Immunohistochemical evaluation of BCL-2 and KI-67 proteins in colorectal adenoma

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Summary:

Background: Development of colorectal adenocarcinoma principally occurs via the adenoma- carcinoma sequence of a multiple step process of tumor progression. This results from an accumulation of genetic changes in cells of intestinal mucosa. The use of molecular biomarkers may further aid in determining the risk of changing to carcinoma.

Objectives: To evaluate the expression of Bcl-2 and Ki-67 in colorectal adenomas and correlate the observed levels with age, gender, histological type, grades of dysplasia as well as to the levels of these two markers with each other.

Materials and Methods: A retrospective stu dy of fifty colorectal biopsies were selected randomly, 40 cases were with different grades of colorectal adenomas and 10 cases with normal mucosa as a control group; covering the period from 2010 till 2012. All the cases collected from the Gastroenterology and Hepatology Teaching Hospital. The biopsies were re-examined histologically and then stained immunohistochemically for Ki-67 and Bcl-2.

Results: There was a significant correlation only between Ki-67 expression and grade of adenoma and there was a significant correlation between Ki-67 and Bcl-2 expression in adenoma. Also there was a significant correlation between age of the patients and histologic type.

Conclusions: The assay of Ki-67 and Bcl-2 is valuable in detecting the risk of early colorectal carcinogenesis in patients with colorectal adenomas. Ki-67 expression increases with progression of the grade of adenoma while BCL-2 decrease with progression of the grade.

Key words: Colorectal adenoma, Carcinomatous changes, KI-67, BCL-2, Immunohistochemical stain.

Introduction:

Adenomas, the benign glandular neoplasms that precede colon cancer development. These are localized proliferation of dysplastic intestinal epithelium. They occur singly or as multiple polyps, pedunculated or sessile (1).The incidence of carcinoma within adenoma is related to size and type, it is rare in tubular adenomas less than 1cm but estimated to occur in 40%-50% of villous lesions larger than 4cm.(2,3) Adenomas with higher degree of dysplasia have a higher incidence of carcinomatous transformation. The overall chance of developing carcinoma in a polyp is estimated at 5 % (4, 5).Adenomas are generally asymptomatic, but a significant number produce microscopic fecal blood loss. They are rarely large enough to cause obstruction in the absence of malignant changes (6).

Patients and Methods:

A retrospective study of fifty colorectal biopsies were selected randomly, 40 cases were with different grades of colorectal adenomas (20 low grade and 20 high grade) and 10 cases with normal mucosa as a control group; covering

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the period from 2010 till 2012.All the cases were collected from the Gastroenterology and Hepatology Teaching Hospital The relevant data were obtained by reviewing the files of histopathological reports including age, gender, histological type and grade of adenomas. Paraffin blocks were cut in 4-micron-thick sections; slides were stained with hematoxylin and eosin and re-evaluated.

Immunohistochemical staining: Colorectal biopsies in this study were prepared from paraffin-embedded blocks. Immunohistochemical staining for Ki-67 and Bcl-2 were performed by (Labeled streptavidin-biotin peroxidase method using automated antigen retrieval device (Danemark) with buffers and detection reagents supplied by the manufacturer. The primary antibodies (Ki-67 and Bcl-2) were diluted and incubated with tissue sections for 30 min. The LSAB+ detection kit (Dako, Danemark) was used for antigen visualization; sections were counterstained with hematoxylin and then coverslipped. Paraffin sections of breast carcinoma and follicular lymphoma were run with each batch to serve as a positive control for Ki-67 and Bcl-2 stains respectively. Immunohistochemical interpretation:

mmunostained slides were scored after the entire slide was evaluated by light microscope.   The criterion for positive reaction confirming the presence of Ki-67 protein was brown nuclear precipitate while for Bcl-2 it was brown cytoplasmic precipitate. The intensity of staining was assessed by semi

quantitative scoring (the percentage of positive cells in 100 adenomatous cells, performed at (x400) total magnification in 25 adenomatous fields that represented the most positive neoplastic area in the slides.

Scoring criteria for Ki-67(7,8)

• None of the epithelial cells revealed positive = score 0
• 5- 10% positive adenomatous cells = score 1
• 10- 25% positive cells =score 2
• 26% - 50% positive cells = score 3
• > 50% positive cells = score 4

Scoring criteria for Bcl-2(9)

• None - < 25% of the epithelial cells revealed positive = score 0
• 25% - < 75% positive epithelial cells = score 1
• 75% -100% positive cells =score 2

Statistical analysis: Statistical analysis was performed with SPSS statistical software program. Associations between categorical variables were assessed via cross tabulation and chi-square. Exact tests were used to calculate the p value. In all statistical analysis, a p value < 0.05 was considered to be significant.

