Bcl_2 overexpression in colorectal carcinoma

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Abstract

Background: Colorectal cancer is a major cause of morbidity and mortality worldwide. Prognostic assessment influences the treatment of patients with colorectal cancer, including decisions about adjuvant therapy. Bcl2 over-expression is a genetic event associated with tumor progression and is a prognostic marker of this disease.

Objective: Colorectal carcinoma is a major cause of morbidity and mortality worldwide. Bcl2 overexpression is a genetic event associated with tumor progression and is a prognostic marker for this disease. The aim of this study is to assess the expression of bcl_2 in colorectal carcinoma and its correlation with other clinicopathological parameters.

Methods: From January 2004- January 2005, thirty –five formalin fixed paraffin embedded tissue samples from patients with colorectal carcinoma were included in this study. Four-micrometer tissue sections were obtained for each case, two of them were stained by H&E and the diagnosis had been revised, and the other two were stained immunohistochemically by using avidin biotin alkaline phosphatase method for evaluating bcl_2 expression. The presence of red cytoplasmic staining in less than 25% of tumor cells was considered a positive expression of bcl_2.

Statistical analysis of all the results were performed using Chi square test at level of significance alpha = 0.05 ($P<0.05$) regarded as statistically significant.

Results: Bcl_2 expression was significantly higher in low grade and early stage colorectal carcinoma .Non mucinous colorectal cancer showed more bcl_2 expression than the mucinous type. An inverse correlation was found between bcl_2 expression with the greatest diameter of the tumor and the lymph node status. Bcl_2 expression was correlated neither with the age nor with the sex of the patient and the tumor location.

Conclusion: Bcl_2 over-expression correlates with many variables as low grade colorectal tumor, early stage, non mucinous type, small tumor size and negative lymph node status.

Key words: bcl2, colorectal carcinoma.

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Introduction

Bcl_2 is a proto-oncogene which codes 26 kd protein that blocks apoptosis and rescues cells from apoptosis [(1)]. Reduction in the capacity of apoptotic cell turnover could be an important step in the development of neoplasia[(2)]. Colorectal carcinoma (CRC) is one of the most common malignancies worlds wide. Several clinical, biological, and genetic parameters have been used to assess the prognosis and to help the clinician in optimizing therapies for CRC patients.

Studies indicate that the most important prognostic variable is the tumor stage [(3)]. however , patients who are apparently at the same pathological stage often have adverse outcome in CRC [(4)], although a lack of correlation have been reported. The role of some cellular oncogenes and tumor suppressor genes in clinical aggressiveness of CRC has been also studied. Point mutations of P53 or K-ras tumor genes occur in about 50% of CRC_s and have been associated with poor prognosis. However, available data are again controversial. Thus recent efforts have focused on prediction of the clinical outcome of CRC patients, with the goal of providing a rational approach for planning specific therapy [(5)].

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Bcl_2 protien expression has been studied in many tumors including CRC. The identification of it as a sensitive prognostic marker may allow the use of adjuvant therapy in a subset of patients with worse prognosis with resultant improvement in their survival [6].

$Bcl_{2\ protien}$ expression is mainly observed in cell populations with along life and/or proliferating ability such as duct cells in exocrine glands, basal keratinocytes, cells at the bottom of colon crypts, and neurons [7].

The bcl2 produces its effect not by increasing the rate of cell proliferation but by reducing the rate of cell death and thus may contribute to tumorigenesis by keeping the cells alive and lending them vulnerable for further accumulation of gene abnormalities [8].

Although **Bcl_2** expression has been shown in **colorectal** neoplasia, their possible impact on the biologic behavior of the **colorectal** carcinomas is still controversial [9].

In the present study we evaluated the expression of bcl_2 in colorectal carcinoma and its correlation with other clinicopathological parameters.

Materials and Methods

Thirty-five cases with **colorectal** carcinomas that had undergone colectomy were included. The clinicopathologic parameters like age and sex of the patient, tumor grade, tumor stage, tumor greatest diameter, anatomic location, histopathologic type and lymph node status were evaluated as **prognostic** indicators. Histological classification of the tumor was done according to the WHO system. The anatomic localizations were grouped as proximal colon meaning the distance from the cecum up to the splenic flexure and as distal colon beginning from the descending colon to the rectum and rectal tumors.

