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**Original Research Article**

# Immunohistochemical expression of BCL-2 in colorectal cancer and its association with clinicopathological parameters: a cross-sectional study from an Indian tertiary care center

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## ABSTRACT

**Background:** Colorectal cancer (CRC) is among the leading causes of global cancer mortality and is steadily increasing in India. Despite advances in multimodal therapy, treatment resistance and recurrence remain major challenges. BCl-2, an anti-apoptotic protein, has emerged as a potential biomarker of tumor aggressiveness and therapeutic response. Objectives were to evaluate BCl-2 immunoexpression in CRC tissues and correlate expression levels with key clinicopathological features.
**Methods:** A cross-sectional observational study was conducted on 60 histopathologically confirmed CRC cases. Formalin-fixed paraffin-embedded samples were stained using standard immunohistochemistry protocols. BCl-2 expression was scored based on percentage of positively stained tumor cells. Clinical data and pathological variables, including tumor location, histological subtype, grade, stage, and lymph node involvement, were recorded. Statistical significance was evaluated using chi-square/Fisher's exact test ($p<0.05$).
**Results:** BCl-2 positivity was identified in 96.7% of CRC cases, with 46.7% demonstrating strong cytoplasmic staining. A significant association was observed between BCl-2 expression and tumor location (rectum) as well as histological subtype (adenocarcinoma NOS). Although higher BCl-2 expression was noted in moderately differentiated tumors, this relationship did not reach statistical significance. No significant associations were found with tumor grade, stage, lymph node status, age, or sex.
**Conclusions:** BCl-2 is frequently expressed in colorectal carcinoma, indicating its role in tumor survival; however, its limited correlation with clinicopathological parameters suggests it is not an independent prognostic marker. Further studies integrating additional apoptotic and molecular markers are needed to clarify its prognostic and therapeutic value.

**Keywords:** Colorectal carcinoma, Bcl-2, Immunohistochemistry, Clinicopathological correlation, Apoptosis

## INTRODUCTION

Colorectal cancer (CRC) is a malignant neoplasm of the colon and rectum and now ranks among the three most common cancers worldwide. Recent WHO estimates show that of the 20 million new cancer cases reported in 2022, CRC accounted for 1.9 million new cases (≈9.6 %) and 900 000 deaths, making it the second leading cause of cancer-related mortality after lung cancer.[1] The burden is rising in low- and middle-income countries due to ageing populations and lifestyle changes; projections suggest global cancer incidence will increase by 77% by 2050. India reflects these trends: CRC is now 4$^{th}$ most incident cancer nationally, with 64863 new cases and 38367 deaths in 2022, and survival remains lower than in high-income nations. Urban regions show higher incidence than rural areas, and risk factors include diets high in red and processed meat, obesity, diabetes, smoking and alcohol use, while protective factors include fibre-rich diets, physical activity and possible benefits from metformin or aspirin.[2] These data underscore the growing public-health challenge posed by CRC in India and globally.

Beyond its mortality impact, CRC and its treatments profoundly impair quality of life. Survivors experience diminished physical function due to pain, altered bowel habits, blood loss with anaemia, weight loss and fatigue, while psychological and social functioning may be hampered by fear, anxiety, sleep disturbances and depression.[3,4] Quality-of-life deficits are particularly severe in rectal cancer; sphincter-preserving procedures can lead to low anterior resection syndrome, causing fecal urgency, pelvic pain, sexual and urinary dysfunction, and nearly 70 % of patients develop some form of this syndrome.[5,6] Ostomies and adjuvant therapies further reduce physical and role functioning and distort body image.[4] These multifaceted impacts highlight the need for treatments that not only prolong survival but also preserve quality of life.

