Original Research Article

A study of expression of BCL-2 in colorectal carcinoma with histopathological correlation

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Abstract

Introduction: Colorectal cancer is the major cause of mortality and morbidity worldwide. Incidence in males - 4.3 /1, 00,000, in females - 3.4/1,00,000. The etiology of colorectal cancer is complex, involving interplay of environmental and genetic factors. Colorectal cancers have been reported to show BCL-2 over expression although, in comparison with adenomas, there is lower intensity of expression in the invasive tumors. There may also be a loss of expression with loss of tumor differentiation and it would appear that the role of BCL-2 is probably more important in the early development of colorectal tumors than in later tumor progression.

Aim and objectives: To perform and interpret BCL-2 immunostaining on all diagnosed colorectal carcinomas, to infer the rate of positivity and negativity of the BCL-2 expression in the colorectal lesions, to correlate the BCL-2 expression with the grades of the colorectal carcinomas, to indicate the prognostic value of BCL-2 expression in colorectal carcinomas.

Materials and methods: A present study was done in MGM Hospital, Warangal for duration of 5 years. A total of 64 cases were studied. All colorectal carcinoma cases are subjected to BCL-2 immunostaining.

Results: In present study, the commonest age group were from 60-69 years with majority of the lesions diagnosed as adenocarcinomas. Males contributed to large number of cases (33) and rectum is the most common site in the present study. Majority of cases (40.6%) in this study were well differentiated carcinomas. BCL-2 overexpression was seen in 42(65.6%) cases, majority were colorectal adenocarcinomas. 96.2% of well differentiated, 75% of moderately differentiated adenocarcinomas and 10% of poorly differentiated adenocarcinomas showed overexpression of BCL-

2 and 12.5% of mucinous carcinomas showed BCL-2 positivity. There was a significant correlation between grade and BCL-2 expression. Higher scores were seen in well to moderately differentiated carcinoma than poorly differentiated carcinoma. In well differentiated carcinoma, 19 cases showed score 3 and in moderately differentiated carcinoma, 5 cases showed score 3 which contained strong BCL-2 expression. In poorly differentiated carcinoma, majority (9 cases) were score 0 and in mucinous carcinoma as well, 69 majority (7 cases) were score 0 which contains negative BCL-2 expression. In rectal cancers BCL-2 overexpression was more common that colon cancers. Rectum shows 47% positivity and left colon 36%. Thus in present study rectal cancers showed high BCL-2 expression. Males (23 cases) showed higher BCL-2 expression than females (20 cases).

Conclusion: There is a significant correlation (P<0.00001) between BCL-2 over expression and grade of colorectal adenocarcinoma, but there is no significant correlation is observed with variables like age, gender, tumor location. BCL-2 over expression serves as good prognostic marker in colorectal adenocarcinomas and it will help to assess the responsiveness of patients to standard treatment.

Key words

BCL-2, Colorectal carcinoma, Histopathology.

Introduction

Colorectal cancer is a malignant epithelial tumor of the colon or rectum. Only tumors that have penetrated through muscularis mucosae into sub mucosa are considered malignant at this site [1]. Globally cancer of the colon and rectum is the fourth most common cancer in males and third leading cause of cancer in females with mortality paralleling incidence [2]. In the developed countries it is the second most common cancer after Lung. Incidence rates range from 25.3 per 100,000 in Eastern Europe to 45.8 per 100,000 in Australia [3]. Incidence in males - 4.3 /1, 00,000, in females - 3.4/1,00,000 [4]. Male sex, increasing age, presence of long standing IBD, and familial predisposition are strong risk factors. The mean age of incidence is 62 years. In high-risk areas 8% of patients are under the age of 50 years. About 60% of all patients diagnosed with colorectal carcinoma will present with locally advanced disease [5]. The incidence is little higher in males than in females. Cancer of right colon is more frequent in females of all ages. In males 40% occur in rectum, 30% in left and right colon. In females 40% occur in right colon, 30% each in left colon and rectum [6]. The etiology of colorectal cancer is complex, involving interplay of environmental and genetic factors. Colorectal carcinoma develops through a multistep process as characterized by histopathological precursor lesions and molecular genetic alterations including adenomatous polyposis coli (APC), KRAS, and p53 [7]. Therefore, considerable interest has focused on the identification of novel tumor-based markers that can more accurately predict the course of this malignancy, as well as determination of optimal adjuvant therapy approaches. BCL-2 is a cytoplasmic protein which localizes to mitochondria, endoplasmic reticulum, and the nuclear envelope. The protein can be identified in many different tissues and is thought to have a role in the inhibition of apoptosis [8]. BCL-2 overexpression has been identified in a large number of epithelial tumors in which its role as an inhibitor of apoptosis is thought to promote tumor growth [9, 10]. Normal epithelium showed strong staining for BCL-2 at the crypt bases with a gradient of diminishing intensity of staining along the crypt axis. Lymphocytes in the stroma and Peyer's patches stained with equal intensity to that of the epithelial cells in the bases of the crypts and served as internal positive controls [11]. Colorectal cancers have also been reported to show BCL-2 overexpression although, in comparison with adenomas, there is lower intensity of expression in the invasive tumors [12]. There may also be a loss of expression with loss of tumor differentiation [13] and it would appear that the role of BCL-2 is probably more

