ORIGINAL RESEARCH PAPER

Pathology

CORRELATION OF BCL 2 ONCOPROTEIN EXPRESSION AND ITS PROGNOSTIC SIGNIFICANCE IN COLORECTAL CARCINOMA WITH AGE, SITE GRADE AND STAGE

KEY WORDS: colorectal carcinoma(CRC0 , histopathology , immunohistochemistry (IHC)

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ABSTRACT

Background & Objective: Colorectal cancer is the third most prevalent malignancy with high mortality rate, necessitating markers that predict survival and guide the treatment. Previous studies have examined the immunohistochemical expression of Bcl-2, anti apoptotic marker, in colorectal carcinoma, but results have been contradictory. To evaluate the histopathological features of colorectal carcinoma, immunohistochemical expression of Bcl-2 must be analyzed to find out statistical association of Bcl-2 expression with certain prognostic factors histopathologic type, grade and TNM staging and also clinical parameters like age gender, site. **Methods:** This prospective study was conducted on the colectomy specimens of colorectal carcinoma, over a period of 2 years 6months From may 2018 to November 2020 The tumor morphology and Bcl-2 status were evaluated by immunohistochemistry in each case, with the inclusion criteria of resected intestinal specimens among which only malignant epithelial lesions and exclusion of all benign lesions. **Results:** The study included 40 cases, with age group of patients 51-60(37.5%) years and male: female ratio of 1.2:1. Bcl-2 positivity was seen in 37.5% of the cases. Weak, moderate, and strong expression of Bcl-2 was seen in 62.5%, 25%, and 12.5% of cases respectively. Even though early stages of colorectal carcinoma showed greater frequency of Bcl-2 expression than advanced stages (25% versus 12.5%), however this association was not statistically significant. **Conclusion:** The expression of bcl 2 out of 40 resected specimens of colorectal carcinoma correlated with various histological and clinical paramaters .bcl 2 positivity was assessed by semiquantitaive method and its expression was decreased with increase in grade and stage of the tumour and increased with the early stages of colorectal cancer

Introduction

Colorectal cancer (CRC) is the third most prevalent malignancy worldwide, with 6,63,904 new cases per year in men and 5,71,204 cases in women and is the second leading cause of death from malignancies in the industrialized nations [1,2]

Results of surgical resection for advanced cancer are still poor, therefore the search for predictors of disease survival and identification of molecular markers are mandatory [3,4] .The most powerful predictors and guides to treatment in CRC are tumor stage and stage- independent factors including histologic grade, tumor type, vascular invasion, and surgical margins (1). Thus, meticulous histopathological examination of the CRC specimen is indispensable.

The pathophysiology of CRC is complex and the accumulation of molecular alterations, including Bcl-2 oncoprotein expression contributes to tumorigenesis (2,5).

colorectal cancer develops either sporadically in 85%of cases or as a part of hereditary cancer syndrome in less than 10% or against background of inflammatory bowel syndrome .Accumulation of molecular alterations including k–RAS P-53 ,BCL 2 and adenomatous polyposis colon contribute to carcinogenesis [22]

As Bcl-2 oncoprotein inhibits apoptosis and its over-expression contributes to neoplastic transformation, it has been studied for its potential impact on disease outcome [2, 5]. Some clinical trials have associated Bcl-2 expression with favourable prognosis and others have shown no statistical correlation between Bcl-2 expression and prognostic factors like tumor stage and grade [2,5]. Thus, the objective of our study is to evaluate the histopathological features of CRC, and to investigate the association of Bcl-2 expression with prognostic parameters like tumor type, stage and grade.

Materials and Methods

Source of Data : This prospective study was conducted on colectomy, low anterior resection and abdominoperineal resection specimens of colorectal carcinoma, received in the Department of Pathology, for routine histopathological evaluation from the Departments of Surgery and Surgical Oncology, CAIMS from may 2018 to November 20210.

Cases where only a biopsy, endoscopic mucosal resection or polypectomy has been performed and cases where there was extensive tumor necrosis without sufficient viable tumor cells were excluded from the study.