Four sections (with four micrometer thickness) from formalin fixed, paraffin embedded tissues were obtained, two of them were stained by H&E and revised, and the other two were stained immunohistochemically with anti **Bcl_2** (Chemicon) monoclonal antibody. The IHC select ® immunophosphatase secondary detection system uses biotin avidin alkaline phosohatase complexed antibodies to detect antimouse IgG in the primary antibody. The sample is then incubated with the streptavidin alkaline phosphatase solution, which binds to the biotin labeled secondary antibody present on the tissue. The chromogenic development reagent, the Red violet is then added and reacts with alkaline phosohatase attached to streptavidin biotin antibody complex. The alkaline phosphatase activity on the chromogenic substrate results in the deposit of the red insoluble precipitates at those antigenic sites containing the specific epitopes recognized by the primary antibody. The sections were counter-stained with hematoxylin. The presence of red cytoplasmic reaction at the site of the target antigen is indicative of positive reactivity. Counter stain will be dark blue coloration of the cell nuclei.

The intensity of the immunostaining was evaluated by dividing the staining reaction in four groups [10]:

Weak cytoplasmic staining intensity

Moderate cytoplasmic staining intensity

Strong cytoplasmic staining intensity

Very strong cytoplasmic staining intensity

The quality of the immunostaining was evaluated as follows

0 no positive immunostaining

1 less than 25% of tumor cells showing cytoplasmic positivity

2 25-50% of tumor cells showing cytoplasmic positivity

3 50-75% of tumor cells showing cytoplasmic positivity
4 >75% of tumor cells showing cytoplasmic positivity
A combined score for immunostaining based on both qualitative and quantitative immunostaining was composed by adding both qualitative and quantitative score, which was then divided into 5 main groups:
*No immunostaining score 0
*Weak immunostaining score 1-2
*Moderate immunostaining score 3-4
*Strong immunostaining score 5-6
*Very strong immunostaining score 7-8

Lymphocytes in the stroma and lamina propria were consistently positive and served as internal control and regarded as very strongly positive according to above scoring system [10] (Figure 1). Bcl_2 expression was also positive in the basal cells of the normal colonic crypts (Figure 2).
Statistical analysis was performed using the chi-square test. At level of significance alpha =0.05 and $p< 0.05$ regarded as statistically significant.

Results

Regarding the sex of the patient, bcl_2 expression was more frequently positive in female cases (55.6%) than male cases (25%) but this was not statistically significant (p=0.129) (Table 1). Concerning the age of the patient, bcl_2 expression was more frequently positive in age group more than 40 years (77.8%) than age group ≤40 years (50%) but the results were also statistically not significant (p=0.127) (Table 1). Considering tumor grade, bcl_2 was expressed in all the well-differentiated Adenocarcinoma (100%) and in the moderately differentiated Adenocarcinoma it was (82.6%) and that is more than the poorly differentiated Adenocarcinoma (33.3%). The results were statistically significant (p=0.013), (Figure 3, 4) (Table 2). Bcl_2 expression was found to be positive in early stages of colorectal carcinoma being (100%) in stage A, (86%)positive in stage B while in stage C it was (66%) and (33%) in stage D (Table 3) . although Bcl_2 expression was not significantly correlated to the tumor stage when comparing its expression among all the stages together (p=0.0999), but when taking bcl_2 expression in stage A and B each apart versus stage D , the results were statistically significant (p=0.035 ,p=0.043) respectively (Table 4,5). Regarding the tumor type, non mucinous adenocarcinoma expressed bcl_2 (77.8%) more than the mucinous type (22.2%) and the results were statistically significant (p=0.031) (fig5). There was no correlation between the tumor location and bcl_2 expression (p=0.651), although the distal colon showed more bcl_2 positivity (92.6%) than the rectum (7.4%) (Table 6). The extent of bcl_2 expression by tumor cells decreased significantly with respect to increasing tumor greatest diameter (p=0.036), so (66.7%) of the tumors of ≤5 cm in greatest diameter were positive for bcl_2 compared to (33.3%) of tumors >5cm in greatest diameter (Table 7).
An inverse correlation was found between bcl_2 expression and the lymph node status. (66.7%) of the tumors with negative lymph node metastasis were positive for bcl_2 compared to (33.3%) of the tumors with positive lymph node metastasis and the results were statistically significant (p=0.036). (Figure 6)