important in the early development of colorectal tumors than in later tumor progression. Loss of heterozygosity of the bcl-2 gene locus on chromosome 18q21.3 occurs in 60% of colorectal cancers [14].

Aim and objectives

- To perform and interpret BCL-2 immunostaining on all diagnosed colorectal carcinomas.
- To infer the rate of positivity and negativity of the BCL-2 expression in the colorectal lesions.
- To correlate the BCL-2 expression with the grades of the colorectal carcinomas.
- To indicate the prognostic value of BCL-2 expression in colorectal carcinomas.

Materials and methods

A present study was done for duration of 5 years (2 years Prospective and 3 years Retrospective) i.e, 2013- 2017 in MGM Hospital, Warangal. All the colorectal biopsies and resection specimens in all age groups, received in the Pathology Department during this period were considered. In present study we have evaluated colorectal resections and few numbers of biopsies in 64 patients between age groups 20 years to 90 years.

Inclusion criteria

- Only samples with definite histopathological diagnosis of carcinoma were considered.
- Representative areas in the biopsies are only included.

Exclusion criteria

- Non neoplastic lesions
- Congenital lesions like Hirschsprungs disease are excluded.
- Inadequate samples are excluded

Specimen handling

Colorectal biopsies and the resection specimens were fixed in 10%formalin and then sent for routine histopathological processing. After a histopathological diagnosis of the lesion was made, the paraffin blocks of the samples which had met the criteria of inclusion are collected. Basic details of the each case like the biopsy no, age and sex, clinical details, histopathological diagnosis are recorded. Sections were made from the paraffin embedded tissue block as follows:

- One 5-micron section taken for staining with H&E.
- Two 5-micron sections taken on the polylysine coated slides stained for BCL-2 IHC.

The results were recorded for individual case.

BCL-2 immunostaining using BCL-2 Antibody (DAKO)

- Sections underwent histologic evaluation to select blocks without necrotic and hemorrhagic areas.
- Consecutive 3-4μm sections were taken on polylysine coated slides and deparaffinized and Antigen-retrieval procedure was performed by trilogy solution using microwave method. Sections are thoroughly washed with buffer in between every step.
- Endogenous peroxidase blocking is done by horse radish peroxidase. Then, monoclonal antibody against BCL-2 protein (clone DO-7; Dako), was applied to the sections and incubated for 30 minutes at room temperature.
- Then, secondary antibody is added and incubated for 20 minutes.
- Then freshly prepared diaminobenzidine (DAB) was added to the sections for 10 minutes and the sections were lightly counterstained with hematoxylin.
- Slides were then dehydrated, cleared and mounted

Interpretation

Immuno reactivity for BCL-2 was evaluated semiquantitatively according to the percentage of positive tumor nuclei, scored as follows:

- None (<5%),
- Weak (+, 5 – 25%),
- Moderate (++, 25 – 50%),

- Strong (+++, >50%).

All tumors showing Bcl2 immunoreactivity (at least +) were considered to be positive (**Photo – 1 to 21**).

