Original Article

Bcl-2 Expression in Colorectal Carcinoma and its Correlation with Clinicopathological Parameters

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Abstract

Background: Bcl-2 gene is a 26-kD protein that blocks apoptosis and inhibits programmed cell death. Its mutation is frequently detected genetic alteration, in human cancers. Its expression can easily be detected by immunohistochemistry (IHC). **Objective:** The objective was to estimate the frequency of Bcl-2 expression in colorectal carcinoma and its correlation with clinicopathologic parameters. **Materials and Methods:** The overexpression of Bcl-2 was studied in 112 paraffin-preserved colorectal carcinoma samples using E-17 clone antibody; Biogenex. The number of cells stained was scored semiquantitatively as none (no immunoreactive cells detectable), weak (1+, <5%), moderate (2++, 5%–50%), and intense (3+++, >50%). All tumors showing Bcl-2 immunoreactivity (at least +) were considered positive. The correlation between Bcl-2 protein expression and clinicopathological parameters was evaluated using Chi-square analysis. **Results:** Bcl-2 staining was positive in 45 of 112 cases. Of these, 15 cases were weak positive (score 1), 17 cases were moderately positive (score 2), and 13 cases were intensely positive (score 3) for Bcl-2 protein expression. Sixty-seven cases were negative for Bcl-2 staining (score 0). There was statistically significant correlation between Bcl-2 staining and sex (49% of females expressed Bcl-2 in tumors; $P = -0.04$), pathological type (mucinous vs. nonmucinous; $P = -0.02$), and tumor stage ($P = -0.04$). There was no significant correlation between Bcl-2 staining and age ($P = -0.38$), site of tumor (left colon vs. right colon; $P = -0.13$), pathological differentiation ($P = -0.73$), and between Bcl-2 scoring and tumor differentiation ($P = -0.91$). **Conclusion:** Bcl-2 protein seems to play an important role in the carcinogenesis of colorectal cancer. Considering the acceptable reliability and feasibility of IHC method for the detection of Bcl-2 immunoreactivity, this technique may be expected to serve as an important diagnostic and prognostic marker in colorectal carcinoma.

Keywords: *Bcl-2 gene, colorectal carcinoma, immunohistochemistry, protein expression*

Introduction

Colorectal carcinoma is the third most common cancer in men (663,000 cases, 10.0% of the total) and the second in women (571,000 cases, 9.4% of the total) worldwide.[1] Almost 60% of cases are encountered in developed countries.[2]

The etiology of colorectal cancer is complex, involving interplay of environmental and genetic factors.[3] The development of malignant colorectal carcinoma is a result of multistep changes including genetic factors, mutations, and epigenetic changes. These mutations occur in many tumor suppressor genes (APC, DCC, and TP53 genes) and proto-oncogenes (K-ras and C-myc gene) which result in inactivation of tumor suppressor genes and DNA mismatch of repair genes or activation of oncogenes.[4-6]

The Bcl-2 gene belongs to Bcl-2 family of proteins, which is a known gatekeeper to the apoptotic response.[7] Bcl-2 gene is a 26-kD protein that blocks apoptosis and thus inhibits programmed cell death.[8-12] Mutation in this gene is a frequently detected genetic alteration in human cancers with apoptosis regulation, playing a key role in this process.[13-15] The biomolecular aspects of colorectal cancer have raised great interest about Bcl-2 gene function.[16,17]

In this study, we described the demographic characteristics of colorectal carcinoma cases diagnosed in Indira Gandhi Medical College (IGMC), Shimla, from April 2014 to April 2016. We studied the pattern of the Bcl-2 protein expression in different histopathological types of colorectal carcinoma and correlated it with clinicopathological parameters. We also studied its utility as a prognostic marker.

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Materials and Methods

The biopsy-proved colorectal carcinoma cases that were diagnosed and treated from April 2014 to 2016 in IGMC, Shimla, were selected for study. The secondary data of these patients including age, sex, family history, past history, histological type, grading of tumor, and stage of disease were obtained. The specimens were grossed and the sections were fixed in 10% formalin and paraffin embedded. Consecutive 4 μm sections were cut from the paraffin blocks. These sections were evaluated microscopically to select blocks without necrotic and hemorrhagic areas. Histopathological diagnosis was established on routine hematoxylin and eosin staining of the sections. Immunohistochemistry (IHC) for Bcl-2 was performed out on the BioGenex Xmatrix fully automated front-end processing system using monoclonal antibody against Bcl-2 protein (E-17 clonal antibody; Biogenex).

Two observers evaluated the immunoreactivity of sections for Bcl-2 semiquantitatively under ×40 magnification and as per the percentage of positive tumor nuclei, scored them as follows:

- None (no immunoreactive cells detectable)
- Weak (1+, <5%)
- Moderate (2++, 5%–50%)
- Intense (3+++, >50%).