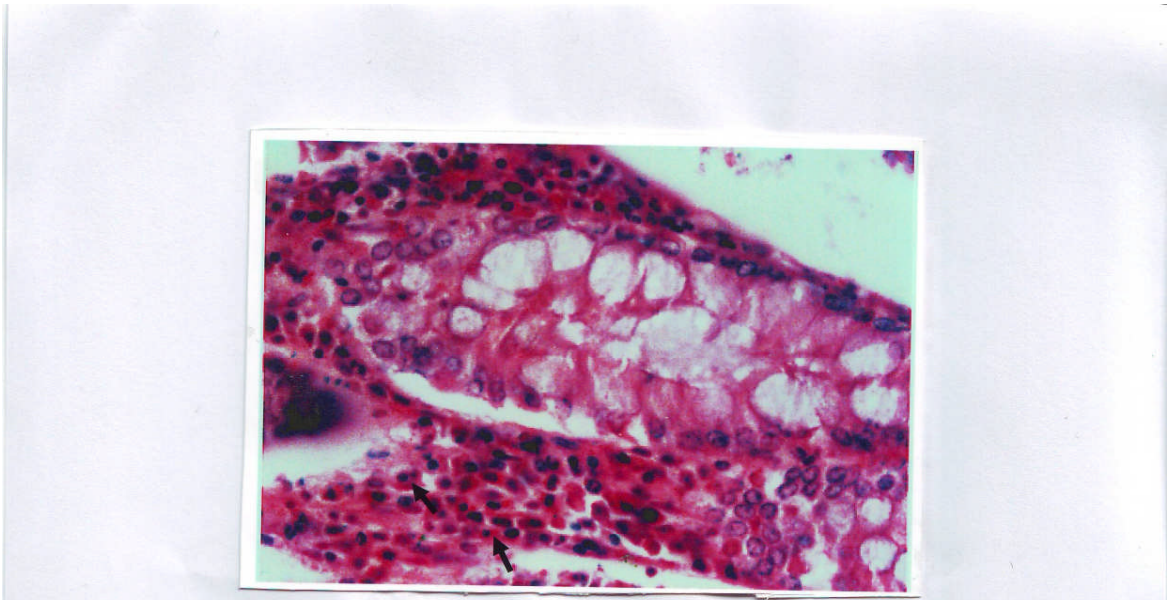

Figure 1: Lymphocytes (arrow) as internal control for bcl$_2$ immunohistochemical staining in a section of colonic mucosal glands (alkaline phosphatase method) (red cytoplasmic staining) 400.

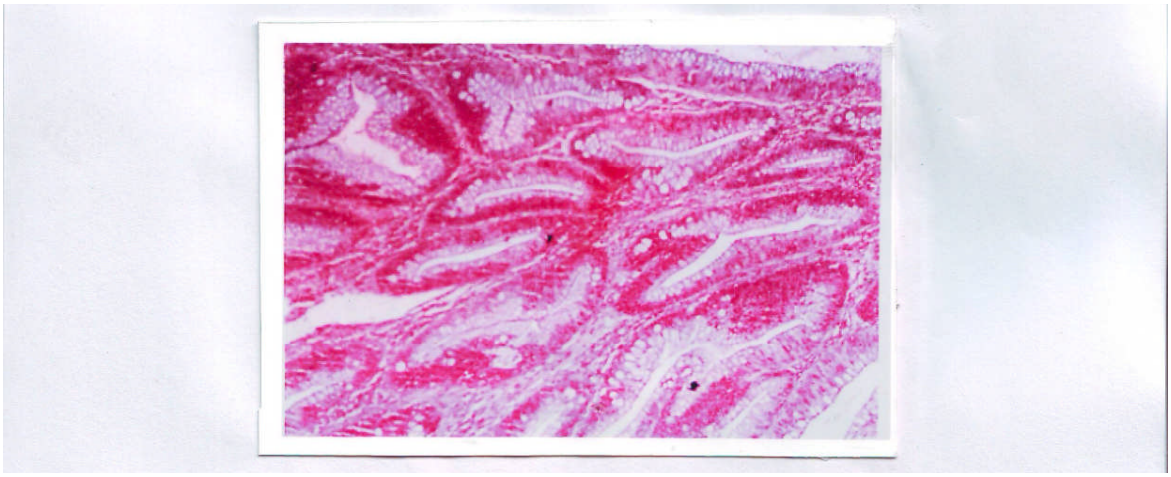

Figure 2: Normal colonic glands showing positive bcl$_2$ expression (alkaline phosphatase method) as red cytoplasmic stain 100.