Photo – 1: Ascending colon growth.

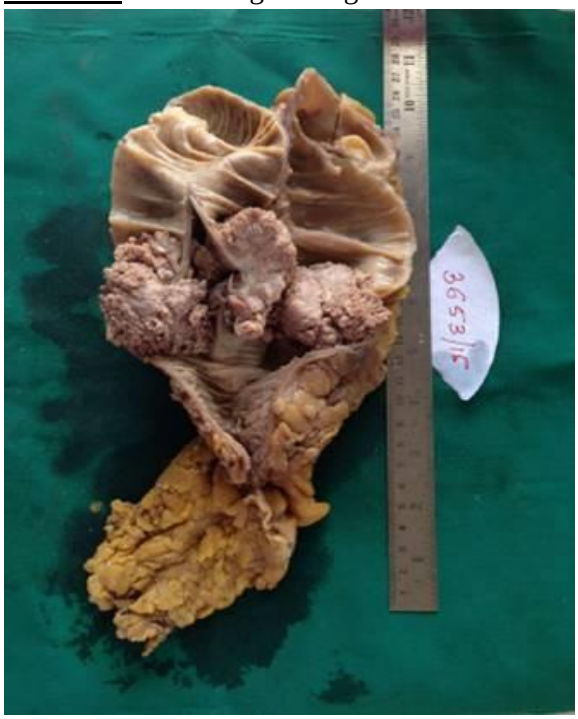


Photo – 2: Rectosigmoid Stricture.

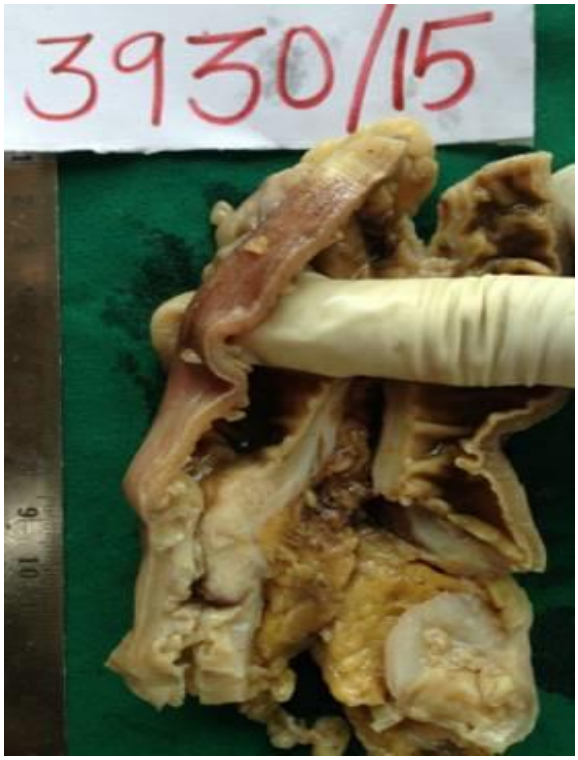


Photo – 3: well differentiated adenocarcinoma.

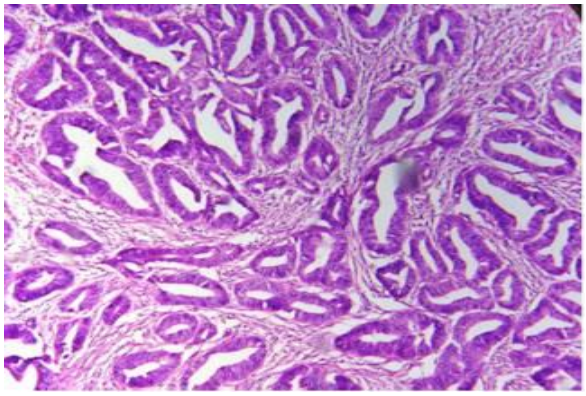


Photo – 4: Moderately differentiated adenoca.

