wnt proteins and Beta-catenin have suggested that aberrant Wnt signaling stimulation after APC loss is responsible for the initiation of intestinal adenoma.[9,12]The aim of the present study was to disclose the paradigm of β -catenin expression in colorectal adenoma cases via exploring β -catenin immunoexpressions in formalin-fixed paraffin-embedded tissue sections of adenomatous polyps and analyze their possible relations with variable clinicopathological prognostic parameters.

Material and methods

A retrospective study was done on 94 formalin fixed paraffin embedded tissue blocks that have been retrieved from histopathology department at Rizgary Teaching Hospital and some private laboratories in Erbil city during the period from January 2014-January 2018 of patients who underwent elective colonoscopy and subsequently polyp removal. The inclusion criteria for the current study were electively resected colorectal adenomas of conventional type based on histopathological results of endoscopically resected polyp specimens and no history of adenomatous polyposis coli or Lynch syndrome. A separate analysis was done on a sub-set data of 22 polyps belonged to eight patients with more than one polyp. Ethical approval was obtained from Research Ethics Committee at Kurdistan Higher Council of Medical Specialties. Data entered and analyzed using Statistical Package for Social Sciences version 25 (SPSS). Descriptive analyses were expressed as frequencies and percentages and the inferential results were compared between the subjects with different variables using a statistical significance level of ≤ 0.05 and analyzed using Pearson Chi square or Fisher's exact tests if necessary. All blocks were examined and the one which represented the best (no necrosis, no hemorrhage) was selected and new sections were reviewed by two experienced pathologists. Five parameters (site of biopsy, type of polyp, degree of dysplasia, size of polyp and grade of expression) were used to find out the variation between different polyps of the same patient The tissue cuts were prepared in 4 µm thick and placed on special salinized slides, samples were deparaffinized and put into a buffer solution (PH 6.0) for 20 min at 95-99°C, cooled in room temperature for 20 min. The method used was Dako Cytomation EnVision + System - horseradish peroxidase (HRP) - which is a two-level IHC staining method. Then ready to use Anti- B-catenin, immunostaining using Dako EnVision+ System-HRP (DAB), (DAKO, Denmark) Code K4006 and Monoclonal Mouse Anti-Human Beta-Catenin Clone -Catenin-1 Code M3539 (DAKO, Denmark). The endogenous peroxidase activity was inhibited by incubation of slides inside peroxidase inhibitor for 10 min. The slides were incubated inside the primary diluted antibody for 30 min and then on labeled polymers for another 30 min. After staining, the slices were put in 3, 3-diaminobenzidine (DAB) + substrate chromogen which detects the antigen in brown color. Then, the stained samples were reviewed under a light microscope

and labeled cells were counted in both pathological and normal tissues for 100 cells in the microscopic field. The majority of primary tumors with normal adjacent colonic epithelium served as positive internal control Figure (1 & 2), while negative control prepared from the same tissue block, but incubated with distilled water instead of the primary antibody.

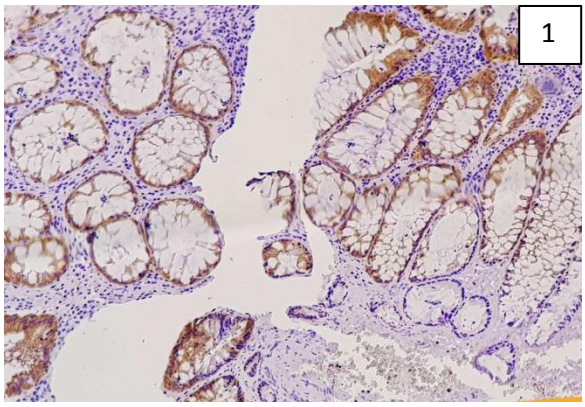


Figure (1): Normal colonic epithelium which is saved as positive control (X200 H&E counter staining).