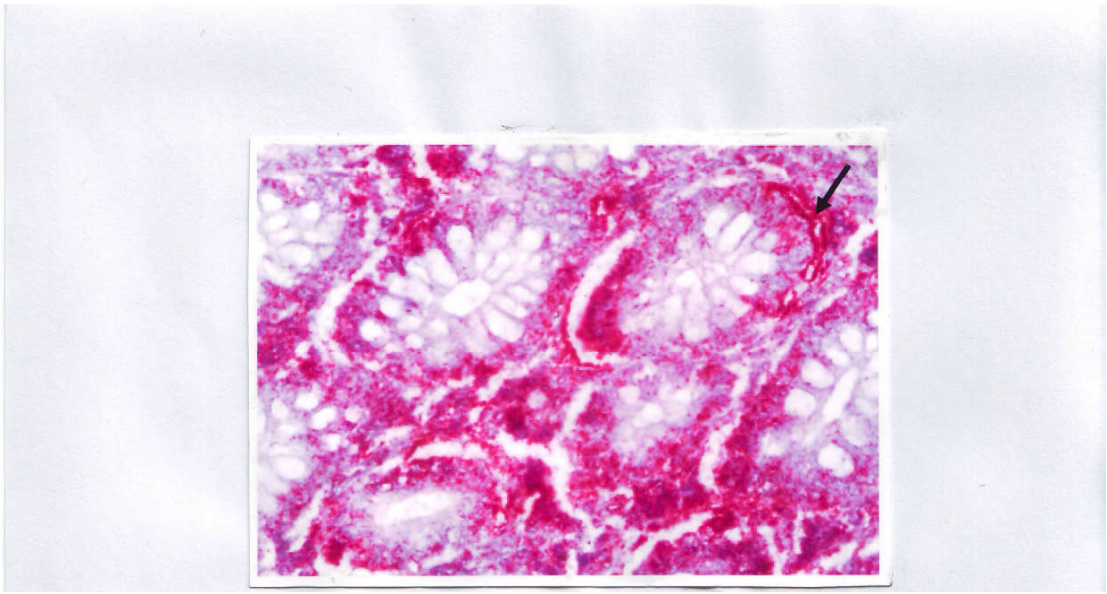

Figure 3: well differentiated colorectal adenocarcinoma showing positive bcl_2 immunostaining (alkaline phosphatase method) (strong reaction) 400.

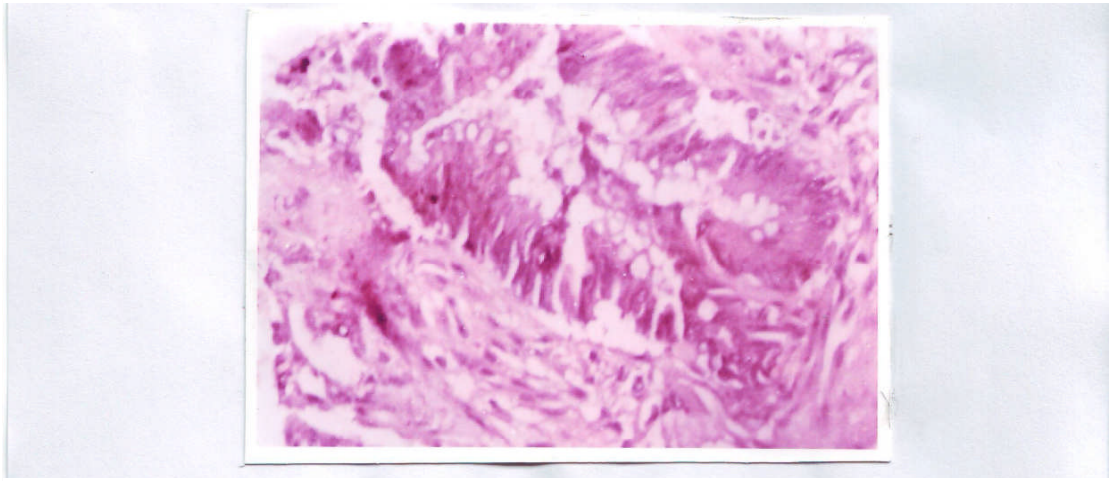

Figure 4: Poorly differentiated colorectal adenocarcinoma showing negative bcl_2 immunostaining (alkaline phosphatase method) 400.

Table 1: Bcl_2 immunostaining in relation to age and sex of the patient

		Bcl_2 immunostaining		P- value
		Negative	Positive	
Sex	Female Male	44.4% 75.0%	55.6% 25.0%	0.125
Age	≤40 y >40 y	50% 22.2%	50% 77.8%	0.127

Table 2: correlation between Bcl_2 positivity and tumor grade in colorectal carcinoma.

