Original Article

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Immunohistochemical Expression of Bcl-2 Protein in Colorectal Adenocarcinoma and Its Association with Grading and Pathological Staging

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Abstract

Background: Colorectal cancer, a leading global malignancy, often involves dysregulation of apoptosis. Bcl-2, an anti-apoptotic protein inhibiting programmed cell death, is overexpressed in early colorectal carcinogenesis, suggesting a key role in tumor initiation. This study aimed to assess Bcl-2 expression in histologically diagnosed colorectal adenocarcinoma and its association with histopathological grade, stage, and other parameters.

Materials and methods: A cross-sectional study of 49 surgically resected colorectal adenocarcinoma specimens was conducted from March 2021 to December 2022 at the Department of Pathology, Chittagong Medical College. Tissue sections underwent Hematoxylin and Eosin (H&E) staining and Bcl-2 immunostaining. Clinical and pathological data were recorded and statistically analyzed.

Results: Overall, 85.7% (42/49) of colorectal adenocarcinoma patients showed positive Bcl-2 expression, predominantly moderate (38.8%) and strong (30.6%) staining, while only 14.3% were negative.Bcl-2 positivity was associated with female sex (p=0.029) conventional histopathological type (p=0.001) tumor grade (93.0% and 33.3% in low-grade and high-grade tumors, respectively, p=0.001) and nodal status (p=0.004). Low-grade carcinomas predominantly exhibited moderate-to-strong Bcl-2 positivity, while high-grade carcinomas were mostly negative, showing a significant inverse correlation between Bcl-2 expression intensity and tumor grade (p=0.001). No significant associations were found with age, tumor location or depth of invasion.

Conclusion: This study demonstrates that reduced or absent Bcl-2 expression in colorectal adenocarcinoma is significantly associated with higher tumor grade and aggressive behavior. These findings suggest Bcl-2 expression profiling holds promise as a prognostic marker and could represent a therapeutic target to promote apoptosis and inhibit early neoplastic growth in colorectal adenocarcinoma.

Key words: Bcl-2; Colorectal adenocarcinoma; Tumor grade; Nodal Status.

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Introduction

Colorectal Cancer (CRC) remains a leading cause of global cancer-related morbidity and mortality, with significant burden in Bangladesh where patients often present at younger ages and advanced stages.[1-3] Despite advances, outcomes for surgically resected advanced CRC are often poor, underscoring the critical need for reliable predictors of survival and novel molecular markers to guide therapy.[4,5] Established prognostic factors like tumor stage, histologic grade, nodal status, vascular invasion, and margin status rely heavily on meticulous histopathological examination.[6] However, the search for complementary biomarkers indicative of tumor behavior and potential therapeutic targets is imperative, particularly as early diagnosis significantly improves survival.[7,8]

Dysregulation of apoptosis, a fundamental process in carcinogenesis, is a hallmark of CRC.[9] The anti-apoptotic protein Bcl-2, located on chromosome 18q21, plays a pivotal role as a gatekeeper, inhibiting programmed cell death and potentially facilitating early tumor development.[10] Several studies have reported Bcl-2 is frequently expressed in CRC, though prevalence varies across studies (Ranging from 28% to 71% in carcinomas).[11-19] A consistent inverse relationship exists between Bcl-2 expression and tumor progression: expression is significantly higher in early-stage tumors (Dukes A/B, TNM I/II) smaller tumors, and well/moderately differentiated adenocarcinomas compared to advanced-stage

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(Dukes C/D, TNM III) larger, poorly differentiated or mucinous carcinomas in previous studies.[11-19] Several studies link Bcl-2 positivity to improved prognosis. It is often associated with better overall survival, particularly in specific subgroups like patients with distal tumors, male patients or RAS (Rat sarcoma)-negative tumors and serves as an independent favorable prognostic factor.[13,14,12,17]

