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Role of Ki-67, P53 and Bcl-2 in Advanced Colorectal Carcinoma (Histopathological and Immunohistochemical Study)

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Abstract: Colorectal cancer accounts for about 10% of all cancers and it is the fourth leading cause of cancer death globally. It is the second leading cause of death from malignancy in the industrialized world. Environmental and genetic factors play an important role in the pathogenesis of colorectal cancer. The aim of the study is to perform a complex morphologic investigation on a series of adenocarcinomas with colonic and rectal location, with comparison of immunohistochemical profiles of ki-67, P53 and bcl-2 markers and evaluation of the predictive value of the investigated markers. This study included 30 archival cases of cancer colon. 14 cases from the right colon, 5 cases from the left colon,7 cases from the sigmoid colon, 1 case from rectosigmoid and 3 cases from the rectum. Immnunohistochemical study of the cases using P53, Ki-67 and bcl-2 antibodies was performed. The immunohistochemical evaluation of Ki-67, P53 and bcl-2 yields refined information on colorectal tumor biology with statistically significant relations with tumor grade and stage. This information could be integrated with the clinical and biologic tumoral framework for good assessment of the studied cases.

Key words: Bcl2 · Colorectal adenocarcinoma, Ki67 · P53 · Stage · Tumor grade

INTRODUCTION

Colorectal cancer accounts for about 10% of all cancers, it is the fourth leading cause of cancer death globally [1] and the second leading cause of death from malignancy in the industrialized world [2]. Environmental and genetic factors play an important role in the pathogenesis of colorectal cancer. Major environmental risk factors for colorectal carcinoma include dietary factors; diets high in red meat, fat, low in fruits and vegetables are associated with an increased risk of colorectal carcinoma [3]. Colorectal cancer develops either sporadically 85% or as a part of a hereditary cancer syndrome in less than 10% or against a background of an inflammatory bowel syndrome [4]. Accumulation of molecular alterations, including K-ras, P53, bcl-2 and adenomatous polyposis coli, contribute to colorectal carcinogenesis [5, 6]. Cellular proliferation is fundamental to maintain tissue homeostasis and is important in oncogenesis. Assessment of tumor cell proliferation may predict tumor behavior. Quantification of cell proliferative activity by Ki-67 is used to assess the relationship between the proliferative index and various pathological findings in colorectal carcinoma including histological grade, type and stage [7-9]. In addition, approximately half of colorectal cancers present mutation in P53 gene with a higher frequency in the distal colon tumors including rectum while a low frequency in proximal tumors [10, 11].

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The aim of the study is to perform a morphologic investigation on a series of adenocarcinomas with colonic and rectal location, comparing immunohistochemical profiles of ki-67, P53 and bcl-2 markers to evaluate the predictive value of the investigated markers.

MATERIALS AND METHODS

This study included 30 archival cases of cancer colon; 14 cases from the right colon, 5 cases from the left colon, 7 cases from the sigmoid colon, 1 case from rectosigmoid and 3 cases from the rectum. Eighteen cases were of glandular type, 12 cases were of mucinous type. As regards the histological grade 16 cases were grade 2, 14 cases were grade 3. According to Dukes' staging; 16 cases were B2, 14 cases were C2. Concerning LN status 15 cases were LN positive and 15 cases were LN negative. The surgical margin was negative in all cases. As regards the markers used they were P53, Ki-67 and bcl-2 antibodies DAKO with streptavidin-biotin method. Semi quantitative analysis of the immunohistochemical reaction for assessment of the proliferative activity, the positivity index Ki-67 index was calculated as number of positive cells from 100 positive and negative cells in microscopic fields investigated with x 40 magnification [12]. Similarly, the expression of p53 was quantified with respect to the percentage of positive nuclei, yielding a P53 index. It is interpreted as having a low, moderate, or marked expression when the percentage of the colored nuclei represented less than 20%, 20% - 50% or more than 50% respectively [13]. Bcl-2 expression was evaluated relative to the percentage of tumor cells with cytoplasmic reaction, the reaction being positive, respectively negative, for the percentage of positive cells, higher, respectively smaller than 5 %.

Statistical Analysis: Data were collected, coded and analyzed by Statistical Package for Social Science (SPSS) software version [14]. The categorical or qualitative data were expressed in terms of percentages. Comparison of the qualitative variables between groups was done using the chi-square test which was used as a test of proportion independence. The categorical or qualitative data as tumor grades, stages and types and the tumor markers as P53, Bcl-2 and Ki-67 expressions were expressed in terms of percentages and cross tabulation. These data were compared by using the non parametric tests, Chi-square and Fisher's Exact test which were used as tests of proportion independence. Two tailed test for significance was used P-value <0.05 was considered statistically significant and P-value <0.01 was considered statistically highly significant [1].