Standard management strategies vary by stage. Surgical resection remains the cornerstone for resectable disease, while chemotherapy, radiotherapy and immunotherapy are employed either alone or in combination for advanced CRC.[7] However, these modalities are non-specific and can be cytotoxic to normal tissues, and nearly half of patients develop recurrent or chemoresistant disease. To overcome resistance, researchers are exploring targeted therapies: immune-checkpoint inhibitors, chimeric antigen receptor T-cell therapy, and RNA-based approaches have shown promise.[8] Additionally, the development of BH3-mimetic drugs such as venetoclax provides proof-of-concept that targeting anti-apoptotic proteins like BCl-2 can yield clinical benefit in haematological malignancies; newer agents and delivery strategies are being investigated to extend this success to solid tumours.[9] Nevertheless, access to these novel treatments remains limited, and robust biomarkers are needed to select patients who may benefit.

Against this background, there is a clear need gap in understanding how anti-apoptotic pathways contribute to CRC progression and therapeutic resistance, particularly in Indian populations where molecular data are scarce. BCl-2 is key regulator of intrinsic apoptosis and a potential predictor of treatment response. This cross-sectional study addresses this gap by examining BCl-2 immunoexpression in 60 histologically confirmed CRC cases and correlating it with demographic, pathological and prognostic parameters. By elucidating patterns of BCl-2 expression in an Indian study, we hope to contribute to the evidence base guiding personalised therapy and future research on targeted anti-apoptotic strategies.

## METHODS

### *Study design and setting*

This cross-sectional observational study was conducted in the Department of Pathology at Ramaiah Medical College and Hospitals, Bengaluru, over an 18-month period from May 2023 to November 2024. The study aimed to evaluate BCl-2 immunohistochemical expression in histologically confirmed CRC cases and to analyze its association with key clinicopathological variables.

### *Study population and sample size*

A total of 60 CRC cases were included, comprising both incisional biopsy and surgical colectomy specimens. The sample size was calculated based on the prevalence of BCl-2 expression in CRC reported by Miya et al (65.6%), using a 95% confidence interval and 12% absolute precision, which yielded a minimum required sample size of 60 cases. Sampling was consecutive, based on all eligible cases received during the study period.

### *Inclusion and exclusion criteria*

Cases included were patients aged 18 years and above with histopathologically confirmed colorectal carcinoma on biopsy or resected specimens. Exclusion criteria comprised specimens with extensive tumor necrosis rendering immunohistochemical interpretation unreliable, and cases that received neoadjuvant chemotherapy or radiotherapy with complete tumor regression, where no viable tumor tissue remained for BCl-2 evaluation.

### *Ethical considerations*

The study was conducted after approval from the institutional ethics committee of Ramaiah Medical College, Bengaluru. The study adhered to the ethical principles outlined in the Declaration of Helsinki, and confidentiality of patient data was maintained throughout. As archived tissue samples were used and no direct patient interventions were involved, informed consent was waived as per institutional guidelines.

### *Specimen handling and histopathological evaluation*

All biopsy and resection specimens received in the pathology laboratory were fixed in 10% neutral buffered formalin for 24-48 hours. Surgical specimens were grossed according to standard oncopathology protocols, documenting tumor site, size, gross morphology, relationship to margins, circumferential resection margin status, and lymph node yield. Representative sections were submitted from the tumor, adjacent mucosa, deepest point of invasion, resection margins, and all identifiable lymph nodes. Tissue sections were processed, embedded in paraffin, sectioned at 4 µm thickness, and stained with hematoxylin and eosin (H and E). Microscopic evaluation included tumor typing and grading per WHO 2019 criteria, depth of invasion (pT), lymphovascular invasion (LVI), perineural invasion (PNI), nodal metastasis, and TNM staging according to AJCC 8$^{th}$ edition.