		Tumor grade			Total
		well	moderate	poor	
negative	No.	0	4	4	8
	%	0	17.4%	66.7%	22.9%
positive	No.	6	19	2	27
	%	100.0%	82.6%	33.3%	77.1%
Total	No.	6	23	6	35
	%	100.0%	100.0%	100.0%	100.0%

Chi-Square	Value	df	P-value
	8.698	2	.013

P<0.05 significant

Table 3: correlation between Bcl_2 positivity and tumor stage in colorectal carcinoma.

		Tumor stage				Total
		A	B	C	D	
negative	No.		2	4	2	8
	%		25.0%	50.0%	25.0%	100.0%
positive	No.	5	13	8	1	27
	%	18.5%	48.1%	26.9%	3.7%	100.0%
Total	No.	5	15	12	3	35
	%	14.3%	42.9%	34.3%	8.6%	100.0%

Chi-Square	Value	df	P-value
	6.265	3	.099

Table 4: Bcl$_2$ expression in stage A versus stage D

		STAGE		Total
		Stage A	Stage D	
positive	No.	5	1	6
	%	83.3%	16.7%	100.0%
negative	No.		2	2
	%		100.0%	100.0%
Total	No.	5	3	8
	%	62.5%	37.5%	100.0%

Chi-Square	Value	df	P-value
	4.444	1	.035

P<0.05 significant

Table 5: Bcl$_2$ expression in stage B versus stage D

		STAGE		Total
		Stage B	Stage D	
positive	No.	13	1	14
	%	86.7%	13.3	100.0%
negative	No.	2	2	4
	%	66.7%	33.3%	100.0%
Total	No.	15	3	18
	%	83.3%	16.7%	100.0%

Chi-Square	Value	df	P-value
	.720	1	.043

P<0.05 significant

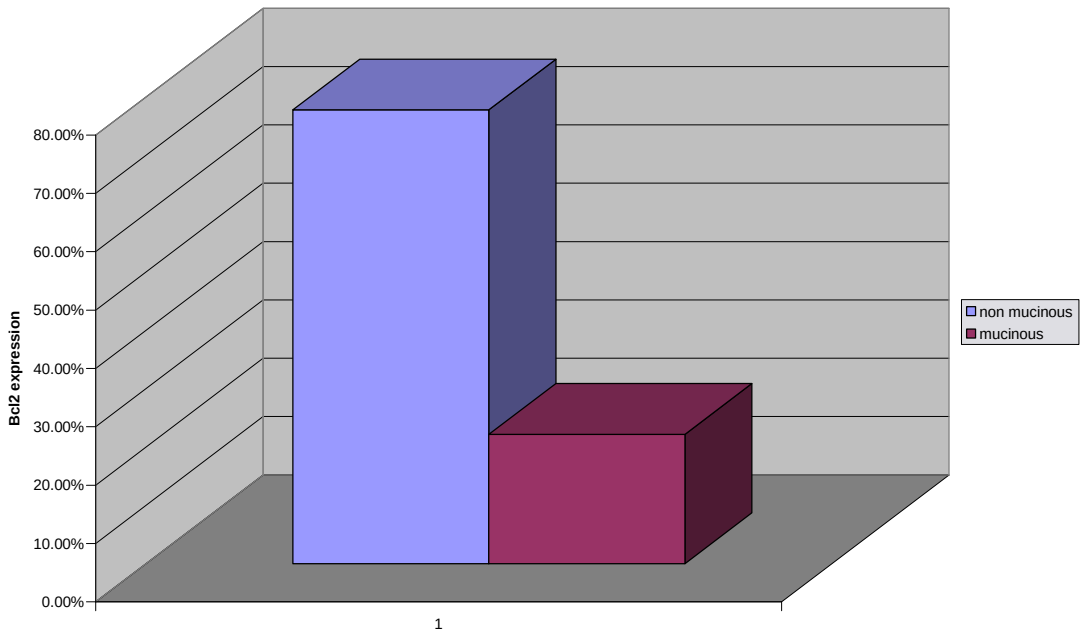

Figure 5: Bar chart of correlation between Bcl$_2$ positivity and tumor type

Table 6: Correlation between Bcl$_2$ positivity and tumor anatomic location in colorectal carcinoma.

