Original Article

Immunohitochemical expression of Bcl-2in human colocrectal carcinoma

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Abstract:

Background: Colorectal carcinoma is the most frequent type of malignancy in terms of incidence,it is the third most common cancer world wide in both sexes . bcl_2 expression has been described as been a better prognostic factor in colorectal carcinoma.

Objcetives: To determine the frequency and the pattern of Bcl-2 expression in colorectal carcinoma by immunohistochemical technique and to correlate this expression with different clinicopathological parameters.

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Material and method: Thirty cases of colorectal carcinoma were studied, these cases were diagnosed in private laboratories in Baghdad / Iraq from January 2015 to Juan 2015. Clinicopathological parameter such as age, gender , pathological diagnosis , tumor site , lymph nodes status , grade and stage of tumor were collected from patients files..Sections of 4µm were stained by hematoxylen and eosin stain, and immunohistochemical stained for Bcl-2.

Results: Nineteen (63.3%) cases were males, 11(36.7%) cases were females, with age distribution ranging from (39-89) years with a mean age of 56.5 years. Histologically the tumor grade range from moderately differentiated in 27 (90%) cases and poorly differentiated in 3 (10%) cases. Ten (33.3%)cases located in the right colon and sigmoid each 6(20%) cases in rectum,8(26.7%) cases in the cecum,3(10%)cases located in right colon and sigmoid each,6(20%) cases in the rectum and 8(26.7%) cases in the left colon. Regarding pathological staging (TNM system),2 (6.7%) cases were stage T2 ,24 (80%) were stage T3 ,4(13.3%) cases were T4. Lymph node involvement found in 14(46%) cases and distant metastasis was found in 9(30%) cases. Bcl-2 expression was present in 14(46.7 %) cases of 30 colorectal carcinoma.There was no significant correlation with age, sex, histopathological grade, location, lymph nodes status and tumor invation.

Conclusion: There was no significant statistical correlation between bcl expression by tumor cell of colorectal carcinoma and different clinicopathological parameters in this study.

Keywords: Colorectal cancer, Bcl-2 expression.

Introduction:

Colorectal carcinoma is the most frequently type of malignancy in terms of incidence, it is the third most common cancer world wide in both sexes (1). Colorectal cancer is believed to result from a series of genetic alterations (2,3). Colorectal cancer is currently one of the major contributors to cancer – related deaths world wide (4,5). Pathophysiology of colorectal carcinoma is complex and developed in multistep process in which several gene mutation will occur and coordinate with each other in genotyping and phenotyping (6). Bcl-2 (B-cell lymphoma 2) encoded in human by BCL2 gene , is the founding member of the Bcl-2 family of regulator proteins that regulate cell death (apoptosis) , by either inducting pro-apoptotic or inhibiting anti-apoptotic (7,8). The bcl-2 proto-oncogene is a known inhibitor of apoptosis and may therefore allow an accumulation of genetic alterations that became propagated by cell division and potentially contribute to neoplastic development (9,10). Bcl-2 expression has also been described as being associated with a better prognosis in cases of colorectal cancer(11).

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Material and methods:

This study is retrospectively designed, a total of 30 cases of colorectal carcinoma diagnosed in private pathology laboratories in Baghdad / Iraq during the period from January 2015 to June 2015, all cases were evaluated in terms of age , gender , pathological diagnosis , including tumor size , location , lymph nodes situation , stage and grade of the tumor.Two Section of 4µm thickness were taken from paraffin blocks of the tumor, one section was stained with hematoxylin and eosin (H and E) and the other slide dewaxed and processed for immunohistochemical staining. Monoclonal mouse anti bcl-2 antibody(dilation:80,colon 124,colon Mo887 Dako,Denmark) was used according to instructions supplied by themanufactores.positive colon used was normal lymph

node sections,for negative control, a normal rabit IgG,instead of the primary antibody was applied(12).Bcl-2 exprssion was evaluation relative to the percentage of tumor cells with cytoplasmic reaction,being positive, respectively negative for the percentage of positive cells, higher respectively smaller than 5%(13,14).

Results:-

The 30 cases that are included in this study are divided into different groups according to each study parameter A comprison has been made among the groups in regards to over expression of bcl-2 immunohitochemically.Table(1) show the clinicopathological parameters.

Table (1): The clinicopathological parameters.