All tumors showing Bcl-2 immunoreactivity (at least +) were considered positive. The observers were kept blind about the report of each other. After the initial evaluation of the results, cases with major discordance in reports were rechecked by both the observers, and after reaching an agreement, the report was submitted. The Bcl-2 scoring was correlated with clinicopathological parameters. The frequency of Bcl-2-positive tumors with each variable was analyzed using χ^2; $P < 0.05$ was considered statistically significant.

Results

Out of total of 112 cases of colorectal cancer, 61 (54%) patients were male and 51 (46%) were female. The mean age of patients was 55 years (range: 26–80 years). The tumor was located in the right colon in 48 (43%) cases, left colon in 57 (51%) cases, and was bilateral in 7 cases. Among 112 cases, 75 (67%) tumors were well-differentiated adenocarcinomas, 33 (29%) cases were moderately differentiated, and four cases were poorly differentiated adenocarcinomas. Among these, 31 (28%) cases were mucinous and 81 (72%) cases were nonmucinous. Lymphovascular invasion was detected in 20 (18%) cases and lymph nodes were involved in 42 (38%) cases. The stage was Duke A in 15 (13%), B in 54 (48%), C in 30 (27%), and D in 13 (12%) cases [Table 1].

Bcl-2 staining was positive in 40% (45 out of 112) of the cases. Of these, 15 cases were weakly positive (score 1) [Figure 1], 17 cases were moderately positive (score 2) [Figure 2], and 13 cases were intensely positive (score 3) [Figure 3] for Bcl-2 protein expression. Sixty-seven cases were negative for Bcl-2 staining (score 0). The relationship between Bcl-2 protein expression and several clinicopathological variables is summarized in Table 1. In our study, 39% of the patients below 60 years of age and 42% of the patients above 60 years of age showed Bcl-2 expression. Bcl-2 positivity was 33% in males and 49% in females. Left-sided tumors showed Bcl-2 expression in 34% of the cases and right side tumors showed it in 45% of the cases. Well-differentiated adenocarcinoma showed Bcl-2 positivity in 40% of the cases, moderately differentiated adenocarcinoma in 42% of the cases, and poorly differentiated adenocarcinoma in 25% of the cases. In pathological type, 26% of mucinous type and 46% of nonmucinous type showed Bcl-2

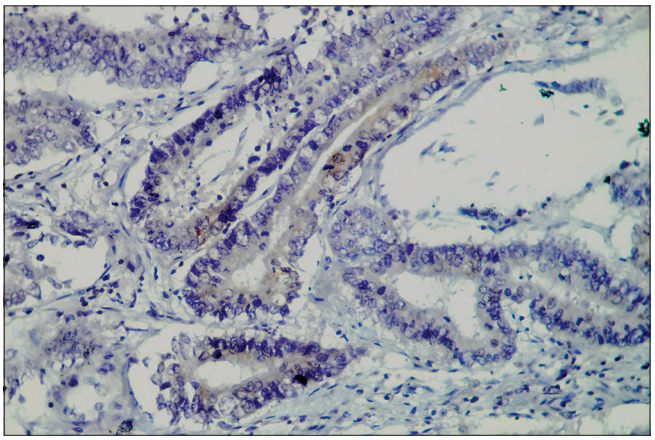

Figure 1: Score 1 weak (1+, <5%), Bcl-2 expression in colorectal carcinoma (IHC, ×400)

Table 1: Clinicopathological correlation with Bcl-2 expression (*n*=112)

Clinicopathological parameters	*n* (%)	Bcl-2 positive, *n* (%)	*P*
Gender			
Male	61 (54)	20 (33)	0.04327
Female	51 (46)	25 (49)	
Tumor location			
Left	59	20 (34)	0.1335
Right	47	21 (45)	
Pathologic grade			
Well differentiated	75	30 (40)	0.7346
Moderately differentiated	33	14 (42)	
Poorly differentiated	4	1 (25)	
Pathologic type			
Nonmucinous	81	37 (46)	0.02873
Mucinous	31	8 (26)	
Stage (Duke)			
A	15	10 (67)	0.04419
B	54	21 (54)	
C	30	10 (33)	
D	13	4 (30)	

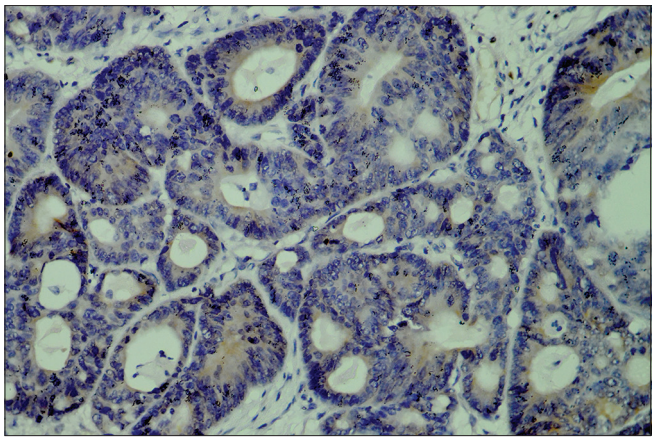

Figure 2: Score 2 moderate (2++, 5%–50%), Bcl-2 expression in colorectal carcinoma (IHC, ×40)