		Tumor location		Total
		Distal colon	Rectum	
negative	No.	7	1	8
	%	87.5%	12.5%	100.0%
positive	No.	25	2	27
	%	92.6%	7.4%	100.0%
Total	No.	32	3	35
	%	91.4%	8.6%	100.0%

Chi-Square	Value	df	P-value
	.204	1	.651

Table 7: Correlation between Bcl$_2$ positivity and tumor greatest diameter in **colorectal carcinoma.**

		Tumor size		Total
		≤5 cm	>5cm	
negative	No.	2	6	8
	%	25.0%	75.0%	100.0%
positive	No.	18	9	27
	%	66.7%	33.3%	100.0%
Total	No.	20	15	35
	%	57.1%	42.9%	100.0%

Chi-Square	Value	df	P-value
	4.375	1	.036

P<0.05 significant

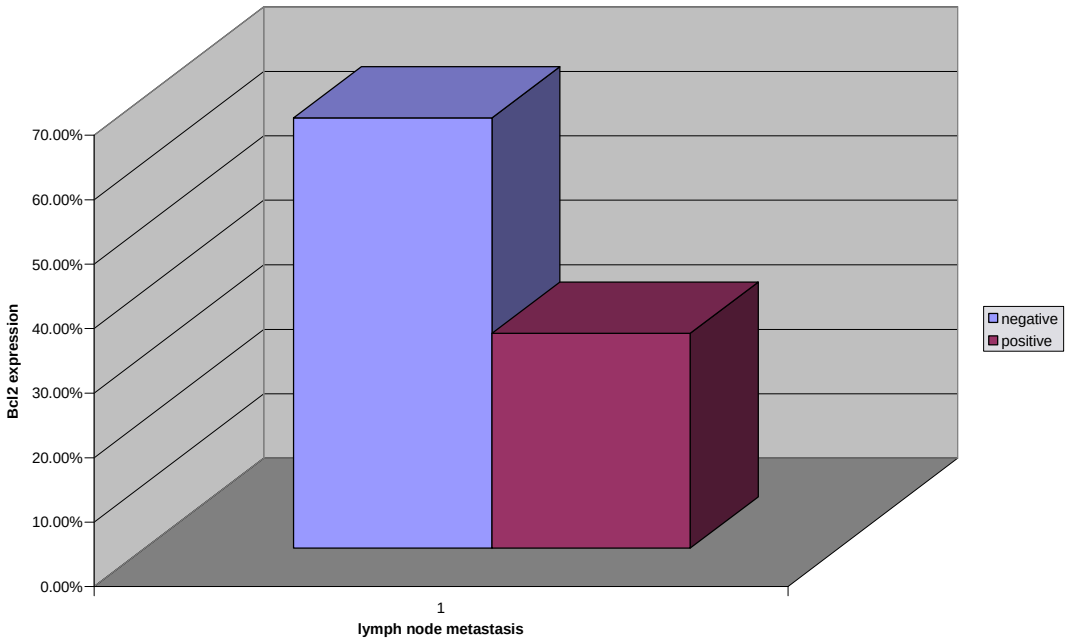

Figure 6: Bar chart of correlation between Bcl$_2$ positivity and lymph node status.

Discussion

In the present study, bcl$_2$ expression was a cytoplasmic staining clearly observed at the base of colonic crypts, as well as in the normal tissue close to the tumor margin.

This matches the observation of Bosari et al., 1995 [11], Barreton et al, 1996 [12], Krajweska et al, 1996[13], Palazzo et al., 1997 [14], and Giatromanolaki et al, 1999 [15].

This may suggest that altered bcl2 expression precedes the development of morphologically recognizable neoplasia.

Regarding bcl$_2$ expression in relation to the age and sex of patients, Bcl$_2$ was found to be more frequently expressed among females and patients of an age group less than 40 years. This agree with the results of Leahy et al., 1999 [16], Tollenaar et al, 1998 [17], Ogura et al, [18], and Ouyang et al, 2005 [19].

The present study revealed that bcl$_2$ expression was significantly correlated with the tumor grade being more frequently expressed in low grade colorectal carcinoma. These results are in agreement with the results of Leahy et al., 1999 [16].

Considering the tumor stage , bcl$_2$ expression was observed in early stages of colorectal adenocarcinoma, although the results were statistically not significant , they agree with the results of Husain et al, 1999 [20] . When we compared bcl$_2$ expression in stage A and stage B each apart versus stage D, a statistically significant correlation was found between high bcl$_2$ expression and early stage tumor. Similarly, Huang et al, 2002 [21], Manne et al, 1997 [22] and Kalklamanis et al, 1998 [23] found a significant correlation of bcl$_2$ expression with the tumor stage. A study made by Meterissian et al, 2001[24] specifically considering bcl$_2$ expression in stage B colorectal adenocarcinoma found that enhanced bcl$_2$ expression in this stage is associated with improved survival. Thus, patients whose tumors do not express bcl$_2$ should be considered for adjuvant therapy.