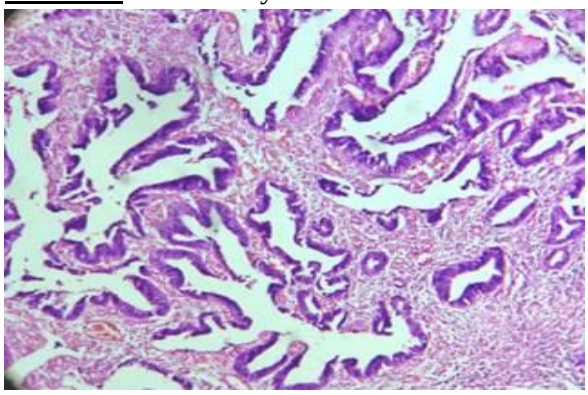


Photo – 5: Poorly differentiated adenoca.

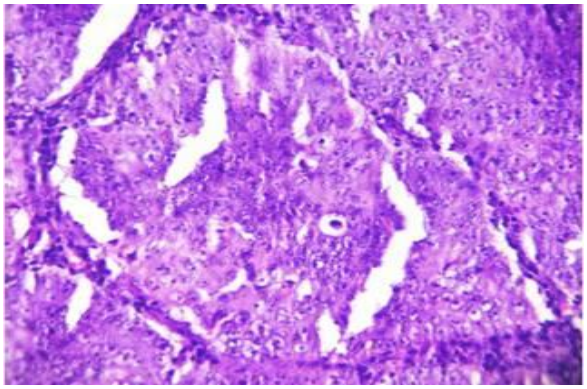


Photo – 6: Adenocarcinoma with signet ring type.

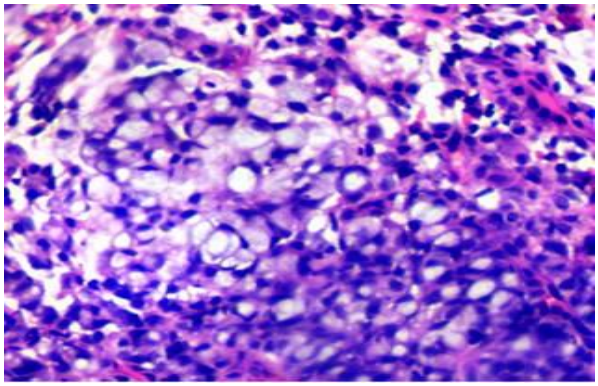


Photo – 7: Mucinous carcinoma.

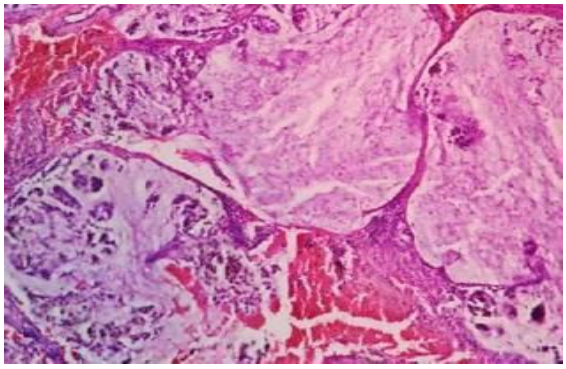


Photo – 8: Papillary adenocarcinoma.

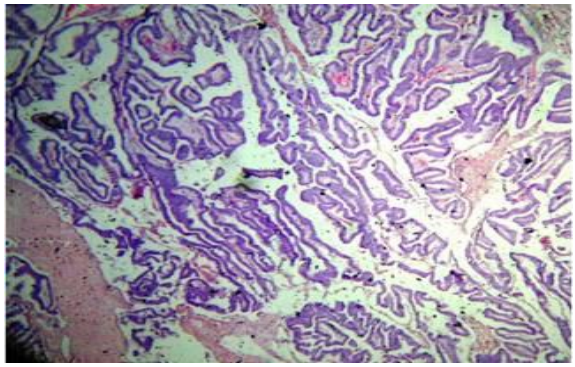


Photo – 9: BCL2 +ve control epithelial cell in basal crypt and lymphocytes.