Variable		Number(%)
Age group	20-39 40-49 50-59 ≥60	2(6.7%) 8(26.7%) 10(33.3%) 10(33.3%)
Gender	Male female	19(63.3%) 11(36.7%)
Site	Cecum right colon left colon sigmoid rectum	10(33.3%) 3(10%) 8(26.7%) 3(33.3%) 6(20%)
Grad	Moderately differatiated Poorly differeatited	27(90%) 3(10%)
TNM stage tumor	T1 T2 T3 T4	0(-) 2(6.7%) 24(80%) 4(13.3%)
Lymph node status(N)	N0 N1 N2	14(53.3%) 9(30%) 5(16.7%)
metastasis	M0 M1	21(70%) 9(30%)

Bcl-2 expression:-Sixteen (53.3%) cases expression a totally negative staining of bcl-2 marker,14(46.7%)cases stained positive for this marker.

Age distribution:-Fourteen (46.7%) cases expressed bcl-2, these cases were distributed among age groups as shown in table(2).

Table(2) Distribution of bcl-2 positive cases according to age group

	Bcl-2 expression		total
	positive	negative	
20-39	1(3.3%)	1(3.3%)	2(6.7%)
40-49	4(13.3%)	4(13.3%)	8(26.7%)
50-59	4(13.3%)	6(20%)	10(33.3%)
≥ 60	5(16.7%)	5(16.7%)	10(33.3%)
total	14(46.7%)	16(53.3%)	30(100%)

There was no significant statistical correlation between age distribution and tumor expressing bcl-2.

Gender distribution: Nineteen (63.3%) cases were males , 11(36.7%)cases were females , only 3(10%) cases of male gender and 2(6.67%)case of females,8(26.7%) cases of male gender and 6(20%) of females expressed bcl-2. No significant statistical correlation was found between gender and bcl-2 expression ($p>0.05$).

Distribution according to the grades of the tumor:-Histologically the tumor grades range from moderately differentiated in 27(90%) cases and poorly differentiated in 3(10%) cases. Twelve (40%) cases of moderately differentiated were bcl-2 positive and 2 (6.7%) cases of poorly differentiated were expressed bcl-2 positive. .No significant statistical correlation was found between bcl-2 expression and tumor grade . ($P>0.05$) as shown in table(3).

Table(3):The incidence and frequency of bcl-2 positive cases in relation to grade of the tumor.

Bcl-2 expression	Grade of the tumor		total	P value
	Moderate differentiated	Poorly differentiated		
positive	12(40%)	2(6.7%)	14(46.7%)	p>0.05 (NS)
negative	15(50%)	1(3.3%)	16(53.3%)	
total	27(90%)	3(10%)	30(100%)	

Topographic distribution of the tumor:-Five (16.7%) cases located in the cecum, and 1(3.3%) cases at right colon, 2(6.7%) cases at sigmoid,3(10%) cases located each at rectum and left colon, all there cases were positive for bcl-2. There was no significant statistical correlation between site of the tumor and bcl-2 expression as shown in table(4).

Table(4):The distribution of cases according to the anatomical site and bcl-2 expression.

Sit of the tumor	Bcl-2 expression		total	P value
	positive	negative		
cecum	5(16.7%)	5(16.7%)	10(33.3%)	p>0.05 (NS)
Right colon	1(3.3%)	2(6.7%)	3(10%)	
Left colon	3(10%)	5(16.7%)	8(26.7%)	
sigmoid	2(6.7%)	1(3.3%)	3(10%)	
rectum	3(10%)	3(10%)	6(20%)	
total	14(46.7%)	16(53.3%)	30(100)	

Distribution according to extent of tumor (T) :-In this study 2 (6.7%) cases were T2 invading the muscular is propria, 24(80%) cases were T3, 4(13.3%) cases were T4 , five (16.67%) cases expressed bcl-2 these cases belong to T3 stege.NO significant statistical correlation was found in this study between stage of the tumor and bcl-2 expression.

Table (5): Distribution of Bcl-2 over expression by the tumor in relation to depth of wall invasion(T) .