RESULTS

In this study we found no statically significant relationship between Bcl-2 marker and the two groups of the tumor type (P=0.8) however, there was statistically significant differences between bcl-2 marker and the tumor grade and the Dukes' stage (P=0.03) for both. As regards Ki67 expression, no significant difference could be detected between the two groups of the tumor type (P=0.55) although, a highly statically significant difference was detected in Ki-67marker between the two groups of the tumor grade as well as Dukes' stage (P<0.001) (Table 1). For immunostaining expression of the 3 markers in relation to tumor types, stages, grades, lymph node status; there was highly significant difference in P53 immunoexpression between the two groups of Dukes stage (P =0.00), between P53 marker and bcl-2 marker (P<0.001), between P53 marker and Ki-67 marker (P=000).

Table 1: Comparison between Bcl-2 and Ki-67 expression grades as regards tumor grades, stages and types.

		Bcl-2			Ki-67		
Items		+%	+++%	P-value	+%	++%	P-value
Grades	Total number	N=27	N=3		N=13	N=17	
	2	59.3	0	S,P=0.03	100	17.6	HS, P<0.001
	3	40.7	100		0	82.4	
Duke stages	B2	59.3	0	S,P=0.03	100	17.6	HS,P<0.001
	C2	40.7	100		0	82.4	
Types	Glandular	59.3	66.7	NS,P=0.8	53.8	64.7	NS, P=0.55
	Mucinous	40.7	33.3		46.2	35.3	

HS: P value is highly significant at the <0.01 level.

S: P value is significant at the <0.05 level.

NS: P value is non significant at the > 0.05 level.

Table 2: Comparison between Tumor stages, grades, types and Lymph node status as regards tumor markers P53, Bcl-2 and Ki-67 expression.

		P53			Bcl-2			Ki67		
		+ve%	-ve%	P-value	+ve%	-ve%	P-value	+ve%Items	-ve%	P-value
Duke stages	Total number	N=10	N=20		N=16	N=14		N=14	N=16	
	B2	0	80	HS,P<0.001	100	0	HS,P<0.001	0	100	HS, P<0.001
	C2	100	20		0	100		100	0	
LN status	-ve	0	80	HS,P<0.001	100	0	HS,P<0.001	0	100	HS.P<P0.001
	+ve	100	20		0	100		100	0	
Grades	2	0	80	HS,P<0.001	100	0	HS, P<0.001	0	100	HS, P<0.001
	3	100	20		0	100		100	0	
Types	Glandular	50	65	NS, P=0.46	62.5	57.1	NS, P=0.765	57.1	62.5	NS, P=0.765
	Mucinous	50	35		37.5	42.9		42.9	37.5	

HS: P-value is highly significant at the <0.01 level.

NS: P-value is non significant at the >0.05 level.

Table 3: Comparison between tumor markers P53 and Bcl-2 as regards Ki-67 marker expression.

		P53			Bcl-2		
Items		+ve%	-ve%	P-value	+ve%	-ve%	P-value
Ki-67	Total number	N=10	N=20		N=16	N=14	
	+ve	100	20	HS,P<0.001	0	100	HS,P<0.001
	-ve	0	80		100	0	

HS: P-value is highly significant at the <0.01 level.

Table 4: The relation between the tumor markers P53 and Bcl-2 expression.

Items		Bcl-2		
		+ve%	-ve%	P-value
P53	Total number	N=16	N=14	
	+ve	0	71.4	HSP<0001
	-ve	100	28.6	

HS: P-value is highly significant at the <0.01 level.

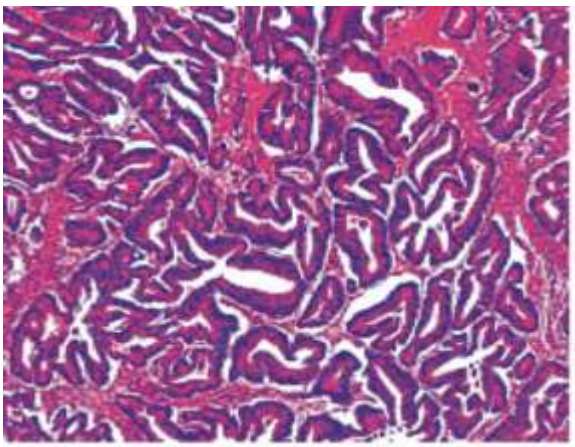

Fig 1: H&E staining in G2 colorectal adenocarcinoma X100.

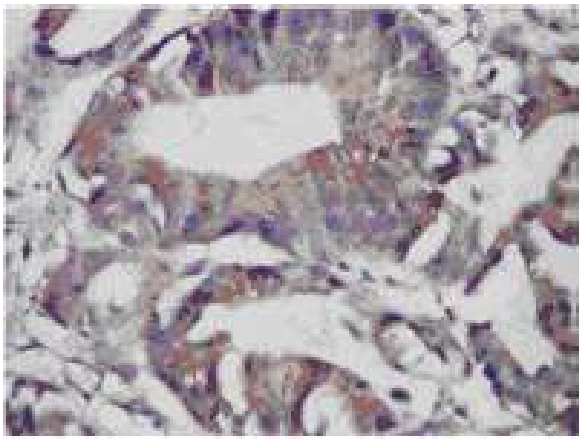

Fig 2: bcl-2staining in G2 colorectal adenocarcinoma X400.

