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Original Article

Immunohistochemical Expression of p53 and bcl-2 in Colorectal Adenomas and Carcinomas Using Automated Cellular Imaging System

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ABSTRACT

Background & Objective: **The current approaches to reduce the risk of colorectal carcinoma are through the detection and removal of the precursor lesion" adenomatous polyps". The study was conducted to evaluate the immunohistochemical expression of p53 and bcl-2 in colorectal adenomas and carcinomas.**

Patients and Methods: **A total of 86 cases, 33 colorectal adenomas, 33 colorectal adenocarcinomas and 20 samples of non -tumerous colonic tissue as control, were included in this retrospective study. Sections were stained immunohistochemically for p53 and bcl-2. Scoring was performed using Digimizer software. Data were analyzed using SPSS program (Statistical Package for Social Sciences) version 16 and Microsoft Office Excel 2007.**

Results: **The frequency of p53 positive cases was significantly higher in carcinoma than adenoma while the frequency of bcl-2 positive cases was significantly higher in adenoma than carcinoma. p53 expression was significantly higher in large sized adenomas, villous configuration, severe dysplasia, and multiple lesions. Bcl-2 expression showed significantly correlated with adenomas of small size, solitary, tubular, and mild dysplasia. There was a significant correlation between bcl-2 expression and non-mucinous carcinoma and a negative correlation with tumor size. There was an inverse relationship between bcl-2 and p53 expression in both colorectal adenomas and carcinomas.**

Conclusion: **p53 overexpression is a late event in colorectal tumorigenesis. bcl-2 may play an important role in the early stage of adenoma-carcinoma sequence.**

Keywords: **p53 Antigen, c-bcl-2 Proto-Oncogene, Adenoma, Colorectal Carcinomas**

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Introduction

The development and progression of colonic carcinogenesis are caused by the accumulation of cancer related genetic alterations, resulting in altered gene expression of oncogenes, tumor suppressor genes, and mismatch repair genes (1). These alterations affect the expression of a variety of downstream genes, including those involved in regulating the cell cycle, apoptosis, adhesion, and angiogenesis (2).

p53 is encoded by the Tp53 gene located on 17p13.1. Its expression is abnormal in more than 50% of human tumors (3). Under normal conditions, the expression of p53 protein is kept at extremely low level. In response to multiple cellular stresses, p53 rapidly accumulates in the nucleus. p53 exerts its proapoptotic function when cellular DNA damage is severe and repair is impossible. On the other hand, p53 promotes G1 cell cycle arrest in the early stage of DNA damage response (4). A p53 mutation is final step in the conversion of adenoma to carcinoma. The frequency of p53 abnormalities increases with the progression of the lesion (3).

Bcl-2 inhibits apoptosis and prolongs the survival of variety of cells, in addition to its role in the progression of cells division. All these actions will potentiate tumor growth (5). In the large bowel, bcl-2 protein has been localized to the epithelial cells at the base of crypts, where stem cell proliferation takes place (6). Studies have shown that bcl-2 expression is higher in colorectal adenomas than adenocarcinoma (5, 7, 8). Many studies have examined the value of the immunohistochemical expression of bcl-2 protein in colorectal cancer, but results have been contradictory (9).

The aim of the study was to evaluate the immunohistochemical expression of p53 and bcl-2 in colorectal adenomas and adenocarcinomas and to correlate this expression with different clinicopathological parameters.

Materials and Methods

The study was retrospectively designed. Out of the total number of 86 cases, 66 paraffin blocks from patients with colorectal tumors were collected from Gastroenterology and Hepatology Center, Gastroenterology unit at Al-Khadhmiya Teaching Hospital and private laboratories for the period 2006-2010, including 33 blocks of colorectal adenoma and 33 blocks of colorectal adenocarcinoma. The control group included 20 samples of non -tumerous colonic tissue taken from autopsy cases from the Institute of Forensic Medicine. These specimens were paraffin embedded in the same center. The clinicopathological parameters were obtained from patients' admission case sheets and pathology reports. An informed consent was taken from patients and relatives of control (autopsy) cases. An absolute confidentiality of the patients' vital information was maintained for ethical purposes and an ethical approval was obtained from institutions in which the study was carried out.