Results:

A total of 50 cases; 32 males and 18 females were included in this study. Age ranged in between 25-75 years. The mean of patients with colorectal adenoma was 50.48 years with standard deviation = 13.01.

There was a statistical significant correlation between the age of patient with adenoma and the histological type, the older age group showed more villous composition.

Table (1): Histologic type distribution of adenoma in relation to age of patients.

Group	No.	Mean Age	SD	P Value
Tubular	5	50.20	15.06	
Tubulovillous	29	49.34	12.08	
Villous	6	56.17	16.57	0.001
Control	10	33.00	5.60	
Total	50	46.98	13.79	

There was no statistical difference between Ki-67 expression and age, sex and histological type of adenoma; but there was a significant correlation between Ki-67 and grade of adenoma as seen in figure (1) and table (2).
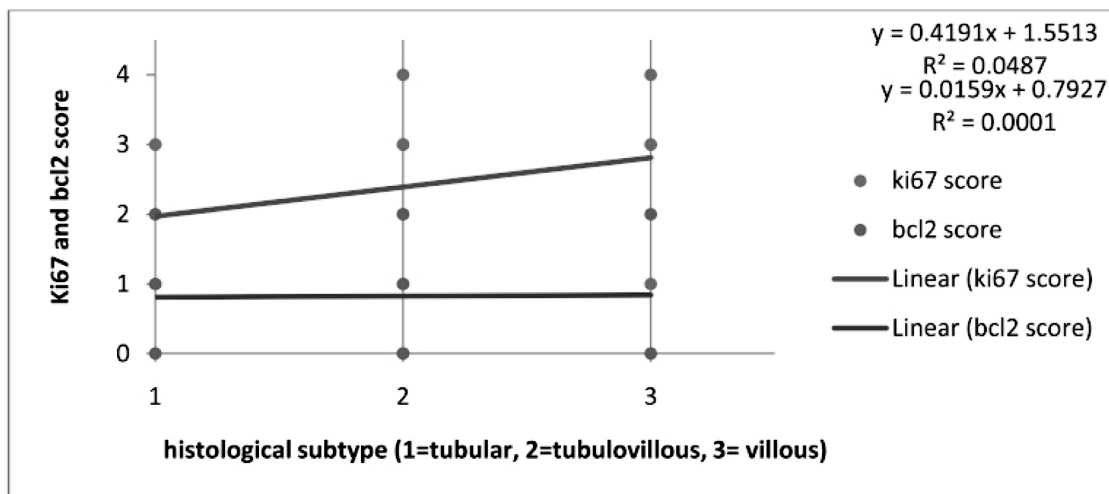

Figure (1): correlation between histological subtype and (ki67 and bcl2 score). (r=0.236, p=0.102) and (r=<0.001, p=1.000) respectively (Kendall's tau-b test)

Table (2): Correlation between Ki-67 and the grade (r = 0.524, p =0.001).

Grade	KI-67 Score											
	0		1		2		3		4		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Low	3	15	4	20	6	30	7	35	0	0	20	50
High	0	0	0	0	3	15	15	75	2	10	20	50
Total	3	7.5	4	10	9	22.5	22	55	2	5	40	100