### *Immunohistochemistry for BCl-2*

Immunohistochemical analysis was performed on 4 µm sections prepared from formalin-fixed paraffin-embedded (FFPE) blocks and mounted on poly-L-lysine coated

slides. Antigen retrieval was performed using citrate buffer (pH 6.0) in a microwave heating system, followed by quenching of endogenous peroxidase activity using 3% hydrogen peroxide. Slides were incubated with mouse monoclonal anti-BCl-2 primary antibody, followed by application of a polymer-based secondary detection system and visualization with diaminobenzidine (DAB) chromogen. Sections were counterstained with hematoxylin. Appropriate positive controls (lymphoid tissue with known BCl-2 expression) and negative controls (primary antibody omission) were included in each staining batch.

### *Scoring and interpretation of BCl-2 expression*

BCl-2 expression was evaluated in tumor cell cytoplasm and quantified based on the proportion of positive tumor cells. Scoring was performed semi-quantitatively as follows: negative (<5% stained cells), weak positive (5-25%), moderate positive (26-50%), and strong positive (>50%). For statistical analysis, expression was further categorized into BCl-2 negative and BCl-2 positive groups, where any score ≥5% was considered positive. Scoring was independently assessed by two pathologists to minimize interpretation bias, and discordant cases were reviewed jointly to reach consensus.

### *Study variables*

The primary study variable was BCl-2 immunoexpression status. Secondary variables included patient demographics (age, sex), specimen type (biopsy vs colectomy), tumor characteristics (anatomical site, size, gross morphology, histological subtype, grade), and prognostic histopathological parameters (lymphovascular invasion, perineural invasion, lymph node metastasis, pathological T stage, presence of metastasis, and overall TNM stage).

### *Statistical analysis*

Data were entered into Microsoft excel and analyzed using IBM SPSS Statistics version 22. Categorical variables were summarized using frequencies and percentages. The association between BCl-2 expression and clinicopathological parameters was analyzed using the Chi-square test or Fisher's exact test, where appropriate. A $p<0.05$ was considered statistically significant. Graphs and tables were generated using Microsoft Excel and interpreted in the context of existing literature.

## RESULTS

### *Clinicopathological characteristics of colorectal carcinoma cases*

Among 60 colorectal carcinoma cases, the mean age was 56.75 years with peak incidence in the $6^{th}$-$7^{th}$ decade (56.6%), and a male predominance (M:F=1.6:1). The rectum was the most common tumor site (48.3%), and adenocarcinoma NOS (90%) was the predominant histological type. Most tumors were Grade 2 (88%) and presented between T3-T4 stages.

**Table 1: Clinicopathological profile of the study.**

| Parameters | Category | N (%) |
|---|---|---|
| **Age group (in years)** | 31-40 | 5 (8.3) |
| | 41-50 | 10 (16.7) |
| | 51-60 | 17 (28.3) |
| | 61-70 | 17 (28.3) |
| | 71-80 | 11 (18.3) |
| **Sex** | Male | 37 (61.7) |
| | Female | 23 (38.3) |
| **Specimen type** | Biopsy | 34 (56.7) |
| | Colectomy | 26 (43.3) |
| **Tumor site** | Rectum | 29 (48.3) |
| **Histological type** | Adenocarcinoma NOS | 54 (90) |
| **Tumor grade** | Grade II | 53 (88) |

### *BCl-2 immunoexpression pattern*

BCl-2 expression was detected in 96.7% (58/60) of colorectal tumors, indicating near-ubiquitous activation of this anti-apoptotic marker in the study. Strong cytoplasmic immunoreactivity was the dominant pattern (46.7%), followed by moderate and weak expression, while only two tumors showed complete absence of staining. The observed staining distribution suggests that BCl-2 upregulation occurs early and persists across tumor subsets, supporting its biological relevance in colorectal tumorigenesis (Table 2).