From each block, three sections of 5μm thickness were taken. One section was stained with Haematoxylin and Eosin for revision and the other 2 section were stained immunohistochemically using three steps- indirect streptavidin method for Monoclonal Mouse Anti-Human p53 protein, clone DO-7 (DAKO, Denmark) and Monoclonal Mouse Anti-Human bcl-2 oncoprotein, clone 124 (DAKO, Denmark). Brown nuclear staining of p53 is considered positive reaction. Brown cytoplasmic and membranous staining of bcl-2 is considered positive reaction. Positive controls for p53 and bcl-2 were lymphoid tissue in Non-Hodgkin's lymphoma. Brown cytoplasmic staining of infiltrating lymphocytes serves as internal positive control for bcl-2. Technical negative control for both markers was obtained by omission of primary antibody.

Scoring of immunohistochemical staining was performed using automated cellular imaging system, Digimizer software, version 3.7.0. Each stained slide was scanned by a light microscope (Proway, China) for the brown immunostaining and three fields reflecting the best of the overall immunostaining were chosen and captured using a Sony digital camera (DSH-H55). To obtain a cut-off value for intensity of immunostaining, photomicrographs presented at the website of NordiCQ showing different grades of brown color intensity were analyzed by Digimizer software, and the digital values of intensity were classed into three categories: weak, moderate and strong.

Statistical Analysis

Data were analyzed using SPSS program (Statistical Package for Social Sciences) version 16 and Microsoft Office Excel 2007. Numeric data were expressed as mean± SEM, frequency was used to express discrete data. ANOVA was used to analyze numeric data while Chi-square was used to analyze discrete data, and Benferroni test was used for multiple comparisons. *P*- Value of less than 0.05 was considered significant.

Results

The frequency of p53 positive cases was significantly higher in carcinoma than in adenoma. The frequency of bcl-2 positive cases was significantly higher in adenoma than carcinoma. Strong p53 staining was significantly higher in seen in carcinoma while strong bcl-2 staining was significantly higher in adenoma (Fig. 1, 2).The mean of the three digital parameters of p53 immunohistochemical expression [Area (A), Number of objects (N), and intensity (I)] were significantly increased in a sequence of control-adenoma-carcinoma while the mean (A, N, and I) of bcl-2 were significantly higher in adenoma than carcinoma and in carcinoma than control groups (Table 1).

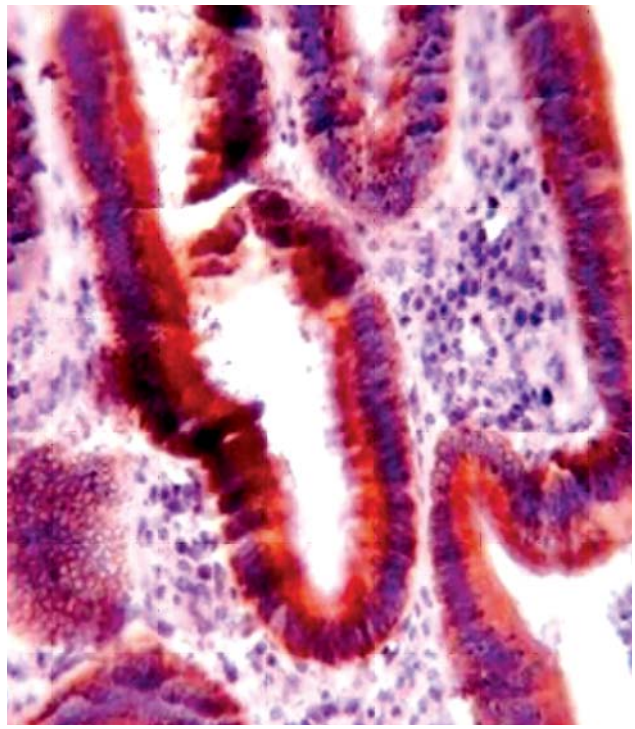

Fig. 1- Well–differentiated colonic adenocarcinoma showing p53-positive brown nuclear immunohistochemical staining with strong intensity (×40)