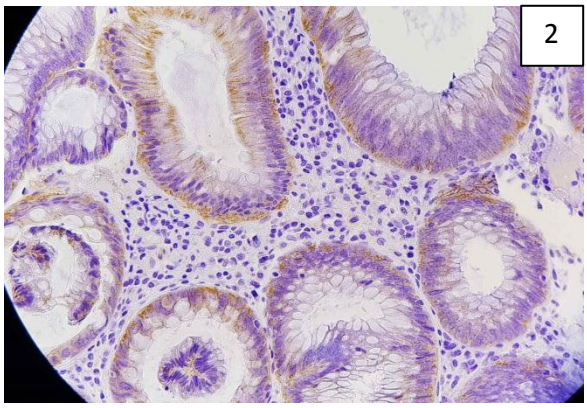


Figure (2): β -catenin distribution within cell membrane (X400 H&E counter staining).

As previously described by Jass *et al*[13] scoring of β -catenin was based upon the distribution of B-catenin within the cell membrane [0-1] Figure(2) , cytoplasmic [0-2] and nuclei [0-2], the authors calculated B-catenin activation score as the sum of nuclear score [+2 = positive strong expression; +1 = weak expression; 0 = no expression] , cytoplasmic score [+2 = positive strong expression; +1 = weak expression; 0 = no expression] and membrane score [0 = positive membrane expression; +1 = negative membrane expression]. Total scores were then collapsed into three grades: grade I [0-1], grade II [2-3] and grade III [4-5], with a total score of [0] reflecting cell membrane staining only, similar to that seen in normal colonic mucosa, up to an aggregate score of [5] for tumors with strong nuclear staining [2], diffuse cytoplasmic staining [2], and loss of cell membrane staining [1]. Tumors were considered positive for nuclear β -catenin staining if ≥5% of cells exhibited nuclear expression in normal tissue, and negative if nuclear β -catenin was expressed in <5% of cells and the staining intensity were weak. For membranous β -catenin, tumors were considered positive if ≥ 50%of the cells exhibited membranous expression of the protein and negative if the expression was below 50%.[14,15]

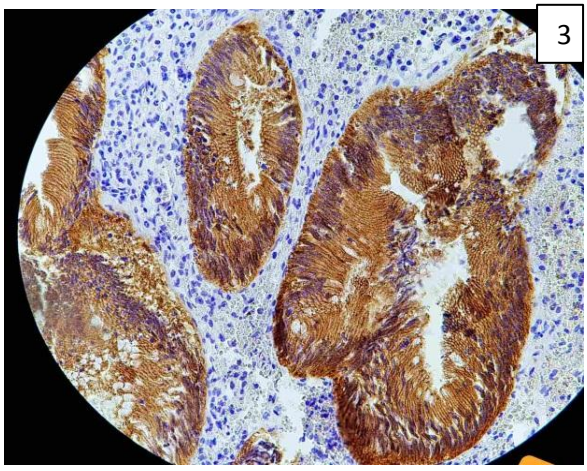
Results

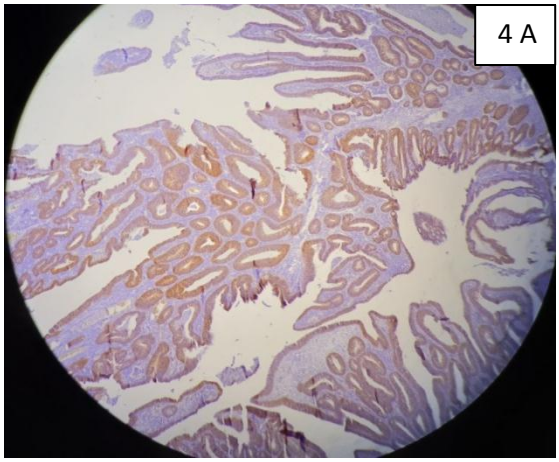
Table (1) represents the descriptive clinicopathological parameter of patients. The expression of β - catenin was seen in 73.4% of the histological samples and 41.5% of them with grade II while less than one third (31.9%) showed grade III and finally nearly one quarter (26.6%) had grade I β -catenin expression.

Table 1: Descriptive parameters of the colorectal adenomas.

