JKBMS, Vol.4, No.1, P.48-53, 2018 https://doi.org/10.56056/amj.2018.42

Significance of B-catenin Immunohistochemical Expression in Colorectal Carcinoma

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Abstract

Background and objectives: Colorectal cancer is the third leading cause of cancer death worldwide and B- catenin has a role in the development of colorectal cancer. The objective of this study was to investigate the B-catenin expression of colorectal carcinoma and assess its correlation with some clinicopathological parameters in Erbil, Kurdistan of Iraq. **Methods:** Retrospective study of 100 patients with colorectal cancer collected between January 2015 and January 2017. Clinicopathological parameters were investigated in relation to nuclear and cytoplasmic B-catenin expression. **Results:** In 100 specimens of colorectal carcinomas was found to be nuclear (21.6%) and cytoplasm (66.1%). B-catenin was expressed more frequently in patients≥ 50 years (67.9%), and more commonly in females (60.7%). Left side colon was more frequently affected (85.7%) than the right side (14.3%) with a significant correlation. Well to moderately differentiated tumors showed higher intensity (89.3%) than poorly differentiated cases (10.7%). Non-mucinous tumors (92.9%) stained more intensely than mucinous tumor (7.1%). 52% of tumor cells were grade I, 37% grade II and 11% grade III. The result of association for B-catenin combined nuclear and cytoplasmic with intensity of nuclear staining was highly significant. Higher staining intensity is observed in patients with positive nodal status (60.7%) and stage III-IV (60.7%) than those of stage I-II (39.3%). **Conlision:** B-catenin was studied in 100 specimens of colorectal cancers, with nuclear staining observed in (21.6%) and cytoplasmic reactivity in (66.1%). Significance was found between intensity of immunoexpression and cellular localization of B-catenin, as well as with clinical parameters of increasing age, female gender, and left sided colonic tumors on another hand.

Keywords: Colorectal cancer; B-catenin; Immunohistochemistry

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, and the 3rd leading cause of cancer death according to WHO and American Cancer Society[1].In Iraq, CRC was the 7th common cancer for both genders, whereas in Kurdistan, CRC constitutes the 4th commonest cancer in both genders[2]. Reports from different cities of Iraq had shown increase the risk of colon cancer from 25% to 50% during 30year period[3]. The pathogenesis of colorectal carcinoma is related to both environmental and genetic factors[4]. CRC has a distinct molecular pathway: this includes chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype (CIMP)[5]. Throughout the chromosomal instability (CIN) pathway, several tumor suppressor genes and oncogenes are mu¬tated, among them, adenomatous polyposis coli (APC), β-catenin, Axin and GSK3B genes, which are the members of the Wnt signaling pathway that plays an important role in colorectal cancer tumorogenesis6. In about 90% of all colorectal cancers have a mutation in individual components of multiple oncogenes in Wnt/β-catenin pathway[6,7]. β-catenin (CTNNB1) is a 92-kDa cellular protein, identified as a protein associated with E-cadherin in maintaining cellular adhesion[8]. Also, β-catenin has an independent role in the Wnt signal transduction pathway, a mutation in components of the APC complex leads to increased lev¬el of cytoplasmic β-catenin and its translocation to the nucleus[9]. Accumulation of nuclear ß-catenin which comes from cytoplasm acts as a transcription factor and binds to T-Cell Factor/Lymphoid Enhancer Factor (TCF/LEF), which leads to activation of target genes including: CyclinD1, c- Myc, CD44 and Survivin, and at the end it results in uncon¬trolled cell proliferation in tumor cells[10]. Thus β-catenin accumulation may be valuable biomarker associated with invasion, metastasis and poor prognosis of CRC[11].

Methods

During period January 2015 -January 2017, a retrospective study of 100 formalin-fixed paraffin-embedded specimens of colorectal cancers were retrieved and randomly selected from histopathology laboratory at Rezgary Teaching Hospital and some private laboratory in Erbil city. All patients had been diagnosed with primary colorectal adenocarcinoma and undergone surgery; none of the patients received chemotherapy or radiotherapy prior to surgery. Exclusion criteria were patients with secondary malignancies, ulcerative colitis, Crohn's disease, patients with familial adenomatous polyposis or hereditary non-polyposis colorectal cancer. All blocks were examined. Two sections of 4 micrometers thickness taken from each block. First sections on the ordinary slide for Haematoxylin and Eosin staining to confirm the diagnosis and histological types and grades of the tumor; second sections were on the charged slide for immunohistochemistry. Demographic data (age at diagnosis, sex) and topography of tumor (location, histo-