Taken into consideration the tumor type, bcl$_2$ expression was significantly

higher in non mucinous tumors compared with the mucinous type and this reflects the well known aggressive behavior and bad prognosis associated with the mucinous type. These results are in disagreement with the results of Dursun et al., 2001[25] who found a significant relation of bcl$_2$ expression with the mucinous type tumor and this may be explained by small sample size in the present study. These results are expected since most of the mucinous tumors which were negative for bcl$_2$ expression were associated with high grade and late stage malignancy.

Taking into account tumor greatest diameter , the extent of bcl$_2$ expression by tumor cells decreased significantly with respect to increasing tumor greatest diameter .This result is in agreement with Ofner et al., 1995[26] . This can be explained by the fact that large tumors may be related to other bad prognostic parameters as poor differentiation, advanced stage, high grade, and positive lymph node status.

Regarding the tumor location, none of the cases in the present study were n the proximal colon, this may be due to the fact that tumors in the proximal colon usually present late in the course of the disease and they attain a large size before clinical detection in addition to that they are far from digital and proctosigmoidoscopic examination and they may be beyond surgical treatment on discovery. The relationship between bcl$_2$ expression in the distal colon and the rectum was not statistically significant .These results are in agreement with others as Dursun et al., 2001[25] , Tollenaar et al, 1998 [17] , Husain et al, 1999 [20] and Huang et al, 2002 [21] .

There was a negative correlation between bcl$_2$ expression and lymph node status. These results are in concordance with the results of Dursun et al, 2001[25] and Goussia et al, 2000[27], that bcl$_2$ expression was more in lymph node negative tumors.

Bcl2 expression in colorectal carcinoma was associated with better clinical course especially when p53 expression was absent suggesting that neoplastic transformation related to inhibition of apoptosis results in less aggressive malignancies than those dependent on other oncogenes as p53[26] . An inverse relation between bcl$_2$ and p53 has been observed in other malignancies suggesting that these proteins may interact through opposite mechanisms: inhibition of apoptosis (bcl$_2$) and promotion of apoptosis (p53) [19] .

Conclusion

Bcl2 expression in colorectal carcinoma is correlated with low grade tumor, early tumor stage, non mucinous type, small tumor size and negative lymph node status.

References

1. Steinert R, Buschmann T, Van-der-Linden M, Fels LM, Lippert H, Reymond MA**:** The role of proteomics in the diagnosis and outcome prediction in colorectal cancer. Technol-Cancer Res. Treat.2002; 4:297-304.

2. Garner EW, Ignatenko NA, Besselsen DG**:** Preclinical models for chemoprevention of colon cancer. Recent Results Cancer Res. Treat.2003; 163:58-71.

3. Grady WM**:** Genetic testing for high risk colon cancer patints. Gastroenterol. 2003; 124:1574-94.

4. Megan M, Garrity L J, Burgart MR, Mahoney HE, Salim M, Wiesenfeld JE, Krook JC**:** Prognostic value of proliferation, apoptosis,defective DNA mismatch repair and p53 overexpression in patients with resected Dukes' B2 or C colon cancer.J.Clin Oncol. 2004; 22(9): 1572-1582.

5. Compton CC, Taylor CK, Welton M, willet C, Fielding LP, Burgart LJ, Hamilton SR, Hammond ME, Henson DE, Hutter RV, Nagle RB, Nielsen ML, Sargent DJ**:** Prognostic factors in colorectal cancer. College of American Pathologist consensus statement, 1999.Arch Path Lab Med. 2000; 124:979-94.

6. Berczi C, Bocsi J, Bartha I, Math J, Balazs G: Prognostic value of DNA ploidy status in patients with rectal cancer. Anticancer Res.2002; 22:3737-41.