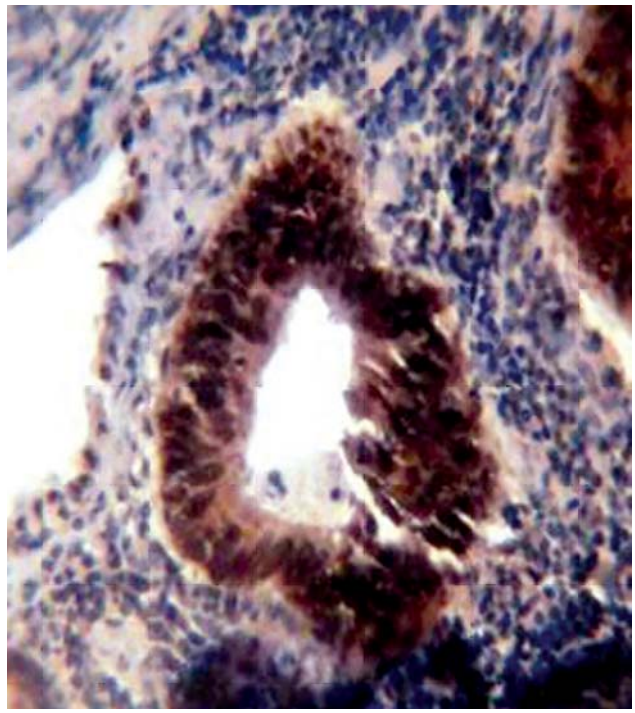

Fig. 2- Tubular colonic adenoma with mild dysplasia showing strong bcl-2 -positive brown cytoplasmic immunohistochemical staining of the dysplastic colonic glands and the infiltrating lymphocytes in the stroma with strong intensity (×40)

Table 1- Comparison of immunohistochemical expression of p53 and bcl-2 among patients and control groups

Marker	Expression	Adenoma No. (%)	Carcinoma No. (%)	Control No. (%)	*P*-value		
p53 frequency	Positive	12(36.36)	26(78.78)	0(0)	<0.001		
	Negative	21(63.63)	7(21.21)	20(100)			
Bcl-2 frequency	Positive	28(84.84)	16(48.48)	4(20)	<0.001		
	Negative	5(15.15)	17(51.51)	16(80)			
	Negative	21.21))7	21(63.63)	100))20			
p53 intensity	Weak	0 (0)	0 (0)	0 (0)	<0.001		
	Moderate	1(3.03)	6(18.18)	0 (0)			
	Strong	25(75.75)	6(18.18)	0 (0)			
	Negative	17(51.51)	5(15.15)	16(80)			
Bcl-2 intensity	Weak	12 (36.36)	0(0)	4(20)	<0.001		
	Moderate	3(9.09)	13(39.39)	0(0)			
	Strong	1 (3.03)	15(45.45)	0(0)			
Comparison of digimizer parameters					A *	N **	I ***
p53	Carcinoma vs. adenoma				<0.001	<0.001	<0.001
	Carcinoma vs. control				<0.001	<0.001	<0.001
	Adenoma vs. control				0.014	0.002	0.007
Bcl-2	Carcinoma vs. adenoma				<0.001	<0.001	0.001
	Carcinoma vs. control				0.009	0.007	0.005
	Adenoma vs. control				<0.001	<0.001	<0.001

* Area, **No. of objects, ***Intensity

The mean (A, N, and I) of p53 were significantly higher in large sized adenoma (≥1cm), multiple adenomas, adenomas with villous configuration and those with severe dysplasia while bcl-2 showed significantly higher levels of mean (A, N, and I) in small sized adenoma (<1cm), solitary adenoma, tubular adenomas and those with mild dysplasia The mean (A, N, and I) of bcl-2 showed a significant negative correlation with the size of the tumor, and a significant positive correlation with non-mucinous type (Table 2).