Expression decreases significantly with worsening tumor stage, size and differentiation grade, correlating with poorer outcomes. Bcl-2 is also more commonly expressed in adenomas than carcinomas, suggesting a role in early carcinogenesis.[11,16] Different forms of association were observed between Bcl-2 expression and clinicopathological features.This expression shows associations with female sex, non-mucinous histology, rectal location and lymphocytic infiltration.[19,16,18,12] Furthermore, Bcl-2 represents a promising therapeutic target, exemplified by BH3 (Bcl-2 homology domain 3) mimetic like ABT-199 (Venetoclax) in hematological malignancies, with preclinical evidence supporting its potential in solid tumors including CRC.[20,21]

Despite this biological and prognostic significance, comprehensive data on Bcl-2 expression patterns and their association with key clinicopathological parameters within the Bangladeshi CRC population is scarce. Understanding these associations in this specific demographic, which exhibits distinct epidemiological features, is necessary.[22] Adenocarcinomas constitute the vast majority, making up over 90% of CRC cases. Specifically, they account for about 93.4% of primary CRC cases.[23] Therefore, this study aimed to assess Bcl-2 protein expression by immunohistochemistry in surgically resected colorectal adenocarcinoma specimens at a tertiary-level hospital in Bangladesh and rigorously evaluate its association with different clinical and histopathological parameters, providing essential insights into the potential of Bcl-2 as a prognostic biomarker and therapeutic target within the Bangladeshi context to inform future management strategies.

Materials and methods

This cross-sectional observational study was conducted in the Department of Pathology, Chittagong Medical College, Chattogram, Bangladesh, over 21 months (March 2021 - December 2022). Ethical clearance was obtained from the Ethical Review Committee of Chittagong Medical College (Memo No: CMC/PG/2022/816, Date: 01/03/2022.) and informed consent was secured from all participants.

Using purposive sampling, 49 surgically resected, formalin-fixed, paraffin-embedded specimens histopathologically confirmed as primary colorectal adenocarcinoma (Excluding patients with prior chemo/radiotherapy) were included in this study. Data on demographic variables (Age, sex) clinical variables (Tumor site) and histopathological variables (Type, grade [Classified per WHO classification system as low/high-grade[24,25]] extent of tumor invasion, nodal status) were collected.

Immunohistochemical staining for Bcl-2 was manually performed at the Immunohistochemistry Laboratory, Bangladesh Medical University (BMU) Dhaka, adhering to the DAKO EnVision™ protocol. Briefly, 3-5µm sections from selected Formalin-Fixed Paraffin-Embedded (FFPE) tumor blocks were mounted on poly-L-lysine coated slides. Following deparaffinization in xylene and rehydration through graded alcohols, antigen retrieval was achieved using microwave heating. Sections were then incubated with the primary monoclonal antibody against Bcl-2 (Dako, Denmark, dilution 1:50) for 30 minutes. Positive control sections from vermiform appendix were included in each run. Finally, Bcl-2 expression (Cytoplasmic staining) was evaluated under light microscopy at ×40 magnification and scored semi-quantitatively according to the percentage of immunoreactive tumor cells: negative (0) weak (+) [<5% cells], moderate (++) [5-50% cells] or strong (+++) [>50% cells] scores ≥ + were considered positive. [19]

Statistical analysis was performed using SPSS version 23.0. Variableswere expressed as frequencies and percentages. Associations between categorical variables were assessed using the Chi-square test. A p-value of less than 0.05 was considered statistically significant.

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Results

The cohort (n=49) demonstrated a predominance of younger patients, with 79.6% (n=39) aged <60 years (Range: 18–75 years). Males comprised 63.3% of cases, yielding a male-to-female ratio of 1.72:1. Regarding tumor location, the sigmoid colon was most frequent (36.7%, n=18) followed by the rectum (28.6%, n=14) transverse colon (20.4%, n=10) ascending colon (10.2%, n=5) and rectosigmoid junction (4.1%, n=2). Histopathologically, conventional adenocarcinoma accounted for 89.8% (n=44) of cases, while mucinous and signet ring subtypes constituted 8.2% (n=4) and 2.0% (n=1) respectively. Staging analysis revealed T3 as the most common depth of invasion (55.1%, n=27) followed by T2 (28.6%, n=14), T1 (8.2%, n=4) and T4 (8.2%, n=4). Most tumors (63.3%, n=31) showed no regional nodal involvement (N0) whereas N1 and N2 spread were observed in 18.4% (n=9) and 16.3% (n=8) of cases, respectively.