**Table 2: BCl-2 immunostaining score.**

| IHC score | Interpretation | N (%) |
|---|---|---|
| **0** | Negative | 2 (3.3) |
| **1+** | Weak | 14 (23.3) |
| **2+** | Moderate | 16 (26.7) |
| **3+** | Strong | 28 (46.7) |

### *Correlation with demographics and tumor site*

BCl-2 expression demonstrated a significant association with tumor anatomical site ($p<0.05$), with rectal cancers showing the highest proportion of positivity, suggesting possible site-specific biological behavior. A significant difference was also seen between biopsy and resection specimens ($p<0.05$), likely reflecting larger tumor representation in colectomy samples. However, no statistical association was observed with age or sex, indicating that BCl-2 activation is independent of demographic influence (Table 3).

### *Correlation with tumor morphological features*

A statistically significant association was found between BCl-2 expression and histological subtype ($p<0.05$), with adenocarcinoma NOS demonstrating the most consistent

and intense expression, suggesting subtype-linked apoptotic resistance. In contrast, tumor grade, size, and gross morphological patterns showed no significant correlation, implying that BCl-2 expression does not escalate linearly with tumor differentiation or macroscopic growth behavior (Table 4).

**Table 3: BCl-2 correlation with demographics and specimen variables.**

| Variables | Key observation | P value |
|---|---|---|
| **Age (in years)** | No association | NS |
| **Sex** | No association | NS |
| **Specimen type** | Higher positivity in colectomy samples | P<0.05 |
| **Tumor site** | Significant enrichment in rectal tumors | P<0.05 |

**Table 4: BCl-2 correlation with tumor morphology.**

| Parameters | Outcome | P value |
|---|---|---|
| **Histological subtype** | Significant (highest in AC-NOS) | P<0.05 |
| **Tumor grade** | No association | NS |
| **Tumor size** | No association | NS |
| **Growth pattern** | No association | NS |

***Correlation with prognostic pathological parameters***

Despite near-universal expression, BCl-2 showed no significant association with established adverse pathological prognosticators, including depth of invasion, lymphovascular or perineural invasion, nodal involvement, distant metastasis, or overall TNM stage. This suggests that while BCl-2 is highly expressed in CRC, it may play a greater tumor-maintenance role rather than a driver of aggressive or metastatic progression in this study (Table 5).

**Table 5: BCl-2 correlation with prognostic indicators.**

| Prognostic parameter | Association | P value |
|---|---|---|
| **pT stage** | No association | NS |
| **Lymphovascular invasion** | No association | NS |
| **Perineural invasion** | No association | NS |
| **Nodal status** | No association | NS |
| **Distant metastasis** | No association | NS |
| **TNM stage** | No association | NS |

## DISCUSSION

Our cross-sectional study evaluated BCl-2 immunoexpression in 60 histologically confirmed CRC cases to explore whether this anti-apoptotic marker correlates with clinicopathological variables. BCl-2 belongs to a family of proteins that govern cell survival by inhibiting apoptosis and autophagy.[10] Its up-regulation in cancer facilitates tumour persistence and may confer resistance to chemotherapy.[11,12] On this premise, we examined the prevalence and intensity of BCl-2 expression in CRC and its association with demographic, morphological and prognostic parameters using a semi-quantitative scoring system. To our knowledge, comparable studies on Indian studys remain scarce, and the heterogeneity of published findings underscores the need for regional data.

The demographic profile in our series (mean age 56.8 years, male-to-female ratio 1.6:1, predominance of rectal tumours) is in keeping with contemporary CRC epidemiology. Globally in 2022, an estimated 1.9 million CRC cases were diagnosed, of which ~1.04 million occurred in males versus ~0.83 million in females.[13] A recent Indian cancer registry analysis reported 40,430 new male cases and 24,433 female cases in 2022 (age-adjusted rates of 5.7 and 3.4 per 100,000, respectively), confirming a clear male predominance.[14] Our male bias parallels these national figures and may reflect higher exposure to risk factors. The median age of onset in our study (sixth decade) is similar to the pattern seen in US data, where more than half of new diagnoses occur after age 65 and the risk is higher in men than women despite similar absolute numbers at older ages. We also observed that almost half of the tumours were located in the rectum, in line with reports that rectal cancers account for 37% of CRC in people younger than 50 but only 24% in those ≥65 years.[15] These similarities suggest our study population is representative of wider CRC demographics.