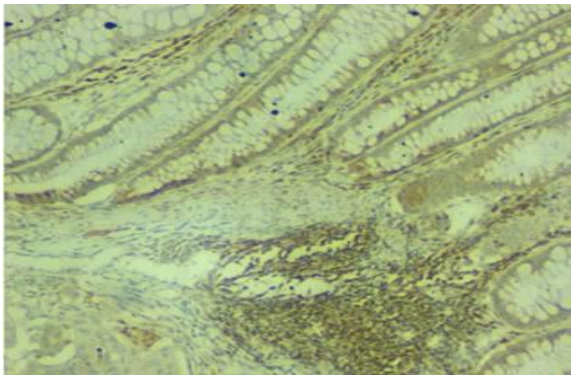


Photo – 10: Grade 1 Adenocarcinoma - BCL2 Positive Score 3.

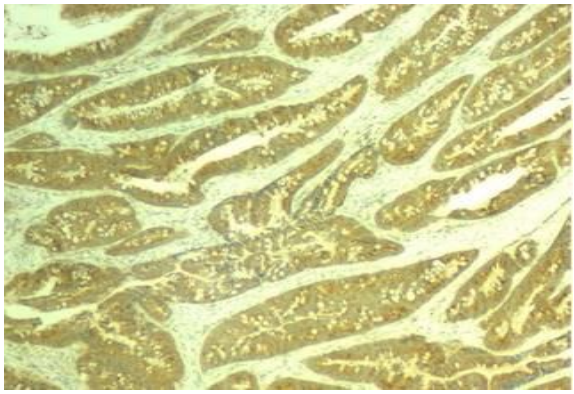


Photo – 11: Grade 1 Adenocarcinoma - BCL2 Positive score 2.

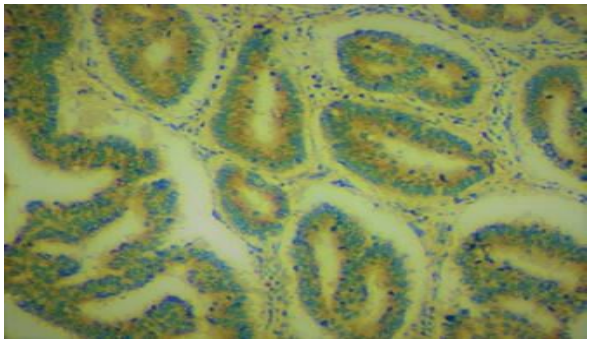


Photo – 12: Grade 1 Adenocarcinoma - BCL2 positive score 1.

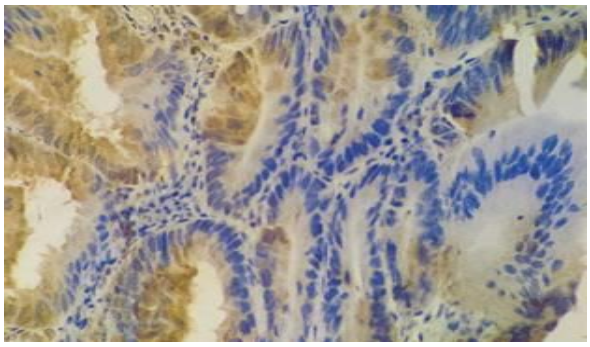


Photo – 13: Grade 1 Adenocarcinoma- BCL2 Negative score 0.

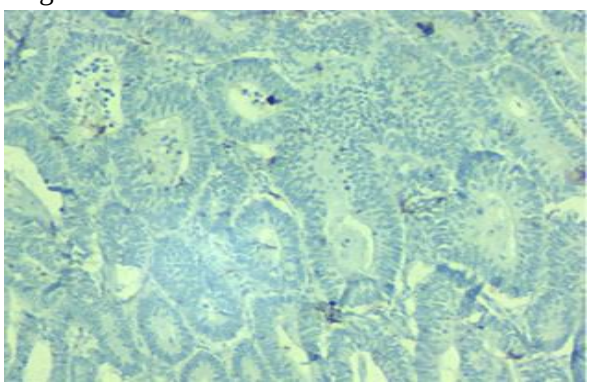


Photo – 14: Grade 2 Adenocarcinoma - BCL2 positive score 3.