logical type, nodal status), were obtained from all patients. The tumors were divided into right, left colon and rectum[12]. Histological grading labeled as G1-2 for well-moderate differentiated and G3 poorly differentiated tumors[13]. And histological tumor type as mucinous and non-mucinous. Staging done according to American Joint Committee on Cancer (AJCC) and the Union International Contre Le Cancer (UICC), by grouping the various TNM components[14], as T1-T2, and T3-T4. Anti-B-catenin, immunostaining using Dako Envision+System-HRP (DAB), Monoclonal Mouse Anti-Human Beta-Catenin Clone-Catenin-1 Code M3539 (DAKO, Denmark) was used in this study. Positive and negative control slides involved in each run of staining. Normal colonic epithelium as a positive control and the majority of primary tumors with normal epithelium adjacent to the tumors served as an internal control. While negative control slides prepared from the same tissue block but incubated with distilled water instead of primary antibody. As previously described by Jass et al. scoring of B-catenin was based upon the distribution of B-catenin within the cell membrane (0-1) cytoplasmic (0-2) and nuclei (0-2)15. In this study also calculated B-catenin activation score as the sum of the nuclear score (+2=strong expression;+1=weak expression;0=no expression), cytoplasmic score (+2=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 the 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 B-catenin staining if more than 5% of cells exhibited nuclear expression. In normal tissue, nuclear B-catenin was expressed in <5% of cells and the staining intensity was weak. For membranous B-catenin, tumors were considered positive if≥ 50% of the cells exhibited membranous expression of the protein and negative if the expression was below 50% 16, 17. All patients 'data entered and analyzed using computerized statistical software, Statistical Package for Social Science (SPSS) version 20. Descriptive statistics presented as(mean Śstandard deviation), frequencies, and percentages, multiple contingency tables conducted and appropriate statistical tests performed. Chi-square used for the categorical variable (Fisher exact test was used when expected variable were less than 20%). In all statistical analysis levels of significance (P value) set at 0.05 and the results presented as tables.

Results

The sampled patients are 57 females and 43 male (with a ratio of 1: 0.78), Their age ranged from 19-85 years with a mean age (± SD) 54.47 ± 14.89. 69% cases had a primary tumor of left colon and rectum while 31 cases had a tumor in (ascending and transverse colon). Only 13 cases have Mucinous adenocarcinoma and 87 cases were non-mucinous, most of the cases had well-moderately differentiated carcinoma comprising 88%, while only 12 % had a poorly differentiated tumor. Regarding the stage and nodal status, 54% had stage III-IV with positive nodal status while 46 % had stage I-II and with negative nodal status as shown in (Table 1).

Table (1): Demographic and Clinicopathological Features of Colorectal Cancer Cases.

Variables	Categories	No.(%)
Age groups	Age groups	
	< 50 years	35 (35%)
	≥ 50 years	65 (65%)
Gender		
	Male	43 (43%)
	Female	57 (57%)
Site of cancer		
	Right	31 (31%)
	Left	69 (69%)
Nodal status		
	Negative	46 (46%)
	Positive	54 (54%)
Stage of cancer		
	I – II	46 (46%)
	III-IV	54 (54%)
Differentiation		
	Well - moderate	88 (88%)
	Poor	12 (12%)
Histopathology Type		
	Non-mucinous	87 (87%)
	Mucinous	13 (13%)
Total		100

In normal tissue adjacent to colorectal carcinoma, B-catenin was mainly localized in the plasma membrane of the cell-to-cell border with a weak expression in the cytoplasm of both the colonic epithelium and goblet cells. No nuclear B-catenin was seen in the normal colonic mucosa as shown in (Figure 1A). 84% of cases lost membranous B- catenin immunostaining, with only 16% showed B-catenin expression as shown in (Figure 1B). B-catenin immune reactivity detected as brownish discoloration in the cytoplasm of the tumor cells in (66.1%) as shown in (Fig.1C). B-Catenin immunostaining reactivity was observed in nuclei of (21.6%) as shown in (Fig. 1D). Only two cases of mucinous adenocarcinoma expressed B-catenin as shown in (Figure 1E) and (Table 2).