Out of the 49 cases evaluated, 42 patients (85.7%) showed positive staining for Bcl-2 protein. The distribution of staining intensity among positive cases showed moderate positivity (++) was the most frequent individual expression level observed (Table I).

Table I Distribution of the patients according to Bcl-2 score

Bcl-2 score	Frequency (n)	Percent (%)
Negative (0)	07	14.3
Weakly positive (+)	08	16.3
Moderate positive (++)	19	38.8
Strongly positive (+++)	15	30.6

Bcl-2 positive expression status had no significant association with age of the patients. However, Bcl-2 expression positivity rate was significantly higher among female (100%) than the male patients (77.4%) (Table II).

Table II Association between age, sex and Bcl-2 expression status

Attributes	Bcl-2 expression status		p value*
	Negative	Positive	
Age			
<60 years	06 (15.4)	33 (84.6)	0.664
≥60 years	01 (10)	09 (90.0)	
Gender			
Male	07 (22.6)	24 (77.4)	0.029
Female	00 (0.0)	18 (100.0)	

Data were expressed as frequency (%). *Chi-square test

Most of the conventional adenocarcinoma (93.2%) showed positive expression for Bcl-2. On the other hand, 75% mucinous type and 100% signet ring type showed negative expression for Bcl-2. Bcl-2 expression positivity rate was higher in colonic tumor (90%) than the tumor in rectum (75%) without any statistical significance (Table III).

Table III Association of histopathological type, site with Bcl-2 expression status

Attributes	Bcl-2 expression status		p value*
	Negative	Positive	
Histopathological type			
Conventional	03 (6.8)	41 (93.2)	0.001
Mucinous	03 (75.0)	01 (25.0)	
Signet ring	01 (100.0)	00 (0.0)	
Tumor location			
Colon	03 (9.1)	30 (90.9)	0.136
Rectum	04 (25.0)	12 (75.0)	

Data were expressed as frequency (%). *Chi-square test

Bcl-2 expression showed significant variation based on tumor characteristics. Positivity was significantly higher in low-grade adenocarcinoma (93%) compared to high-grade (33.3%). While Bcl-2 positivity was observed in all T1 (100%) and T4 (100%) tumors, negativity was more frequent in T2 (14.3%) and T3 (18.5%) tumors; however, this association with tumor invasiveness (T-stage) was not statistically significant. In contrast, a significant association was found with nodal status (N-stage): positivity was highest in N1 tumors (100%) followed by N0 (93.5%) and significantly lower in N2 tumors (55.6%) (Table IV).

Table IV Association between histopathological grade and Bcl-2 expression

Attributes	Bcl-2 expression status		p value*
	Negative	Positive	
Tumor grade			
Low grade	03 (7.0)	40 (93.0)	0.001
High grade	04 (66.7)	02 (33.3)	
Tumor invasiveness			
T1	0 (0)	04 (100.0)	0.631
T2	02 (14.3)	12 (85.7)	
T3	05 (18.5)	22 (81.5)	
T4	0 (0.0)	04 (100.0)	
Nodal status			
N0	2 (6.5%)	29 (93.5)	0.004
N1	0 (0.0)	08 (100.0)	
N2	04 (44.4)	05 (55.6)	

Data were expressed as frequency (%). *Chi-square test

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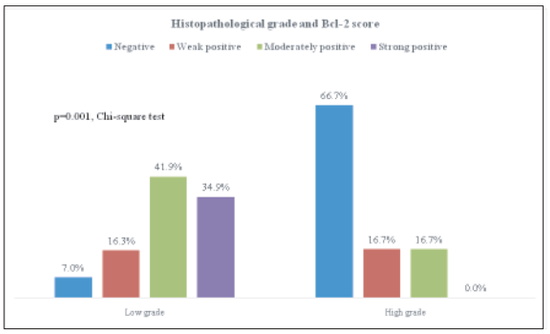

Figure 1 Association between histopathological grade and Bcl-2 score

Bcl-2 expression intensity was significantly higher in low-grade adenocarcinoma compared to high-grade carcinoma (Figure 1). Among low-grade tumors, strong positivity (+++) was most frequent (34.9%) followed by moderate positivity (++: 41.9%), weak positivity (+: 16.3%) and negativity (0: 7.0%). In stark contrast, the majority of high-grade tumors were negative (66.7%) with the remainder showing only weak (16.7%) or moderate (16.7%) positivity, no high-grade tumors exhibited strong Bcl-2 expression.