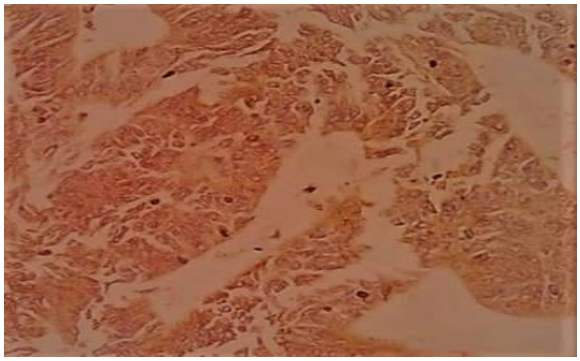


Photo – 15: Grade 2 Adenocarcinoma - BCL2 positive score 2.

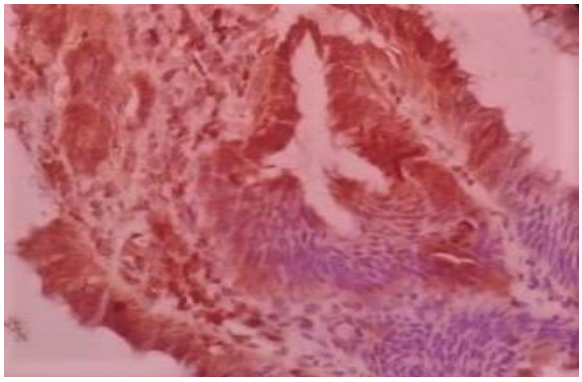


Photo – 16: Grade 2 Adenocarcinoma - Bcl2 Positive score 1.

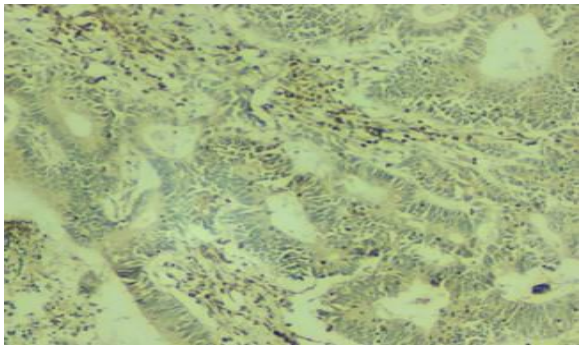


Photo – 17: Grade 3 Adenocarcinoma - BCL2 positive score 1.

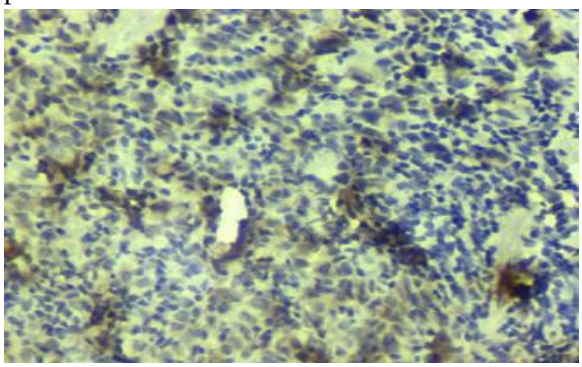


Photo – 18: Grade 2 Adenocarcinoma - BCL2 positive score 0.

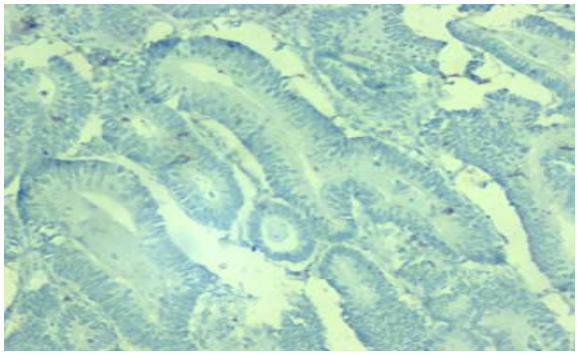


Photo – 19: Moderately differentiated adenoca.

