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Bcl-2 Analysis in Colorectal Carcinoma- An Immunohistochemical Study

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Abstract **-** ***Introduction:*** *Bcl-2 is an oncogene which inhibits apoptosis. The overexpression of Bcl-2 may play an important role in predicting the prognosis of colorectal cancer.* ***Aim:*** *To evaluate the expression of Bcl-2 oncoprotein with clinicopathological variables in colorectal carcinoma.* ***Materials and Methods:*** *Thirty resected specimens of colorectal carcinomas which included ten cases each of carcinomas in stages I, II and III were selected for the study. The Bcl-2 immunostaining pattern was correlated with the patient's age, gender, site, size, grade, nodal status and pathological stage.* ***Results:*** *The age of the patients ranged from 18 to 75 years with 12 males and 18 females. The sizes of the tumours ranged from 1.5 to 9cms.Twelve cases were right sided and 18 cases were left sided. Twelve cases were low grade and 18 were high grade. Lymph nodes were positive in ten out of the thirty cases. Bcl-2 was positive in 56% of the cases. The Bcl-2 expression was 65% in stages I and II carcinomas and only 40% in stage III carcinomas. However, there was no statistically significant correlation with the clinicopathological variables.* ***Conclusion:*** *Analysis of Bcl-2 protein expression may have a potential use in the management of colorectal cancer.*

Keywords: ***Bcl-2, Colorectal cancer.***

Introduction

Colorectal cancer is a major cause of morbidity and mortality worldwide, being the third most common cancer in men and the second most common in women.

Recently, in India, there has been a disproportionate rise in the incidence of rectal cancers.[1]

Colonic carcinogenesis is a multi-step process; being characterised by an accumulation of diverse genetic alterations that cause disruption of the control of normal cell growth mechanisms.[2] Regulation of balance between cell proliferation and cell death are very critical for normal and neoplastic tissue homeostasis.[3]

The Bcl-2 family of proteins play a central role in regulating cell death, of which the Bcl-2 gene is an important component.[4]

The Bcl-2 gene is located at chromosome 18q21 and is present at the mitochondrial level, the endoplasmic reticulum and the nuclear envelope. It codes for an oncoprotein which serves as a programmed cell death inhibitor (anti-apoptotic), thereby increasing the life span of the cell[2,3]

In the normal colon, Bcl-2 has been found to be positive in the cells at the base of the crypts.[2] It has been reported that the expression of Bcl-2 is both frequent and abnormal, early in the carcinogenesis of colorectal carcinomas.[5]

The aim of the present study was to evaluate the expression of Bcl-2 oncoprotein in colorectal carcinoma and the association with clinicopathological variables.

Materials and Methods

Thirty resected specimens of colorectal carcinomas were selected for the study. Histological analysis of tumour tissue samples were processed after formalin-fixing and paraffin-embedding. Ten cases each of carcinomas in stages I, II and III were included.
Immunohistochemical staining with Bcl-2 was done using the anti-bcl-2 antibody (EP-36, rabbit monoclonal) at 1:50 dilution (Pathnsitu).As a part of the secondary kit the PolyExcel HRP/DAB detection system was used.

Cytoplasmic staining of Bcl-2 was semi-quantitatively assessed separately by two pathologists and expressed as a percentage of positive tumour cells. Tumours displaying more than 25% cytoplasmic staining with Bcl2 was taken as positive. Those showing negative immunodetection as well as less than 25% staining as were considered negative.

Statistical Analysis

Data was analysed using SPSS version 22. The Chi Square test was performed and a p value of less than 0.05 was considered significant.

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Results

The expression of Bcl-2 was correlated with clinicopathological variables such as age, gender, site, size, differentiation, nodal status and pTNMstage (AJCC-pathological TNM stage) the findings are shown in Table 1.