Variables	Categories	Frequency	Percentage
Gender	Male	55	58.5
	Female	39	41.5
Age groups	< 40 years	21	22.3
	40 – 50 years	16	17.0

	> 50 years	57	60.6
Size of tumor	< 1 cm	25	26.6
	1-2 cm	52	55.3
	> 2 cm	17	18.1
Site of biopsy	Right colon	20	21.3
	Left colon	74	78.7
Dysplasia	Low grade	50	53.2
	High grade	44	46.8
Type of polyp	Tubular	19	20.2
	Tubulo-villous	66	70.2
	Villous	9	9.6
Advanced adenoma	Yes	38	40.4
	No	56	59.6
Expression of β-catenin	Negative	25	26.6
	Positive	69	73.4
Grade of expression of β-catenin	I	25	26.6
	II	39	41.5
	III	30	31.9
Total		94	100





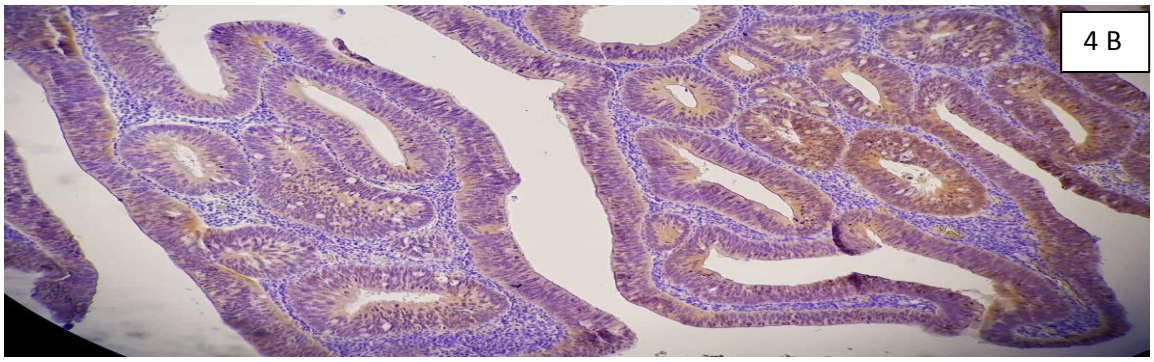


Figure (3): Nuclear and cytoplasmic expression of B-catenin distribution within villous adenoma with high grade dysplasia (X200 H&E counter staining). **Figure (4-A)** Low power, **Figure (4-B)** high power: Nuclear and cytoplasmic expression of B-catenin distribution in advanced adenoma (X200 H&E counter staining).

Table (2) reveals association between β -catenin and the clinicopathological .Forty percent of male patients had grade III B-catenin expression, nearly one third (36.4%) with grade II, and less than quarter (23.6%) with grade I B-catenin immune expression, less than half (48.7%) of female patients had grade II, 30.8% with grade I and 20.5% with grade III expression (p-value = 0.187). Regarding age groups, , patients less than 40 years had grade II B-catenin expression in 42.9% of adenomas , more than one third had grade III and only 19% had grade I expression. Among 40-50 year old patients, half of adenomas had grade II, less than one third (31.3%) had grade III expression and (18.8%) had grade I expression. Of patients more than 50 years, 38.6% of adenomas had grade II, followed by (29.8%) with grade III and less than one third (31.6%) had grade I B-catenin expression (p-value =0.720). Half of right sided adenomas had grade II B-catenin expression, (30%) grade I and (20%) grade II. In the same manner 39.2% of left sided adenomas had grade II expression, (35.1%) with grade III and (25.7%) with grade I β -catenin expression (p-value 0.431). Concerning size of the polyp: 44% of the adenomas with less than 1 cm had grade I Beta catenin expression, nearly one third (32%) of them had grade II and the rest 6 (24%) with grade III expression, while less than half (48.1%) of adenoma between (1-2 cm) had grade II, (32.7%) of them with grade III followed by (19.2%) with grade I expression (p-value = 0.187). Most (51.5%) of tubulo-villous adenomas had grade II Beta catenin expression while 42.1% of the tubular polyps had grade III expression in contrary two thirds (66.7%) of villous polyps had grade I Beta catenin expressions, with statistically significant association (p-value = 0.006). More than one third (38%) of adenomas with low grade dysplasia express grade II β -catenin, 34% expressed grade III followed by (28%) with grade I , similar to that 45.5% of the adenomas with high grade dysplasia express grade II B-catenin, (29.5%) with grade III and (25%) with had grade I expression (p-value = 0.764). Exactly half (50%) of advanced adenoma express grade II , nearly one quarter (26.3%) of them had grade III and 23.7% of them had had grade I while (35.7%) of the non-advanced adenomas had grade II and the same percentage of adenomas had grade III expression and 28.6% of those polyps had grade I Beta catenin expression (p-value 0.378).