The specimens were received in 10% formalin. In every case, the standard protocol for surgical grossing of resected specimens was followed. After a detailed gross specimen examination, multiple representative tissue bits were taken from the tumor, surgical margins, mesentery, and all the lymph nodes.These were processed. The latter were processed as per standard protocol and paraffin embedded tissue blocks were cut and stained by hematoxylin and eosin (H & E). The H & E stained slides were studied for the tumor histology and grade.The tumor was staged according to AJCC cancer staging.

Processing for Immunohistochemistry Immunohistochemical detection of Bcl-2 was done on 4µm thick sections, cut from a paraffin block of tumor tissue. The technique for IHC includes antigen retrieval in citrate buffer in a-microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse anticlonal antibody against Bcl-2 (Anti-bcl-2 oncoprot), linking with rabbit anti mouse secondary antibody (DAKO), enzyme labelling with streptavidin- horseradish peroxidase, developing chromogen with deaminobenzidine (DAB) and counterstaining with hematoxylin 7Staining was defined as positive for Bcl-2 protein whenever any specific cytoplasmic staining was detected. In each case, the percentage of positive staining tumor cells (the number of positive tumor cells over the total number of tumor cells) was

evaluated. A semi-quantitative assessment of staining was done as follows:

- Negative (0) – No Bcl-2 immunoreactivity detectable
- Weak positive (1+) – less than 5% of tumor cells showing Bcl-2 positivity.
- Moderate positive (2+) - 5-50% of tumor cells showing Bcl-2 positivity.
- Strong positive (3+) - More than 50% of tumor cells positive for Bcl-2 (8).

Differences in the proportion of expression between different grades, types, etc. were tested for statistical significance by Chi- square test significance/ Fisher`s Exact test.

Results

During the study period, 40 resected specimens of colorectal carcinoma were received in the department of pathology. The Age range: 51 to 60 years). Males i.e 18 cases (55%) were found to be highly susceptible to CRC when compared to females i.e 12cases (45%) with a male to female ratio of 1.2:1. The most common site for colorectal carcinoma was left sided colon i.e rectosigmoid colon. The ulceroinfiltrative pattern was the dominant pattern seen in the gross specimen, followed by annular constricting and diffuse infiltrating pattern Adenocarcinoma –NOS (ACa-NOS)i.e 36cases (90%) was the predominant histologic type of colorectal carcinoma, followed by mucinous adenocarcinoma 4 cases (MACa; 10%). Majority of the tumors were grade 2 (moderately differentiated, 18cases 45%) followed by grade 1 (well differentiated;18 cases 45%), and grade 3 (poorly differentiated ;4cases 10%) on morphology. Stage I and II(45% each) were the most common presentation, followed by stage III (10%). Well and moderately differentiated CRC were associated with greater expression of Bcl-2 compared to poorly differentiated CRC. Early stage (stages I and II) tumors showed greater expression of Bcl-2 in contrast to advanced stage tumors (stages III) However, there was no statistically significant association between the Bcl-2 expression and tumor stage (P=0.5) and grade (P=0.58).

Statistical Analysis of Data:

Descriptive statistics were employed to express quantitative parameters such as age, duration of the disease etc. and were summarized in terms of percentage with 95% confidence interval. Differences in the proportion of expression between different grades, types, etc. were tested for statistical significance by Chi- square test significance/ Fisher`s Exact test.

Table 1. expression of bcl 2 among study

Expression of bcl 2 score	No of cases	Percentage
0	25	62.5
1+	10	25
2+	05	12.5

Among the cases with positive bcl 2 expression most of the cases scored 1+ i.e 5-50% of malignant cells showed positive histological study.

Table 2. Bcl-2 Expression in relation to tumour grade

Tumour grade	No of cases	Bcl2 positive %	Bcl 2 negative %
0	18	9(50%)	9(50%)
1	18	5(27.7%)	13(72.7%)
2	4	1(25%)	3(75%)
3	40	15(37.5%)	25(62.5%)

The expression of bcl 2 decreased with increase in grade of the tumour .there is no statistical correlation was noted in the expression of bcl 2 in grade of the tumour (p =0.7)

TABLE 3 –bcl 2 expression in relation to tumour stage

Tumour stage	No of cases	Bcl 2 positivity	Bcl 2 negative
1	14	10(71.4%)	04(28.5%)
2	17	4(25.5%)	13(76.4%)
3	9	1(11.1%)	08(88.8%)
Total	40	15(37.5%)	25(62.5%)

The expression of bcl 2 decreased with increasing stage of the tumour. p <0.05)

Table 4 – comparison of distribution of tumour with gender.