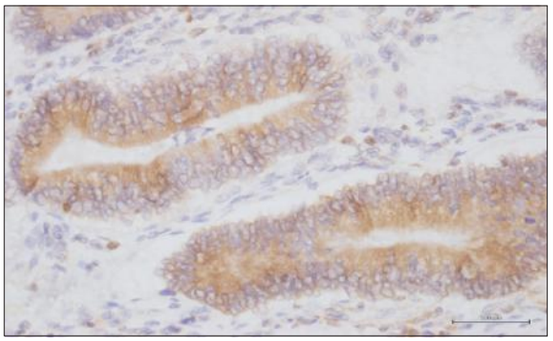

Figure 2 Bcl-2 expression strongly positive (Score +++) in well differentiated colorectal adenocarcinoma, IHC (40X)

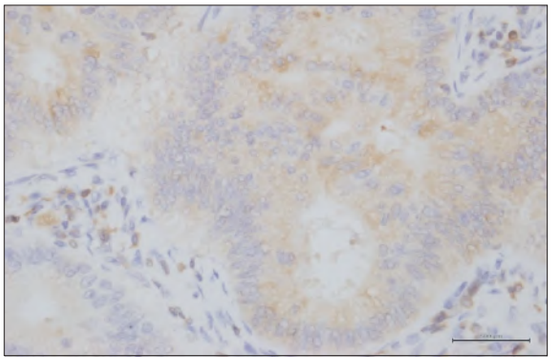

Figure 3 Bcl-2 expression strongly positive (Score +++) in moderately differentiated colorectal adenocarcinoma, IHC (40X)

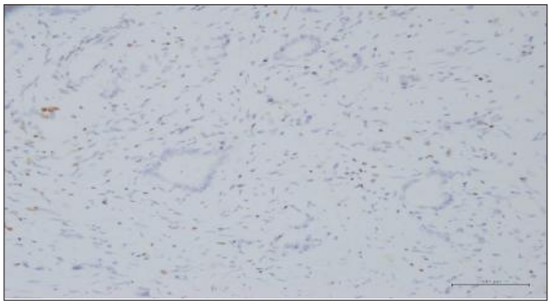

Figure 4 BCL-2 expression negative (Score 0) in poorly differentiated colorectal adenocarcinoma, IHC (40X)

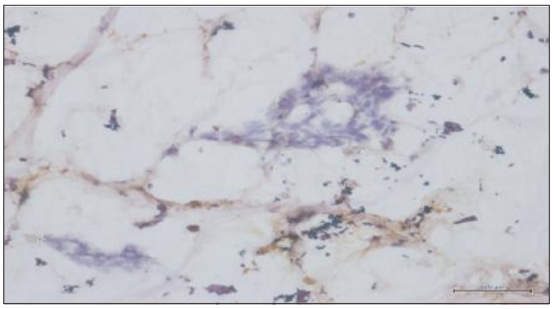

Figure 5 BCL-2 expression negative (Score 0) in mucinous colorectal adenocarcinoma, IHC (10X).