7. Lu QL, Poulson R, and Wong L, Hangby A.M: Bcl-2 expression in adult embryonic nonhemopoietic tissues. J. Pathol.1993; 169(4): 431-7.
8. Xu AG, Li SG, Lin JH, Gan AH: Function of apoptosis and expression of bcl-2, P53 and c-myc in the development of gastric cancer. World J gastroenterol 2001; 7(3): 403-406.
9. Matsuda K, Masaki T, Watanobe T, Kitawama J, Nagawa H, Muto T, Ajioka Y. Clinical P53 protein expression in colorectal carcinoma . Japanese Journal of Clinical Oncology 2000; 30:89-94.
10. Soini Y, Kinnula V, Kaarteenaho-Wiik R, Kurttila E, Kaija L, Paä P: Apoptosis regulating proteins bcl-2, Mcl-2, bcl-x and bax in malignant Mesothelioma. Clinical Cancer Res.1999; 5(11): 3508-15.
11. Bosari S, Monechini L, Graziani D, Lee A.KC, Murray JJ, Coggi G, Viale G: Bcl-2 oncoprotein in colorectal hyperplastic adenomas and adenocarcinomas. Hum Pathol 1995; 26:534-540.
12. Barreton GB, Diebold J, Chrisroforis G: Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomas and carcinomas. Cancer 1996; 77:255-264.
13. Krajewska M, Moss SF, Krajewsky S, Song K, Holt PR, Reed JC: Elevated expression of Bcl-2 and reduced Bak in primary colorectal adenocarcinoma. Cancer Res 1996; 56:2422-2427.
14. Palazzo JP, Kalka NJ, Grasso L. The role of P53, P21 and bcl-2 in radioresistant colorectal carcinoma. Hum Pathol 1997; 28:1189-1195.
15. Giatromanolaki A, Stathopoulos GP, Tsiobanou E, Papaclimitrium C, Georgonlies V, Gatter KC, Harris AL, Koukourakis MI. Combined role of tumor angiogenesis, bcl-2 and P53 expression in the prognosis of patients with colorectal carcinoma. Cancer 1999; 86(8): 1421-30.
16. Leahy DT, Salman R, Mulcary II, Sheahen K, O'Donoghue DP, Parfrey NA: Bcl-2 protein expression is associated with better prognosis in colorectal cancer. Histopathology 1999; 35(4): 360.
17. Tollenaar RA, Van Krieken JH, Van Slooten HJ, Bruinvels DJ, Nelemans KM, Van Den Broek LJ, Hermans J, Van dierendonck JH: Immunohistochemical detection of P53 and bcl-2 in colorectal carcinoma: no evidence of prognostic significance. Br J Cancer 1998; 77(11): 1842-7.
18. Ogura E, Senzaki H, Yamamoto D, Yoshida R, Takada H, Hioki K, Tsubura A.: Prognostic significance of bcl-2, bcl-XL/S, bax and Bak expressions in colorectal carcinoma. Oncol Rep 1999; 6(21): 365-9.
19. Quyang M, Zhang G.Y, Xu MH: Expression of PGE, bcl-2 and Bax in a genesis of colorectal mocosa. World Chin Digestol 2005; 13(11): 1305-1309.
20. Husain AS, Jarkson H, Khatib GH, Banerjee M: Correlation of bcl-2 oncoprotein immunohistochemical expression with proliferation index and histopathologic parameters in colorectal neoplasia. Pathol Oncol Res 1999; 5(4): 273-279.
21. Huang W, Yao X: The expression of the apoptosis suppressive gene bcl-2 in the tissue of colon carcinoma. Technolog Med Sci 2002; 22(3): 218-20. (Medline).
22. Manne Y, Myers RB, Moron C, Poczatek RB, Dillard S, Weiss H, Brown D,Srivastava S, Grizzle WE: prognostic significance of bcl-2 expression and P53 nuclear accumulation in colorectal carcinoma. Int J Cancer 1997; 74(3): 346-58.
23. Kaklamanis L, Savage A, Whitehouse R, Doussi-Anagnostopoulou I, Biddolph S, Tsiotos P, Mortensen N, Gatter KC, Harris AL: Bcl-2 protein expression associated with P53 and prognosis in colorectal cancer. Br J Cancer 1998; 77(11): 1864-9.
24. Meterissian SH, Kontagiannea, Al-Sowaid M, Linjawi A, Halwani F, Jamison B, Edwardes M: Bcl-2 is a useful prognostic marker in Dukes' B colon cancer. Ann Sur Oncol 2001; 8(6): 533-537.
25. Dursun A, Poyroz A, Süer O, Sezer C, Akyol G: Expression of bcl-2 and c-ErbB-2 in colorectal neoplasia. Pathol Oncol Res 2001; 7(1):24-27.
26. Ofner D, Rieheann K, Maier H, Riedmann B, Nehoda H, Totsch M, Bocker W, Jasani B, Schmid KW: Immunohistochemically detectable bcl-2 expression in colorectal carcinoma: correlation with tumor stage and patient survival. Br J Cancer 1995; 72(4): 981-5.
27. Goussia AC, Ioachim E, Agnantis NJ, Mahera M, Tsianos EV: Bcl-2 expression in colorectal tumors correlation with P53, mdm-2, Rb protein and proliferative indices. Histol Histopathol 2000; 15(3): 667-72.