Out of thirty cases Bcl2 was positive in 17 cases (56%) and was negative in 13 cases (44%). The age of the patients ranged from 18 to 75 years with 12 males and 18 females. The sizes of the tumours ranged from 1.5 to 9cms.Twelve cases were right sided and 18 cases were left sided. Twelve cases were low grade (well differentiated) and 18 were high grade (moderate and poorly differentiated). Lymph nodes were positive in ten out of the thirty cases.67% cases had negative nodal status out of which majority (65%) showed positive Bcl 2 staining.

40% of the cases were well differentiated adenocarcinomas, out of which 58% showed positive Bcl2 staining.The moderate and poorly differentiated cancers constituted 60% of the total cases and showed 55% Bcl-2 positivity.

67% cases were low stage i.e stage I& II (Figures 1.a, 1.b, 2.a, 2.b), whereas 33% cases where high stage i.e stage III. (Figures 3.a & 3.b).

The Bcl-2 expression was 65% in stages I and II carcinomas and only 40% in stage III carcinomas. However, there was no statistically significant correlation with the clinicopathological variables.

Table 1 showing the clinicopathological features and Bcl-2 expression

Variables	Bcl2 positive	Bcl 2 –ve negative
Age		
<_50 years	6	6
>50 years	11	7
Gender	6	6
Male	11	7
Female		
Size of tumour	3	7
<_4 cm	14	6
>4cm		
Site of tumour	10	8
Left colon	7	5
Right colon		
Differentiation	7	5
Well	10	8
Moderate and poor		
Nodal status	13	7
Negative	4	6
Positive		
Stage (pTNM)	13	7
Low stage(stage I&II)	4	6
High stage(stage III)		

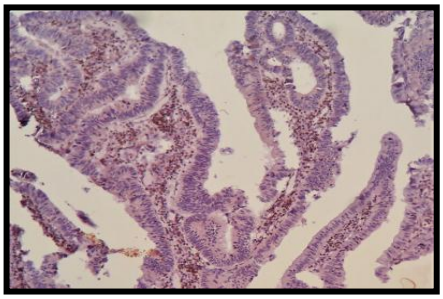

Figure 1.a

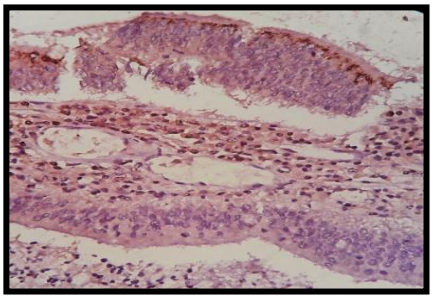

Figure 1.b

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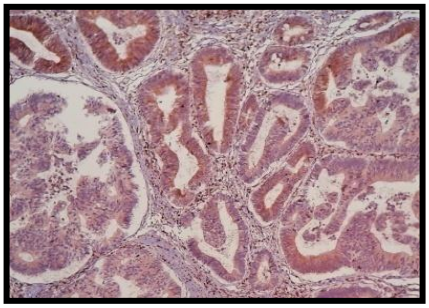