Discussion

CRC pathogenesis involves dysregulated apoptosis, a process critically modulated by the Bcl-2 protein family. Our study investigated Bcl-2 expression patterns in 49 CRC cases, revealing significant associations with key clinicopathological variables. We observed a high overall Bcl-2 positivity rate (85.7%) with staining intensity distributed as strong (30.6%), moderate (38.8%), and weak (16.3%). This prevalence aligns with several previous studies reporting rates of 56-67%, though contrasts with lower rates of 35.7% in the study of Al Temimi et al. and 40% in the study of Bhardwaj et al. potentially reflecting methodological or cohort differences.[11,17,18,15,19]

A highly significant inverse relationship between Bcl-2 positivity and histological grade emerged in this study. Positivity was markedly higher in low-grade tumors (93%) versus high-grade (33.3%). Expression intensity was also significantly greater in low-grade tumors (Strong: 34.9%; moderate: 41.9%) compared to high-grade tumors (Negative: 66.7%; weak/moderate only: 33.3%; no strong positivity). This robust correlation, reinforcing findings by Miya et al. and Al Temimi et al.

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strongly suggests Bcl-2 is more active in well-differentiated neoplasms and decreases as the degree of differentiation worsens, supporting its role in early carcinogenesis.[13,15]

Present study demonstrated that Bcl-2 positivity was significantly higher in female patients (100%) compared to males (77.4%). This finding concurs with Bhardwaj et al. who reported higher female positivity (49% vs 33%, p=0.04) although other studies found no association, indicating potential population-specific effects.[19,15,17,26]

A significant association existed between Bcl-2 positivity and histopathological type with conventional adenocarcinoma showed high positivity (93.2%) while mucinous (75% negative) and signet ring (100% negative) subtypes were predominantly negative. This aligns with studies demonstrating significant correlation with non-mucinous histology.[16,19]

Regarding lymph node status, present study found highest Bcl-2 positivity was in N0 (93.5%) and N1 (100%) tumors but significantly lower in N2 tumors (55.6%). This pattern of reduced expression with advanced nodal spread corroborates Kumar et al. suggesting Bcl-2 loss may facilitate metastatic progression.[18]

In the present study, no significant link was found between Bcl-2 expression and patient age (p=0.664), consistent with multiple studies.[15,17,19,26] No significant association was observed between colonic (90% positive) and rectal (75% positive) tumors supported by the study of Al Temimi et al, Abdul et al. Miya et al., and Bhardwaj et al. Despite 100% positivity in T1 and T4 tumors and higher negativity in T2 (14.3%) and T3 (18.5%) no statistically significant association was established, consistent with Abdul et al.[15,26,19,26]

The strong inverserelation between Bcl-2 expression (Both positivity and intensity) and tumor grade, coupled with its association with less advanced nodal disease, supports its role as an indicator of favorable prognosis. The significant variation in expression intensity further enhances its potential utility. Integrating Bcl-2 immunohistochemical phenotyping – particularly strong expression indicating low-grade potential and negativity (0) suggesting high-grade aggressiveness alongside conventional grading could significantly refine prognostic stratification in CRC. Furthermore, given its fundamental anti-apoptotic function and distinct expression patterns, Bcl-2 represents a compelling therapeutic target, especially in tumors where its expression persists.

Limitations

Key limitations of the present study include the single-institute sampling design, which may limit generalizability to broader populations, and a constrained sample size due to resource limitations. Additionally, reliance on external facilities for immunohistochemistry introduced logistical and financial challenges.

Conclusion

This study demonstrates a strong inverse association between Bcl-2 expression and tumor grade in colorectal adenocarcinoma, where reduced or loss of Bcl-2 correlates significantly with higher-grade, aggressive tumors. These findings establish Bcl-2 as a valuable prognostic biomarker and highlight its potential as a therapeutic target for modulating apoptosis in CRC progression.

Recommendations

Future multi-center studies with larger cohorts, extended follow-up and integrated biomarker panels are recommended to validate the role of Bcl-2 in early carcinogenesis. Implementing Bcl-2 scoring could enhance prognostic stratification in clinical practice. Establishing local IHC facilities would improve feasibility and accessibility for biomarker assessment.

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Contribution of authors

JR-Design, acquisition of data, data analysis, drafting & final approval.

RAM-Acquisition of data, data analysis, inteerpretation of data, drafting & final approval.

ZS-Data analysis, interpretation of data, critical revision & final approval.

AS-Acquisition of data, data analysis, drafting & final approval.

PB-Conception, design, interpretation of data, critical revision & final approval.

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Disclosure

All the authors declared no conflict of interest.

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