BCl-2 was expressed in 96.7% of our tumours, with strong cytoplasmic staining in 46.7%. This prevalence is higher than the 59.8% positivity reported by Jamai et al in Tunisian colorectal adenocarcinoma and far exceeds the ~87 % overall positivity seen in a Romanian series assessing BCl-2/p53 co-expression.[16,17] Our high detection rate may be due to methodological differences; we considered ≥5 % of tumour cells with cytoplasmic staining as positive and included both biopsy and resection specimens. The distribution of staining intensities in our study resembles that described by Kunac et al who found strong BCl-2 expression in lamina propria cells of low-grade CRC and moderate expression in high-grade tumours. However, our results differ from that study's observation of decreasing BCl-2 expression with advancing grade-in our series, BCl-2 positivity remained high across all grades. Rahadiani et al likewise reported universal BCl-2 positivity in low-grade tumours and no correlation with tumour stage, aligning with our finding of abundant expression irrespective of histological grade. Variability across studies highlights the influence of scoring systems, antibodies and population differences on BCl-2 detection.[18]

We identified significant associations between BCl-2 expression and tumour location as well as histological

subtype. Rectal tumours showed higher positivity than colonic tumours, echoing observations that rectal cancer may harbour distinct molecular features; a recent transcriptomic study noted higher BCl2 mRNA levels in mucinous CRCs compared with non-mucinous cancers.[19] Our finding that adenocarcinoma NOS had the highest BCl-2 expression corroborates this and may reflect tumour biology rather than degree of differentiation. In contrast, BCl-2 expression was not associated with age, sex, tumour grade, size or gross morphology. Importantly, no significant correlation was seen with depth of invasion, lymphovascular or perineural invasion, lymph node metastasis, distant metastasis or TNM stage, despite the high overall positivity. This lack of prognostic linkage mirrors results from Rahadiani et al where BCl-2 did not correlate with stage or lymph node status, but contrasts with the Tunisian study in which BCl-2 positivity was associated with distant metastasis and, together with other markers, predicted poorer survival.[18,19] Such discordance suggests that BCl-2 alone may not be a reliable prognostic marker and that its impact might depend on interaction with other apoptotic regulators or tumour microenvironment factors.

The implications of our findings are two-fold. First, the near-ubiquitous BCl-2 expression in CRC supports its role in tumour cell survival and raises the possibility of therapeutic targeting. BCl-2 inhibitors are currently being evaluated in haematological malignancies, and there is growing interest in extending these approaches to solid tumours. Our data indicate that most CRCs express BCl-2 strongly enough to be potential candidates for such therapy. Second, the absence of association with conventional prognostic parameters suggests that immunohistochemical BCl-2 status alone has limited utility in predicting tumour behaviour. Future studies should evaluate the combined prognostic value of BCl-2 with other apoptotic markers (e.g., Bax, BCl-xl) and correlate expression with patient outcomes. Our study is limited by its small sample size, single-centre design and lack of survival data, which preclude definitive conclusions about prognostic significance. Additionally, the subjective nature of immunohistochemical scoring and the inclusion of both biopsies and resections may have influenced results. Nevertheless, the study contributes valuable data from an under-represented population and underscores the importance of multi-marker panels and molecular profiling in understanding CRC biology.

## CONCLUSION

Our study highlights that BCl-2 expression is frequent in colorectal carcinoma, supporting its potential role in tumor cell survival. However, its lack of significant association with established clinicopathological parameters suggests that BCl-2 alone may not serve as a reliable independent prognostic marker. Future large-scale, multicentric studies integrating BCl-2 with other apoptotic and molecular markers are warranted to establish its combined prognostic and therapeutic significance in colorectal carcinoma.

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