Figure 2.a

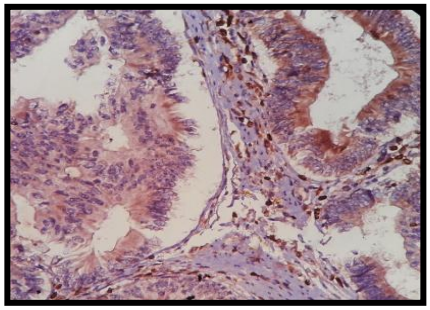

Figure 2.b

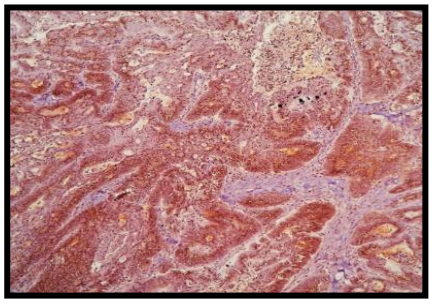

Figure 3.a

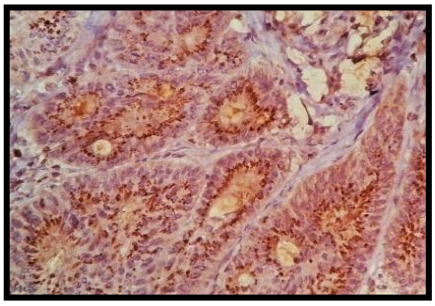

Figure 3.b

Figure 1.a Bcl2, showing less than 25% positive staining **(10x)**; **Figure1.b**. Bcl2, showing less than 25% positive staining **(40x)**; **Figure2.a**.Bcl2, showing 25% to 50% positive staining **(10x); Figure 2.b**.Bcl2, showing 25% to 50% positive staining**(40x)** ; **Figure 3.a.**Bcl2, showing more than 50% positive staining**(10x)**; **Figure 3.b**.Bcl2, showing more than 50% positive staining **(40x).**

Discussion

Bcl 2 is a marker of high interest and research is ongoing to determine its role in carcinogenesis as well as tumour cell proliferation in a variety of cancers. It was initially discovered in B-cell lymphomas, associated with translocation(t14;18) but is also found in other tumours such as breast cancer, follicular carcinoma of the thyroid, hepatocellular carcinoma, neuroblastoma and non small- cell lung cancer.[2]

Previous studies have shown that Bcl-2 expression seems to correlate with favourable clinicopathological parameters and is expressed in approximately 25-50% of colorectal adenocarcinomas.[2,6,7,8] Also reported are similar results of Bcl2 expression in breast cancer[9] and non small cell lung carcinomas.[10]

In our study, Bcl-2 expression was more in the low stage carcinomas(stages I&II-65%) as compared to the higher stage (stage III- 40%); suggesting a possible role of Bcl-2 in the early part of tumorigenesis.Bcl-2 also showed a greater percentage of positive expression in lymph node negative cases (65%) as compared to lymph node positive cases (30%). Well differentiated adenocarcinomas showed only a slightly increased percentage of Bcl-2 positivity (58%) when compared to the moderate and poorly differentiated adenocarcinomas (55%). These findings point towards a favourable role of Bcl 2 in colorectal cancer.

There was, however no statistically significant correlation with any of the clinicopathological parameters with Bcl-2 expression probably due to the small number of cases

Bcl-2 is normally expressed in the bases of the crypts which correspond to the progenitor cells, where it protects the regenerative compartment from cell death.[2,11,12]

The mechanism of Bcl-2 in cells without t (14;18) translocation is still unknown but it could be due to a disturbance in the post-translational regulation of Bcl-2 in carcinomas.[11]

The role of Bcl-2 in the development of colorectal carcinomas is uncertain but is believed to be in the early stages of carcinogenesis.[12,13]

Sinicrope et al. (1995) reported the first data concerning Bcl-2 expression in colorectal carcinomas, and found a significant correlation of relapse free survival rates with higher fraction of Bcl-2 positive tumour cells.[14]

One study found a statistically significant correlation with reducing Bcl-2 expression and increasing stage and poorer clinical outcome.[15] Other studies have reported increased proportion of Bcl-2 expression in adenomas than in carcinomas, again indicating the role of Bcl-2 early on in neoplastic transformation.[16,17]

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Bcl-2 has been found to be significantly associated with low proliferative activity; therefore could be associated with slower tumour growth rates and thereby a favourable prognosis.[14]

One study showed that the rates of apoptosis increased during neoplastic transformation, with the highest apoptotic index being found in a colon carcinoma.[18] This could explain the decreasing expression of an anti-apoptotic oncogene such as Bcl-2 with increasing stage, in our study.

Conclusion

In conclusion, this study shows increased percentage of positive Bcl-2 expression in the lower stages as well as lymph node negative cases of colorectal carcinoma. We believe that Bcl-2 plays a key role in early colorectal carcinogenesis. However, larger studies with prospective evaluation of the same are needed to confirm the contribution of Bcl-2 in colorectal carcinoma.

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