Also a highly statistically significant relationship between Ki-67; marker and Bcl-2 marker (P=0.00). As regards Dukes' stage there was highly statistically significant relationships with bcl-2 and Ki67 marker (P=0.00) for both.

Moreover, highly significant relationship between LN status and the tumor markers (P53, Ki-67 and Bcl-2) (P =0.00) was also notable (Table 2). There was highly significant relationship between tumor grade and the

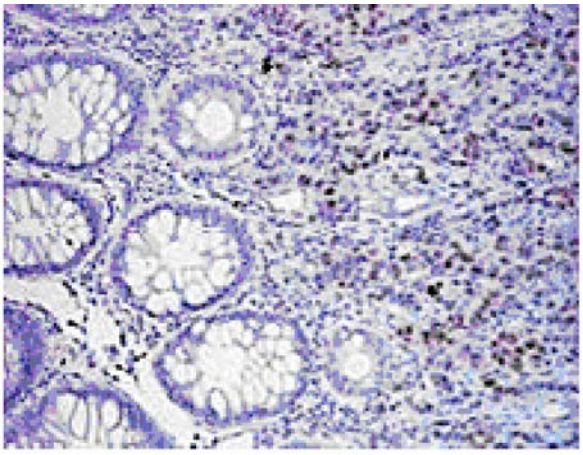

Fig 3: P53 staining in G3 colorectal adenocarcinoma diffuse distribution (right), negative benign cells of mucosal glands (left) X100.

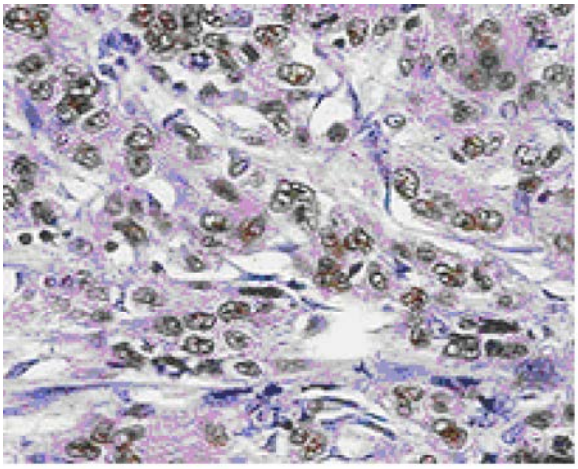

Fig4: Ki-67 staining in G3 colorectal adenocarcinoma X400.

tumor markers (P53, bcl-2 and Ki-67) (P=0.0000) for the three markers. However there was no significant difference or relationship between the tumor types and the tumor markers P53, bcl-2 and Ki-67; (P=0.461), (0.765), (0.765), respectively (Tables 3, 4 and Figs 1, 2, 3, 4).

DISCUSSION

Ki-67 is a nuclear protein that is present in all cell cycle phases except the Go phase and early G1 phase making it a good marker for cell cycling [15, 16], Ki67; has a prognostic and/or predictive value in different tumor types [16]. Recent studies, established the fact that an increased expression of Ki67 indicates a better survival in rectal and recto sigmoid cancer as these tumors have better response to radiotherapy [17-19], as irradiation destroys preferentially the quick dividing cells [20, 21]. There was no statistically significant relation in our studied cases between proliferation activity and the pathological subtypes of the studied tumors, this was in agreement with those reported by Petrisor *et al.* [2] and Clauia *et al.* [13]. However in agreement different studies there was a highly significant relation between Ki67 marker, the tumor grade and stage. This ascertains is in consensus with the literature data, underlying the concept of colorectal cancers as a whole [7, 22]. P53 is a tumor suppressor gene, approximately half of colorectal cancers present mutation in P53 gene with a higher frequency in the distal colon. Our study showed a highly significant difference in P53 marker between the two groups of Dukes' stage and tumor grade (P=0.000). This is in agreement with Claudia *et al.* [13] stating that bcl-2 is a gene involved in the cell cycle regulation by inhibiting apoptosis, anti apoptotic oncoprotein in some cell systems under physiological and neoplastic condition. The role of bcl-2 in colorectal tumor genesis is believed to be in the early stage of carcinogenesis. A decrease in the levels of bcl-2 can lead to cell death by apoptosis while it's over expression protects against programmed cell death. A significant association was found between bcl-2 expression in our studied cases and tumor grade and stage; this was in agreement with Schwandnero *et al.* [14].

CONCLUSION

The evaluation of Ki-67, P53 and bcl-2 yields refined information on colorectal tumor biology as we noticed statistically significant relations with tumor grade and stage. This could be integrated with the clinical and biologic tumoral framework for good assessment of the studied cases.

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