Table (2): Clinicopathological parameters and their association with Beta catenin expression.

Parameters	Variables	Beta catenin grades			p-value
		I	II	III	
Gender	Male	13 (23.6%)	20 (36.4%)	22 (40%)	0.187
	Female	12 (30.8%)	19 (48.7%)	8 (20.5%)	
Age groups	< 40 years	4 (19%)	9 (42.9%)	8 (38.1%)	0.720
	40 – 50 years	3 (18.8%)	8 (50%)	5 (31.3%)	
	> 50 years	18 (31.6%)	22 (38.6%)	17 (29.8%)	
Site of biopsy	Right colon	6 (30%)	10 (50%)	4 (20%)	0.431
	Left colon	19 (25.7%)	29 (39.2%)	26 (35.1%)	
Polyp size	< 1 cm	11 (44%)	8 (32%)	6 (24%)	0.187
	1-2 cm	10 (19.2%)	25 (48.1%)	17 (32.7%)	
	>2 cm	4 (23.5%)	6 (35.3%)	7 (41.2%)	
Type of polyp	Tubular	7 (36.8%)	4 (21.1%)	8 (42.1%)	0.006
	Tubulo-villous	12 (18.2%)	34 (51.5%)	20 (30.3%)	
	Villous	6 (66.7%)	1 (11.1%)	2 (22.2%)	
Degree of dysplasia	Low grade	14 (28%)	19 (38%)	17 (34%)	0.764
	High grade	11 (25%)	20 (45.5%)	13 (29.5%)	

Advanced adenoma	Yes	9 (23.7%)	19 (50%)	10 (26.3%)	0.378
	No	16 (28.6%)	20 (35.7%)	20 (35.7%)	
Total		25 (26.6%)	39 (41.5%)	30 (31.9%)	

Studying the parameters of patients with multiple polyps, Table (3), reveals that eight patients had multiple polyps; four of them had only two polyps, three of them with three polyps and finally one patient with five polyps. There was no difference in the site of biopsy i.e. the multiple polyps were located at the same side of colon, either right or left side except for two patients. No variation was observed regarding the type of polyp; all of the patients were tubulo-villous adenomas alone and only one of the two patients had tubular and tubulo-villous types while the other had villous and tubulo-villous polyps in contrary. Among the eight cases only one patient had a different degree of dysplasia in contrast to majority of cases in which they had the same degree of dysplasia. A part from one patient, the multiple polyps in majority of the cases (seven cases) had different grades of expression and it was ranging from grades I-III.

Table 3: Patients with multiple polyps and their parameters.

Patients	Polyps	Site of biopsy	Type of polyp	Size of polyp	Degree of dysplasia	Grade of expression
1	First	Left colon	Tubulo-villous	1-2 cm	High grade	I
	Second	Left colon	Tubulo-villous	1-2 cm	High grade	II
	Third	Left colon	Tubulo-villous	1-2 cm	High grade	II
	Fourth	Left colon	Tubulo-villous	1-2 cm	High grade	III
	Fifth	Left colon	Tubulo-villous	1-2 cm	High grade	II
2	First	Left colon	Tubulo-villous	>2 cm	High grade	I
	Second	Left colon	Tubulo-villous	1-2 cm	High grade	II
3	First	Left colon	Tubulo-villous	>2 cm	High grade	II
	Second	Left colon	Tubulo-villous	>2 cm	High grade	III
	Third	Left colon	Tubulo-villous	1-2 cm	High grade	II
4	First	Left colon	Tubular	1-2 cm	High grade	II
	Second	Left colon	Tubulo-villous	1-2 cm	High grade	III
	Third	Left colon	Tubulo-villous	>2 cm	High grade	II
5	First	Right colon	Tubulo-villous	< 1 cm	High grade	I
	Second	Right colon	Villous	1-2 cm	High grade	I
6	First	Left colon	Tubulo-villous	1-2 cm	Low grade	I
	Second	Left colon	Tubulo-villous	1-2 cm	Low grade	II
7	First	Left colon	Tubulo-villous	1-2 cm	Low grade	I
	Second	Left colon	Tubulo-villous	1-2 cm	Low grade	III
	Third	Left colon	Tubulo-villous	>2 cm	High grade	I
8	First	Left colon	Tubulo-villous	1-2 cm	Low grade	III
	Second	Left colon	Tubulo-villous	1-2 cm	Low grade	I