There was no significant correlation between Bcl-2 and age, gender and histological type. Our study shows that Ki-67 expression is increase from normal looking mucosa to low grade then high grade adenoma, while for Bcl-2 there was weak expression in normal looking mucosa then it increase in low grade adenomas and showed lower expression in high grade adenomas. This difference was statistically significant (p value = 0.001) as seen in figure (2).
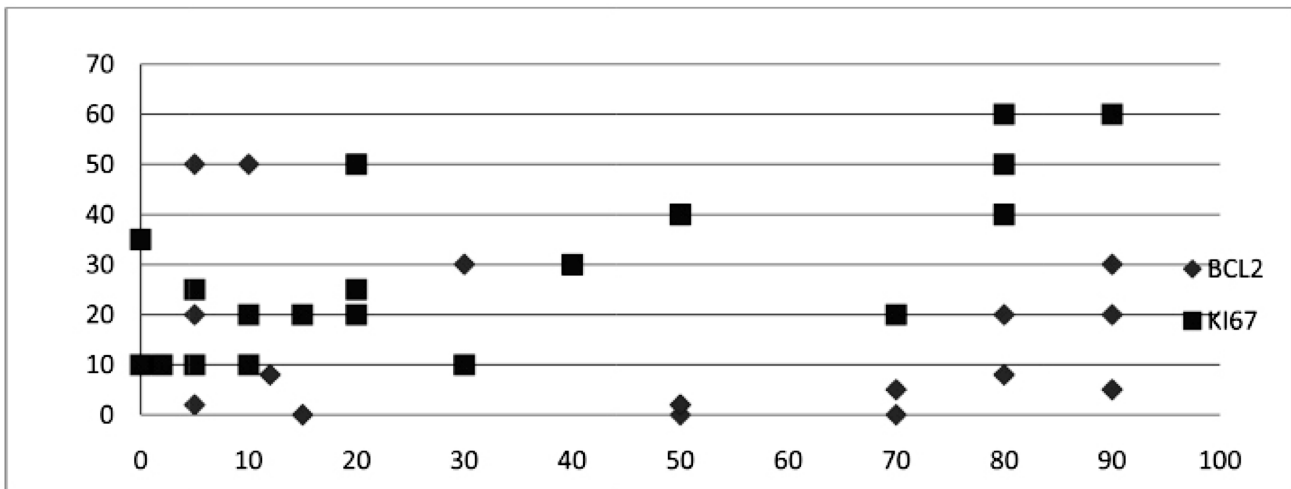
Figure (2): Correlation between Bcl-2 & Ki-67 expression in adenomatous group (low and high grade adenomas).
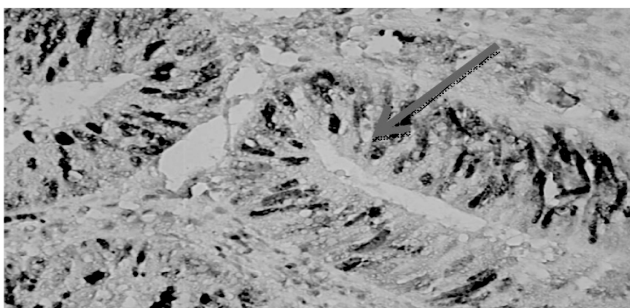

Figure (3): High grade adenoma stained by Ki-67 showing strong Ki-67 staining (score 3), arrow shows brownish stained nuclei of dysplastic cell IHC{x400}
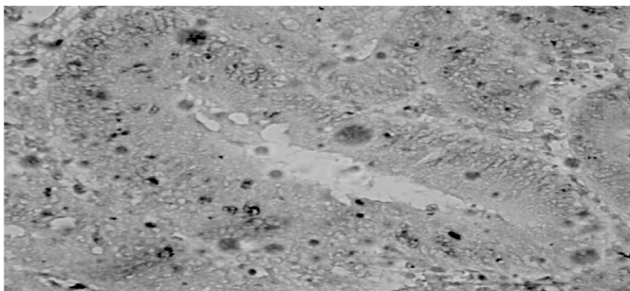

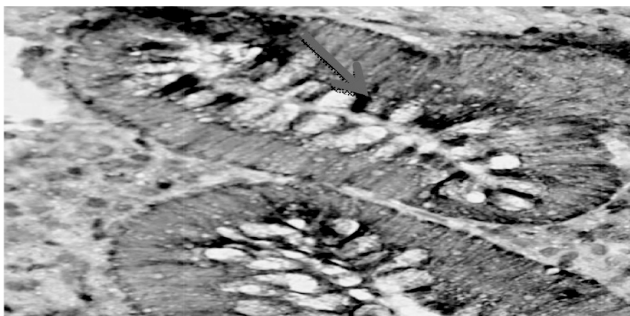**Figure (4) Low grade adenoma stained by Ki-67 showing (score2). IHC (x400)**

Figure (6): Low grade colorectal adenoma stained by Bcl-2 (score 2), arrow shows dark brown cytoplasmic staining. IHC (x400)

Discussion:

Knowledge of factors associated with the genesis and progression of colorectal cancer is of great importance in the development of strategies for prevention and treatment. Polypectomy currently constitutes the best strategy for prevention of colorectal cancer. The identification of parameters reflecting the biological behavior of adenomas, correlated with the severity and degree of evolution, is an important determinant of prognosis and improvement in cancer therapy.Our study revealed a highly significant correlation between Ki-67 expressions and grade of adenoma which is in concordance with the results obtained in Serbia (10). High grade adenomas show a lower Bcl-2 expression than low grade adenoma. However, such correlations were not statistically significant. These results agree with those of Raem T (11) and Zahraa M (12).There was no statistical difference between KI-67 and Bcl-2 expression and other parameters (age, sex, histological type). Vernillo R, Zahraa M ,Yinghao S and Raem T obtained similar results (13, 12, 14, 11) In our study there was significant correlation between the histological type and the age of the patients, these results are in agreement with that of Konishi and Morson (15).In conclusion there was an inverse correlation between the expression of Ki-67 and Bcl-2 in relation to the grade of adenoma and hence the potential for carcinomatous transformation. Such evaluations could be quite valuable for selecting high risk patients with adenomas who would need close follow up in the course of management.

Author contriutation:

Areege Nema collect the cases with their data and stain them and interpreted them under the supervision of. Khitam Al-Khafagi.

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