Table (2): B-catenin immune expression in the sampled cases

Variables	-ve No. (%)	+ve No. (%)
Membrane staining	84 (49.4%)	16 (12.3%)
Cytoplasmic staining	14 (8.2%)	86 (66.1%)
Nuclear staining	72 (42.4%)	28 (21.6%)

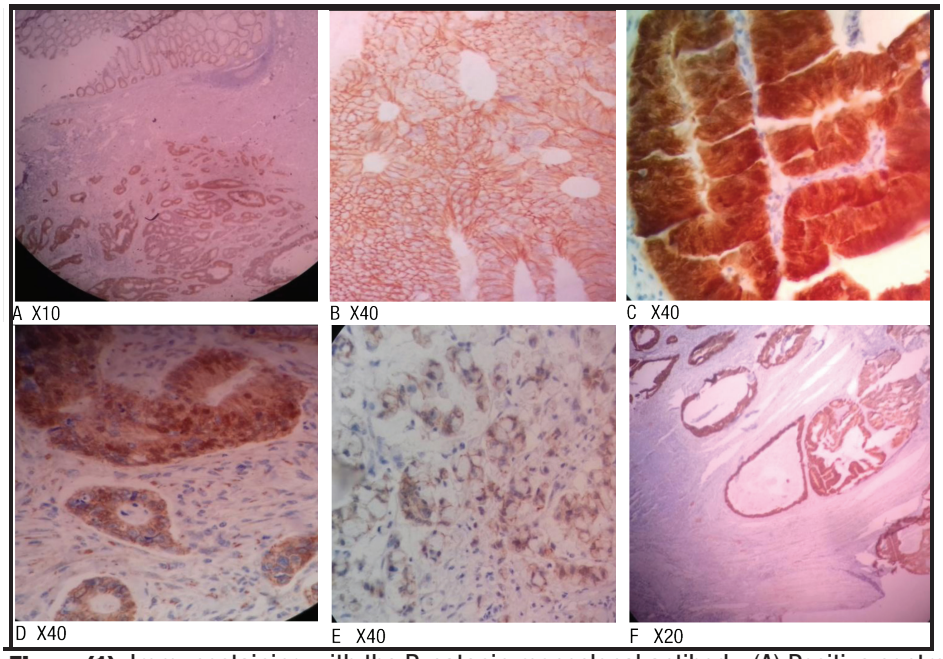

Figure (1): Immunostaining with the B-catenin monoclonal antibody. (A) Positive control of B-catenin expression of normal intestinal epithelial cells adjacent to tumor tissue. (B)Membranous staining of tumor tissue (Grade I). (C) Cytoplasmic staining of tumor tissue (Grade II). (D) Nuclear staining of tumor tissue(Grade II).(E) CRC signet ring adenocarcinoma with cytoplasmic B-catenin.(F) Invasion of smooth muscle by tumor glands.

Nuclear B-catenin expression was higher in patients ≤50years (67.9%), with a higher percentage in female (60.7%) than male. Left side colonic tumor showed a higher percentage (85.7%) than right side tumors (14.3%) with statistically significant correlation (P value of 0.02). Higher staining intensity detected in a patient with positive nodal status (60.7%) than negative nodal status (39.3%), however, the level of statistically significant difference was not reached. Cases with stage III-IV showed more immunostaining (60.7%) than stage I-II (39.3%). Well-moderate differentiated tumors showed higher staining intensity (89.3%) than poorly differentiated cases (10.7%). Non-mucinous tumors (92.9%) had a higher intensity than mucinous tumor (7.1%). Association for B-catenin nuclear staining with cytoplasmic staining and grading of nuclear staining was highly significant with (P value of 0.001), but there was no significant association between nuclear staining and membranous staining as shown in (Table 3).

Table (3): Frequency of the Distribution of Nuclear B-catenin Immune Expression and its Correlation with some Clinico-Pathological Parameters.

Variable	Categories	Nucleus		P value
		-ve expression	+ve expression	
Age	≥ 50 years	46 (63.9 %)	19 (67.9 %)	0.70
	< 50 years	26 (36.1%)	9 (32.1%)	
Gender	Male	32 (44.4%)	11 (39.3%)	0.64
	Female	40 (55.6%)	17 (60.7%)	
Site of tumor	Right	27 (37.5%)	4 (14.3%)	0.02
	Left	45 (62.5%)	24 (85.7%)	
Nodal status	Negative	35 (48.6%)	11 (39.3%)	0.40
	Positive	37 (51.4%)	17 (60.7%)	
Stage	I – II	35 (48.6%)	11 (39.3%)	0.40
	III-IV	37 (51.4%)	17 (60.7%)	
Differentiation	Well - moderate	63 (87.5%)	25 (89.3%)	0.80
	Poor	9 (12.5%)	3 (10.7%)	
Histopathology type	Non-mucinous	61 (84.7%)	26 (92.9%)	0.27
	mucinous	11 (15.3%)	2 (7.1%)	
Membrane expression	Positive	61 (84.7%)	23 (82.1%)	0.75
	Negative	11 (15.3%)	5 (17.9%)	
Cytoplasmic expression	-ve expression	4 (5.6%)	10 (35.7%)	0.001 **
	+ve expression	68 (94.4%)	18 (64.3%)	
Grade	1	45 (62.5%)	7 (25%)	0.001 **
	2	27 (37.5%)	10 (35.7%)	
	3	0 (0%)	11 (39.3%)	