Table 2- Correlations of p53 and bcl-2 expression with clinicopathological parameters of colorectal tumors

Parameter	p53 (*P*-value)			Bcl-2 (*P*-value)		
	A *	N **	I ***	A	N	I
Adenoma						
Age (yr)	0.141	0.225	0.183	0.869	0.320	0.785
Gender	0.321	0.521	0.343	0.900	0.288	0.359
Site	0.450	1.000	0.731	0.158	0.333	0.706
Size	0.004	0.003	0.012	0.047	0.037	0.013
No. of adenomas	0.019	0.021	0.017	0.040	0.045	0.039
Histopathological types	0.023	0.036	0.035	0.031	0.036	0.048
Degree of dysplasia	<0.001	<0.001	<0.001	0.046	0.034	0.046
Carcinoma						
Age (yr)	0.762	0.612	0.864	0.825	0.928	0.859
Gender	0.842	0.360	1.000	0.268	0.573	0.508
Site	0.925	0.558	0.312	0.191	0.345	0.148
Size	0.409	0.632	0.636	0.032	0.036	0.027
Gross morphology	0.302	0.882	0.539	0.631	0.421	0.473
Histopathological types	0.593	0.353	0.655	0.001	0.018	0.014
Grade	0.617	0.634	0.621	0.420	0.449	0.393
Stage	0.838	0.071	0.826	0.516	0.564	0.364
Lymph node involvement	0.561	0.371	0.604	0.328	0.473	0.205
Lymphovascular permeation	0.213	0.500	0.090	0.699	0.943	0.303
Lymphocytic infiltration	0.264	0.679	0.265	0.862	0.807	0.815

* Area, ** No. of objects, *** Intensity

The correlation between p53 and bcl-2 was significantly negative in adenoma (r= -0.324, P= 0.032) and carcinoma(r= -0.456, P= 0.002).

Discussion

The present study has shown that p53 expression was significantly higher in carcinomas than adenoma. The three digital parameters of p53 expression (A, N, and I) were significantly higher in carcinoma than adenoma. Strong p53 staining was mainly seen in carcinoma. This is comparable to the literature, which stated that the frequency of p53 abnormalities in colorectal carcinogenesis increases with the progression of the lesion, thus alterations are found in 4%-26% of adenomas, 50% of adenomas with invasive foci, and in 50%-75% of colorectal cancer (CRC) (3).

Advanced colorectal adenomatous polyps are identified based on size ≥10 mm, high-grade

dysplasia, and/or villous histology (10). The current work found that p53 overexpression is significantly higher in adenomas measuring≥ 1 cm, adenomas carrying villous configuration, those with severe dysplastic change and in multiple adenomas. These results collectively indicate that p53 overexpression is associated with advanced colorectal adenomatous polyps supporting the notion that p53 overexpression is a late event in colorectal tumorigenesis, in keeping with results reported previously (10-14). These results suggest that p53 immunohistochemical profile may be useful adjuncts in detecting adenomas with a malignant potential and p53 protein may be prognostic indicator useful in follow-up of patients with severely dysplastic colorectal adenomas. In addition, villous change may indicate increased malignant potential and may be useful to consider when assigning surveillance guidelines (10).

Concerning Iraqi studies, Nussrat *et al.* (15) and Abdulamir *et al.* (16) demonstrated that p53 immunoreactivity is significantly correlated with grade of dysplasia and size of adenomas in agreement with the present study.

Regarding other parameters of adenomas including age, gender and site, the current work revealed no significant correlation with p53 overexpression similar to the findings of other authors (15, 17).

The present work showed that 78.78% of CRC displayed p53 overexpression .Studies found that the prevalence of p53 mutations in colorectal cancer is highly variable among different series ranging from 59%- 85.4% (5, 18, 19). This wide variation in the results could be due to the choice of methods used, sensitivity, and specificity of different antibodies used, and different modes of scoring systems and interpretations of the results.