Depth of wall invasion (T)	Bcl-2 expression		total	P value
	positive	negative		
T2	2(6.7%)	0(0)	2(6.7%)	p>0.05 (NS)
T3	10(33.3%)	14(46.7%)	24(80%)	
T4	2(6.7%)	2(6.7%)	4(13.3%)	
total	24(46.7%)	16(53.3%)	30(100%)	

Distribution according to lymph nodes status (N of TNM):- Sixteen (53.3%) cases showed no nodal involvement, while 14(46.7%) cases presented with nodal involvement. These cases were as follows:- seven(23.3%)cases from N0,5(16.7%) cases of N1 and 2(6.6%) cases from N2.No significant statistical correlation was seen between bcl-2 expression and nodal status,as shown in table(6).

Table (6): Frequency distribution of Bcl-2 over expression in relation to lymph node status.

Lymph node status	Bcl-2 expression		total	P value
	positive	negative		
N0	7(23.3%)	9(30%)	16(53.3%)	p>0.05 (NS)
N1	5(16.7%)	4(13.3%)	9(30%)	
N2	2(6.7%)	3(10%)	5(16.7%)	
total	14(46.7%)	16(53.3%)	30(100%)	

Discussion :

The bcl-2 oncogene is a known inhibitor of apoptosis that may allow the accumulation and propagation of cell containing genetic aterations (15).Usually expression of bcl-2 in colorectal cancer has been shown as being a favorable prognostic factor (16). Immunohistochemistry proved to be powerful method to detect the tissue expression of bcl-2 (17). In this study,bcl-2 is found to be positive in 14(46.7%) cases of colorectal carcinoma .This result agree with many other studies and disagree with others (18,19,6).Studies on bcl-2 status in colorectal carcinoma have presented controversial results with a variable inter-observer reproducibility,This may explain the wide range rates reported in the literature(9.6%to 94%).(13,14).

In this study colorectal carcinoma tends to be more common in elderly patients , the age ranges (39-89)years with mean 56.5 years, 19(63.3%)male , 11(36.7%) female . these results agree with many other studies(20,21).There was no significant statistical correlation between patients sex and expression of bcl-2 oncoprotein in this study,these results agree with many other studies (22,23,24). The role of bcl-2 in colorectal tumor genesis is believed to be in the early stages of carcinogenesis.Adecrease in the level of bcl-2 can lead to cell death by apotosis,while its over expression protects against programmed cell death. This study showed that bcl-2 expression is more in well differentiated – moderated differentiated carcinoma than in poor differentiated , but no significant relation between bcl-2 expression and grade of tumor found , this result agree with many other studies (25,26 ,27). And disagree with others(28). Asignificant association was found between bcl-2 expression in Hegazy et.al.studies cases and tumor grade and stage, This was in agreement with Schwandero et.al.(24).According to the anatomical site of colorectal carcinoma this study show high number in cecum more than other sites including rectum, the expression was 5(16.7%) cases in the ceacum,while 3(10%) cases of the rectum, but no significant statistically correlation was found between tumor site and bcl-2 expression, this result agree with other studies (29 ,30).According to stage of tumor(T) in this study, , T3 showed highest percentage of bcl-2 expression in 10(33.3%) cases.,However,.no significant statistical correlation between stage of tumor(T) and bcl-2 expression was found . these results agree with other studies (31,32,33) .Bosari et.al.(43) investigated by immunohistochemistry bcl-2 expression in normal colonic mucosa ,hyperplastic polyps, adenomas and adenocarcinomas of large bowel in large us population.In colorectal carcinoma bcl-2 expression 76.9%was higher than that in adenomas 59.0%, but bcl-2 expression in colorectal cancer and adenomas was not correlated with relevant clinicopathological parameters,and had no prognostic significant. This study shows highest expression of bcl-2 was in (N0) in 7(23.3%) cases regarding lymph node status.and no significant correlation was found between bcl-2 expression

and lymphatic metastasis, these results agree with many other studies (34,35).

Conclusion:

Bcl-2 oncogene is a known inhibitor of apoptosis that may allow the accumulation and propagation of cells containing genetic alteration. There was no significant statistical correlation between bcl-2 expression by tumor and different clinicopathological parameters in this study.

Author contributions:

Tharwa Hadi Hassan AL-Tai: Acquisition of data analysis,interpretation of data and drafing of manuscript.

Kifah Hamdan AbdulGhafour: Study conception,design,interp retation of data and critical revision

Hind Hamid Shaker: Acquisition of data analysis and critical revision

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