Similar to nuclear staining, cytoplasmic immune expression was higher in patients' ≥ 50 years old (64%) and higher in female (57%) than male patients (43%).

Left side colonic tumor showed a higher percentage (69.8%) with positive nodal status (51.2%) rather than right side tumors (30.2%) with negative nodal status (48.8%) with no significant correlation. Stage III-IV tumors showed more cytoplasmic expression (52.3%) than stage I-II (47.7%). As well-moderate differentiated nuclear tumors showed higher expression (88.4%) than poorly differentiated cases (11.6%). Non-mucinous cases comprising (86%) of B-catenin cytoplasmic expression while mucinous tumor showed (14%). No statistically significant correlation was observed between B-catenin immune reactivity with membrane staining and grade of the staining intensity as shown in (Table 4).

Table (4): Frequency of the Distribution of Cytoplasmic B-catenin Immune Expression and its Correlation with some Clinico-Pathological Parameters.

Variable	Categories	Cytoplasm		P value
		-ve expression	+ve expression	
Age	≥ 50 years	10 (71.4%)	55 (64%)	0.58
	< 50 years	4 (28.6%)	31(36%)	
Gender	Male	6 (42.9%)	37 (43%)	0.99
	Female	8 (57.1%)	49 (57%)	
Site of tumor	Right	5 (35.7%)	26 (30.2%)	0.68
	Left	9 (64.3%)	60 (69.8%)	
Nodal status	Negative	4 (28.6%)	42 (48.8%)	0.15
	Positive	10 (71.4%)	44 (51.2%)	
Stage	I – II	5 (35.7%)	41 (47.7%)	0.40
	III - IV	9 (64.3%)	45 (52.3%)	
Differentiation	Well - moderate	12 (85.7%)	76 (88.4 %)	0.77
	Poor	2 (14.3%)	10 (11.6%)	
Histopathology type	Non-mucinous	13 (92.9%)	74 (86%)	0.48
	Mucinous	1 (7.1%)	12 (14%)	
Membrane expression	+ ve expression	10 (71.4%)	74 (86%)	0.16
	-ve expression	4 (28.6%)	12 (14%)	
Grade	1	11 (78.6%)	41 (47.7%)	0.07
	2	3 (21.4%)	34 (39.5%)	
	3	0 (0%)	11 (12.8%)	

Discussion

Colorectal cancer (CRC) is a multi-step carcinogenic process, with etiology comprising genetic factors, environmental exposures, and inflammatory bowel disease[4]. Bowel cancer is strongly related to age. In this study the mean age of patients were 54.47 ± 14.89; this is in agreement with the study done by Aldrubiet al, 2015; where mean age in his study was(52.85 ± 11.74) years[18]. Another study in Iraq showed that the mean age of patients was 56.88 ±1.99 years[19]. The highest frequency of cases was observed in patients ≥ 50 years. This is in agreement with another study in Iraq where the highest frequency was among 60-69 years of age[20]. This study revealed a slight preponderance of female with a male: female ratio (0.78:1) which is similar to what has been reported in a study by Hamilton et al, 2010 reported high cancer incidence rate especially among female compared to male[21].In agreement with the results of this study. The most frequent histological grade was well-moderately differentiated adenocarcinoma (88%), Hana et al, observed the same results in her study[22]. Left colonic and rectal tumors showed a higher frequency than right side tumor, with higher stage III-IV; this is in agreement with previous results[18,22]. B-catenin localization was different between cancer cells and normal mucosal cells in which we observed a strong uniform membranous staining at the normal cell-cell junction. This localization is consistent with results of Iwamoto et al, where they found the cytoplasmic tail of B-catenin binds to E-cadherin and, indirectly to the cytoskeleton, so it is localized to the junction of cell-to-cell plasma membrane[24]. Multiple protein complexes will be formed of APC, axin and B-catenin which make B-catenin a target for degradation so that no cytoplasmic or nuclear B-catenin will be detected in normal tissue[25]. This agrees with the results of this study where normal colonic mucosal cells showed no nuclear and cytoplasmic staining shown in (Figure 1A&1B). This study revealed alteration in B-catenin expression in all 100% colorectal carcinomas (maintained by most membranous + cytoplasmic or nuclear positivity), where nuclear expression was in (21.6%), cytoplasm in (66.1%) and membrane (12.3%) sampled cases. In a study done in Iraq by