The following classical factors predict the prognosis in CRC: grade, stage, tumor size, lymph node involvement, blood vessel invasion, gender, and age of patients (18). In the present study, no significant correlation was found between p53 expression and prognostic factors of CRC in accordance with results obtained by other authors (5, 19, 20).

Some studies have found discordant results to the current study. A significantly higher rate of p53 mutation in left colorectal cancer was reported by Soong *et al.* (21) and Gervaz *et al.* (22). Other studies showed higher frequency of p53-positive expression for non-mucinous (20, 23). p53 immunostaining was decreased with grade of carcinoma and was inversely correlated with depth of invasion but not with lymph node involvement and p53 was also correlated with the age of patients (18). Such discordant results may be due differences in antibody types, detection methods, variations in statistical analyses in addition to racial and geographical factors.

Bcl-2 gene activation, which has been shown to occur earlier in the adenoma-carcinoma sequence, might represent a marker involved in tumor initiation. The localization of the bcl-2 to the dysplastic cells in colorectal adenomas confirms its role in imitation of tumor growth by inhibiting apoptosis and consequently the accumulation of genetic alterations (24).

In the present study, the expression rate and the three digital parameters expression (A, N, and I) of bcl-2 and strong bcl-2 staining were significantly higher in adenomas. In normal colonic tissue (control cases), bcl-2 expression was restricted to the base of colonic glands indicating that basal epithelial cells of the normal colonic crypts uniformly express the bcl-2 protein. Given that the crypt cell population arises from basally located stem cells, the immunolocalization of bcl-2 suggests that it protects stem cells from apoptosis. The finding of a high level of diffuse homogenous bcl-2 expression in dysplastic and malignant cells, in contrast to non-neoplastic cells, suggests that abnormal bcl-2 gene activation is an early event in neoplastic development or progression of colorectal carcinoma (25).

Several studies on the role of bcl-2 in colorectal carcinogenesis reported higher rates of bcl-2 expression in adenoma than carcinoma (7, 8, 26).

In the current work, bcl-2 expression in colorectal adenoma was significantly correlated with mild glandular dysplasia, tubular type, solitary lesion, and small size (<1cm). The results of the present work are in a good accordance with the observations published by other authors and suggest that bcl-2 may play an important role in the early stage of the adenoma-carcinoma sequence followed by other genetic changes, e.g., p53 accumulation. Down-regulation of bcl-2 is associated with the risk of malignant transformation for colorectal adenoma (27).

In colorectal carcinoma, the present study detected a statistically significant correlation between bcl-2 expression with decreasing tumor size and non-mucinous type in agreement with an Iraqi study (28).

Investigations on clinicopathological significance of bcl-2 in colorectal carcinoma have yielded conflicting results. Bcl-2 expression was not significantly correlated with clinicopathological parameters (29). Bcl-2 oncoprotein expression in well-differentiated carcinoma was significantly higher than in moderately differentiated carcinoma (7). The expression of bcl-2 protein was related only to lower tumor stage (6). Bcl-2 expression was not related to tumor grade (8). There was no statistically significant correlation between the expressions of bcl-2 in relation to the grade, staging, disease-free interval, survival, and mortality (5). These conflicting data are partly due to different populations, different staging systems, different statistical analyses, as well as primary factors regarding the immunohistochemical technique and the evaluation of the results. Although immunohistochemistry has been used in most studies, the results have been interpreted using a wide variety of protocol variables and scores, which makes the comparison between studies rather difficult.

Conclusion

p53 overexpression is a late event in colorectal tumorigenesis, being associated with advanced colorectal adenomatous polyps can be used as an ancillary marker for the risk of malignant transformation. Bcl-2 expression is significantly higher in adenoma than carcinoma indicating that bcl-2 may play an important role in the early stage of the adenoma-carcinoma sequence followed by other genetic changes, e.g., p53 accumulation.

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