Study	Males%	Females%
RAEM tollenaar et al[27]	54%	46%
Petros c.papagiorgis[3]	58%	42%
Nicholas FS Watson et al[24]	58%	42%
Present study	55%	45%

Table 5 – comparison of grading of tumour with various studies.

Study	Grade 1	Grade 2	Grade 3
AC gouissea et al[26]	24%	67.6%	8.3%
Petros c.papagiorgis[3]	6%	86.5	6.5%
Kavitha mardi et al[28]	51.6%	40%	8.5%
Present study	45%	45%	10%

Table 6– comparison of staging of tumours among various studies

Tumour stage	Mihalache et al[29]	Petrous c.papagiorgis[3]	Present study
1	4.16%	10%	35%
2	26.6%	42%	42.55
3	37.5%	37%	22.5%
4	31.6%	11%	00%

Table 7– comparison of bcl 2 expression among various studies.

Study	Incidence of bcl 2 expression.
AJM Watson et al [21]	36.5%
Hunaldo lima de menezes et al[25]	31.75
Balzi et al[22]	47.6%
Cai et al[23]	29%
Present study	37.5%

In our study bcl 2 expression was positive in 37.5% which is similar to study done in AJM Watson et al36(36.5%)

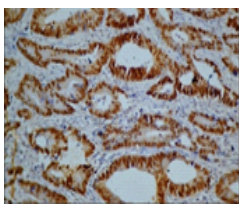
Table 8 – bcl 2 expression with clinicopathological parameters of colorectal carcinoma.

No of cases	No of bcl 2 positive cases
Age	
11-20:1(2.5%)	00(0%)
21-30:1(2.5%)	00(0%)
31-40:1(7.5%)	00(0%)
41-50:10(25%)	03(30%)
51-60:15(37.5%)	08(53.3%)
61-70:10(25%)	04(40%)
Site	
Proximal:14(35%)	03(21.4%)
Distal :26(65%)	12(46.1%)
Histopathological	
Adenocarcinoma :37(92.5%)	15(40.5%)

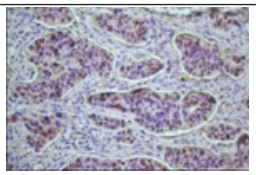
Mucinous :03(7.5%)	00(0%)
Grade	
G1:18(45%)	09(50%)
G2:18(45%)	05(27.7)
G3:04(10%)	01(25%)
Dukes stage	
A:28(70%)	14(50%)
B:03(7.5%)	00(0%)
C:09(22.5%)	01(11.1%)
TNM staging	
Stage 1:14(35%)	10(71.4%)
Stage 2:17(42.5%)	04(23.5%)
Stage 3:09(22.5%)	01(11.1%)

Table 9 – comparison of bcl2 expression with various studies and its prognostic significance

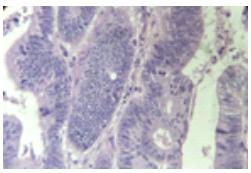
study	Sample size	Bcl 2 expression	Prognostic value	P value
Pity IS et al[2]	52	9.6%	Low bcl2 expression was observed with advanced tumour stage ;however this association did not reach the level of significance	0.27
Ofner D et al[10]	104	47.1%	Bcl 2 oncoprotein appears to be associated with favourable clinical outcome since its expression decreased with the increasing stage.	0.001
Zhao et al[12]	93	57%	Bcl 2 expression had no prognostic significane	>0.005
Current study	40	37.5%	statistical prognostic correlation between bcl 2 and stage was present.	<0.005



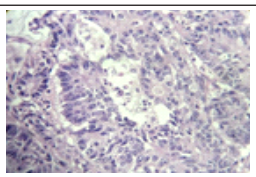
WELL DIFFERENTIATED ADENOCARCINOMMA (BCL-2 EXPRESSION)