Aldrubi et al, he found that the nuclear localization of B-catenin detected by IHC in (42.86%) cases of carcinoma group, while cytoplasmic expression of B- catenin was detected in (92.86%) cases[18], in the same year a study done in Iran by Nazemalhosseini et al, (19.39 %) cases ex¬pressed nuclear β-catenin and (80.6 %) cases were negative for nuclear B-catenin expression17. Wong et al. clearly showed that the expres¬sion of nuclear B-catenin increased notably during the development of normal mucosa to carcinoma form[23]. In a study done by Kobayashi et al, he has found that nuclear over-expression of B-catenin was observed in 35% of intramucosal cancers and 42% of invasive cancers, while the cytoplasmic B-catenin was frequently expressed at high levels in invasive cancers, it was not correlated with nuclear expression of B-catenin[26]. In contrast to this study where a highly significant correlation was observed between nuclear B- catenin and cytoplasmic expression and with the grade of B-catenin staining intensity (P value= 0.001). This can be explained by stabilization of B-catenin in the cytoplasm and its subsequent entry into the nucleus where it interacts with transcription factors and activate target genes. It is, therefore, likely that cytoplasmic accumulation of B-catenin starts at early stages of colorectal tumorigenesis, thus cytoplasmic accumulation of B-catenin is a cause rather than a result of the progression from adenoma to carcinoma[26]. This abnormal distribution of B-catenin in form of cytoplasmic or nuclear expression reflects either an ineffective B-catenin or loss of B-catenin connection to the cytoskeleton. This was in agreements with other observers[27-29] where they thought that an abnormally high amount of B-catenin in the cytoplasm and not in the membrane seems to indicate a B-catenin protein with oncogenic potential. This cytoplasmic and nuclear accumulation of B-catenin stimulates 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 [28,29]. The result of this study revealed a higher level of B- catenin in the female patient than male patient this disagrees with the results of Cesar Wong et al and Kawasaki et al[30,31]. On the other hand, we did not find any correlation between B-catenin and patient's age. A significant association between left-sided colonic tumors and stronger B-catenin expression was noted in this study (P-value 0.02), a finding which was reported by others[17,18,31]. High expression of B-catenin was observed in advanced tumor stage with deeper invasive tumors showing higher grade of B-catenin than superficial tumors, similar findings have been reported previously both in CRC and in other types of human carcinoma[30, 32]. In the present study also and in agreement with others[33] high grade expression of B-catenin was associated with lymph node metastasis, The same results reported by Tóth et al,[34]and Lugli et al, in a study based on tissue microarray analysis which showed that increased expression of nuclear B-catenin and loss of membranous E-cadherin were related to tumor stage and lymph node invasion, the presence of vascular invasion and worse survival[35]. Also B- catenin was higher in low-grade tumor rather than in high-grade tumor this is in agreement with others[30-31] those reported lower expression of B-catenin in high-grade tumors and disagrees with other[36] that showed higher expression with deteriorating tumor grade. Aldrubi et al, revealed that the overall expression of β-catenin showed an association with better differentiation[18]. This study revealed a low level of B-catenin nuclear staining in mucinous carcinoma constituting (7.1%) this is in agreement with Wong et al, who found low level of nuclear B- catenin IHC scores in colorectal mucinous adenocarcinoma implies that this tumor does not exploit the B- catenin related pathway in carcinogenesis [23].

Conclusions

In this study, B-catenin expression observed in all (100%) primary colorectal carcinoma where both nuclear (21.6%) and cytoplasmic expression (66.1%) were noted. A significant correlation was found between alterations in immunoexpression and cellular localization of B-catenin in one hand and increasing age, female sex and left side colonic tumor on the other hand. Also, a rising trend of B-catenin was observed with lymph node metastasis and higher stage of CRC of the sampled cases but not reached a significant level. These findings may support the idea that B-catenin adds prognostic information to standard clinicopathological parameters that can be used in selecting candidates for closer follow-up and aggressive adjuvant therapy.

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