Discussion

The interaction of the adenomatous polyposis coli (APC) tumor-suppressor protein and the intracellular cell-adhesion protein β-catenin is crucial for the development of colorectal tumors, Since functional nuclear complexes of β-catenin with transcription factors have been identified, the knowledge of level and distribution of β-catenin in sporadic colorectal adenomas will give important insights into the intracellular mechanism of sporadic colorectal tumor initiation and

progression.[11,17] Many investigators thought that B- catenin expression may have a pattern in colorectal adenomas and the nuclear accumulation in single cells of small adenomas can be considered as the first visible sign of the loss of APC function, thus the immunohistochemical detection of β-catenin distribution could serve as a criterion for estimating the malignant potential in the clinicopathological evaluation of colon tumors during their early progression. The current study was trying to probe more on this query and uncover the paradigm of β -catenin expression in patients with colorectal adenomas and its relation to clinicopathological parameter in Northern part of Iraq. We found that 73.4% of the examined histological slides showed positive expression for β - catenin while 26.6% did not show any signs of B-catenin expression. These findings were very close to Bourroul et al results who discovered that 74.6% of colorectal adenomas showed positive β - catenin expression,[13] similarly the findings were in agreement with Dai et al observations who noticed that 70% of the colorectal adenomas had positive expression.[16] In contrary our finding was not in line with Wong et al who revealed a much higher percentage of positive expressions and stated that 92% of the adenoma cases had positive staining.[17] The positive expression in colorectal adenomas seemed to be very different from that of colorectal carcinomas i.e. the adenomas had lower percentages of positive expression than carcinomas as it was observed by Dai et al (95.2%).[16] and Wong et al (100%).[17] Both nuclear and cytoplasmic expression of β-catenin may explain that the stabilized β-catenin passes from cytoplasm into the nucleus to interact with transcription factors and activate target gene, therefore, it is likely that cytoplasmic accumulation of β-catenin starts at early stages of colorectal tumerogenesis, thus cytoplasmic accumulation is a cause or a result of the progression from adenoma to carcinoma.[18] This abnormal distribution of β -catenin in from of cytoplasmic or nuclear expression reflects either an ineffective β -catenin or loss of β -catenin connection to the cytoskeleton. This was in agreements with other observers.[19-21] where they thought that an abnormally high amount of β -catenin in the cytoplasm and not in the membrane seems to indicate a β -catenin protein with oncogenic potential. This cytoplasmic and nuclear accumulation of β -catenin suggest enhanced transcription and activation of the target genes (such as c-myc, cyclin D1 and matrilysin) which are responsible for tumor formation and malignant progression through interaction with members of the TCF/LEF DNA-binding family.[20, 21] Except for the type of adenoma; there was no statistically significant association between β - catenin expression and the clinicopathological parameters namely gender of participants, age groups, site of biopsy, type of polyp, degree of dysplasia, size of polyp and grade of expression. Compared with results of colorectal carcinoma studies, our findings were similar to the results reported by Ahmad and Stromberg who found that there was no significant association between β-catenin expression and gender, age of subjects and location of biopsies parameters.[22] Gao et al also discovered alike results and concluded no statistically significant correlations between expression levels of β-catenin and the clinicopathological characteristics including age, gender of participants, tumor size and site.[23] On the other hand, our results were against the findings of Brabletz et al who confirmed a very strong correlation of β- catenin-immunoreactive scores with tumor size.[24] In the current study a total of eight patients had multiple polyps, four only two polyps, three with three polyps and the last patient were diagnosed with five polyps. There was no any variation in the site of biopsy, the polyps of only one patient showed different degree of dysplasia.

Conclusions

There was no statistically significant association between β - catenin expression and the clinicopathological parameters except for the type of adenoma (p-value of 0.006). For the patients diagnosed with multiple adenomas, two of their parameters which were size of polyp and grade of expression were remarkably different between the various polyps of the same patient. This outcome was demanding a careful and meticulous histopathological examination of each polyp separately without omitting any single adenoma.

Conflicts of interest

The author reports no conflicts of interest.

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