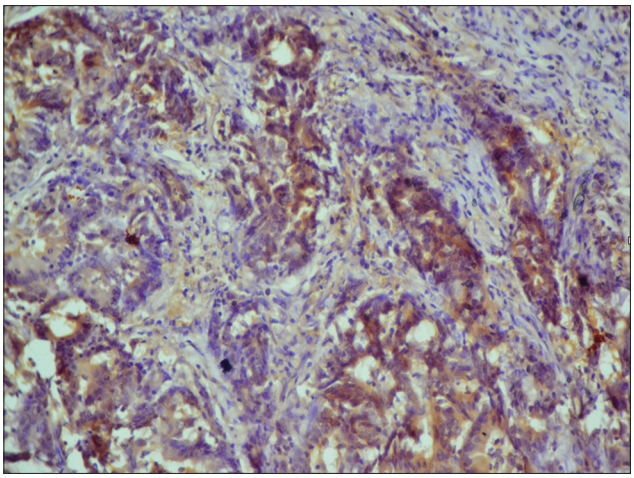

Figure 3: Score 3 intense (3+++, >50%), Bcl-2 expression in colorectal carcinoma (IHC, ×40)

expression. In tumor stage, Bcl-2 positivity was 67% in Duke Stage A, 54% in Stage B, 33% in Stage C, and 30% in Stage D.

Discussion

The Bcl-2 oncogene is a known inhibitor of apoptosis which may allow the accumulation and propagation of cells containing genetic mutation.[18] It is closely related to carcinogenesis in most malignant diseases. In the present study, Bcl-2 expression in colorectal carcinomas was found using IHC and was correlated with clinicopathological parameters to assess the utility of Bcl-2 expression in the diagnosis and prognosis of colorectal carcinoma.

In our study, age of the patients ranged between 26 and 80 years, which was comparable to study done by Sinicrope *et al*.[19] and Ghavam-Nasiri *et al*.[20]. The male-to-female ratio was 1:1, which was comparable to the study done by Ofner *et al*.,[21] Ghavam-Nasiri *et al*.,[20] and Sinicrope *et al*.[19] (1:1).

Among the 112 cases in our study, 45 (40%) cases were Bcl-2 positive, which was comparable with Bcl-2 positivity in the studies by Ofner *et al*. (45%),[21] Watson *et al*. (45%),[22] and Kaklamanis *et al*. (33%).[23] In this study, 39% of the patients who were below 60 years of age and 42% of the patients above 60 years of age expressed Bcl-2 immunoreactivity, which was similar to the study done by Manne *et al*.[24] Our study revealed Bcl-2 immunoreactivity in 33% of all male patients and 49% of all female patients. The results were comparable for males in the study done by Kaklamanis *et al*.[23]) and were comparable for females in studies done by Zhao *et al*.[18] and Manne *et al*.[24] Statistically significant correlation was seen between Bcl-2 positivity and sex of the patient ($P = -0.04$), which was not seen in any of the previous studies done.

Out of 75 (67%) of well-differentiated carcinoma, 30 (40%) revealed Bcl-2 positivity. Similar finding was noted by Kaklamanis *et al*.[23] in 36% of well-differentiated carcinoma. Nonmucinous tumors were more (81, 72%) than mucinous (31, 28%) tumors in our study. Comparable observations were made by Das *et al*.[25] and Ghavam-Nasiri *et al*.,[20] where the percentage of nonmucinous tumors was 72% and 78%, respectively. Bcl-2 was positive in 46% of nonmucinous adenocarcinomas in our study. There was a statistically significant correlation between Bcl-2 staining and pathological type (mucinous vs. nonmucinous; $P = -0.02$). However, no other previous studies revealed such correlation.

In our study, Bcl-2 positivity decreased with tumor stage ($P = -0.04$) and was comparable to studies done by Zhao *et al*.[18] There was no significant correlation between Bcl-2 staining and age ($P = -0.38$). This result was similar to that seen in studies done by Al-Temimi SMA *et al*.,[6] Goussia *et al*.,[26] Zhao *et al*.,[18] and Manne *et al*.[24] The site of tumor (left colon vs. right colon; $P = -0.13$) and pathological differentiation ($P = -0.73$) showed no correlation with Bcl-2 positivity.

Conclusion

Bcl-2 protein seems to play an important role in the carcinogenesis of colorectal cancer and its expression decreased with increasing Duke stage, and there was a significant correlation of Bcl-2 positivity with sex and pathological type (mucinous versus nonmucinous). Considering the reliability and feasibility of IHC method for the detection of Bcl-2 immunoreactivity, this technique may be expected to serve as an important prognostic marker in colorectal carcinoma. However, more studies are required to establish the utility of Bcl-2 as a reliable prognostic marker in colorectal carcinomas.

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Conflicts of interest

There are no conflicts of interest.

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