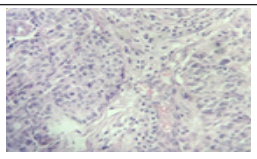
POORLY DIFFERENTIATED (BCL-2 EXPRESSION)



WELL DIFFERENTIATED ADENOCARCINOMMA (H&E)



MODERATELY DIFFERENTIATED ADENOCARCINOMA(H&E)



POORLY DIFFERENTIATED ADENOCARCINOMA (H&E)

Discussion

In the current study, Bcl-2 marker was targeted for immunohistochemical analysis for its role in cancer prognosis. Bcl-2 family members play important roles in tumor initiation and progression. Immunohistochemical expression of Bcl-2 was found in 37.5% of 40 cases. The prevalence of Bcl-2 immunohistochemical expression in CRC varies greatly from one study to another [9-14] These conflicting data are partly due to different populations, different scoring systems, different statistical analyses, as well as primary factors regarding the immunohistochemical technique and the evaluation of the results.

comparison of site distribution of the tumour with Hunaldon lima de menses et al[25], Michalche et al [29] and present study on left and right side of colon were 55% and 45% ,72.5% and 27.5% ,62.5% and 37.5% respectively.

Intensity of Bcl-2 Expression:

Intensity of Bcl-2 expression in our study differs from other studies due to varying sample sizes and different scoring system. Other studies have used different scoring systems, which makes the comparison between the studies difficult [15,16]

Bcl-2 and Histopathologic Type:

In the present study, Bcl-2 positivity was found mainly in ACa-NOS compared to MACa, SCa. No statistical correlation between Bcl-2 positivity and histopathologic type was found (P>0.05). Qasim *et al*. found statistical significance between Bcl-2 and non- mucinous histopathologic type [16]. Some studies have demonstrated Bcl-2 positivity in more than 30% of a mucinous adenocarcinoma [17]

Bcl-2 Expression and Histologic Grade:

In the current study, well differentiated and moderately differentiated cases had greater Bcl-2 expression than the poorly differentiated cases, which was not statistically significant. One author had demonstrated that Bcl-2 overexpression seems to be associated with advanced histologic grade, resulting in a more aggressive tumor (15). Some studies had failed to demonstrate any correlation between Bcl-2 expression and histologic grade, in agreement with our studies (16). Petrisor *et al.* demonstrated that the proportion of Bcl-2(+) expression in poorly differentiated lesions is significantly lower than that in the other grades,

which could explain a better prognosis for Bcl-2 positive cancers (18).

Bcl-2 Expression and TNM Stage:

In the present study, Bcl-2 was expressed mainly in lower stages compared to advanced stages. There was no statistically significant correlation between the Bcl-2 expression and tumor stage. This finding was also demonstrated by many other studies [19, 20]. One study has reported increased proportion of Bcl-2 expression in adenomas than in carcinomas, indicating the role of Bcl-2 in early neoplastic transformation (18). Another study had demonstrated statistically significant correlation with reducing Bcl-2 expression and increasing stage and poorer clinical outcome (21). Results concerning the role of Bcl-2 in relation to stage and survival are conflicting.

Conclusion

In the present study, Bcl-2 positivity was expressed in 37.5% of CRC cases. Even though well and moderately differentiated CRC were associated with a greater expression of Bcl-2 compared to poorly differentiated CRC, this association was not statistically significant. Similarly, even though early stage tumors (stages I and II) were associated with greater expression of Bcl-2 than advanced stage tumors (stages III and IV), this association was not statistically significant. This lack of statistically significant correlation between Bcl-2 immunohistochemical expression and prognostic parameters like tumor grade and stage, suggests that Bcl-2 immunoexpression may not be a significant prognostic marker in CRC. However, follow up studies, with correlation between Bcl-2 expression and cancer specific 5-year survival statistics, need to be done for definite assessment of the prognostic value of Bcl-2 in CRC.

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Conflict of Interest

The authors declared that there is no conflict of interest regarding the publication of this article.

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