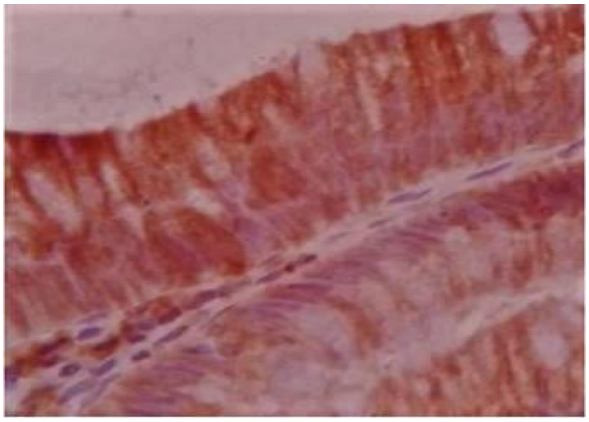


Photo – 20: Grade 3 Adenocarcinoma - Bcl2 Negative score 0.

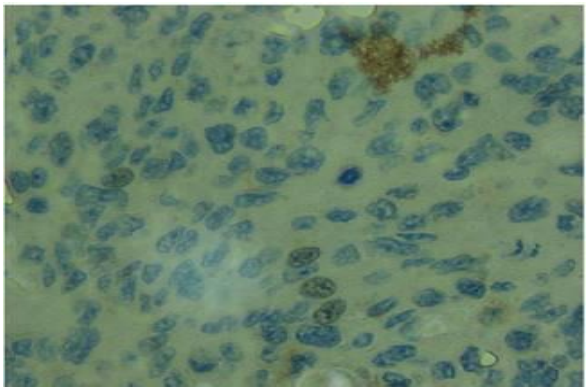
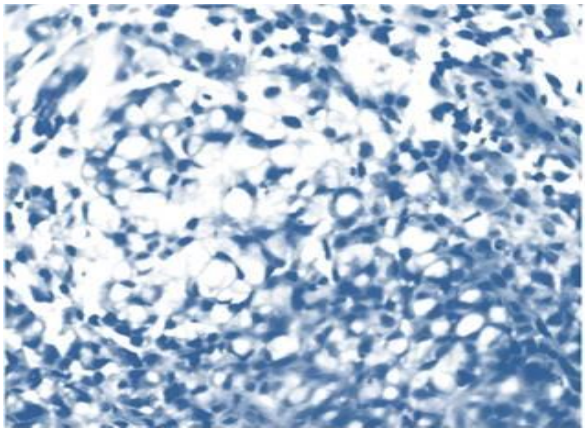


Photo – 21: Poorly differentiated adenocarcinoma with signet ring and BCL2 negative.

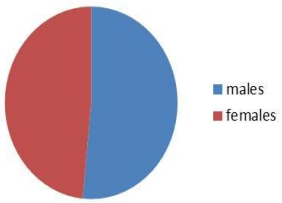


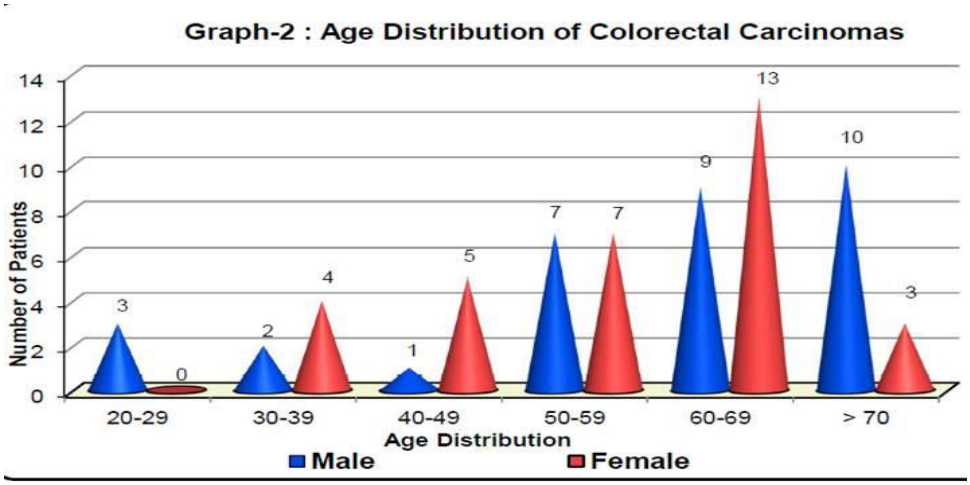
Results

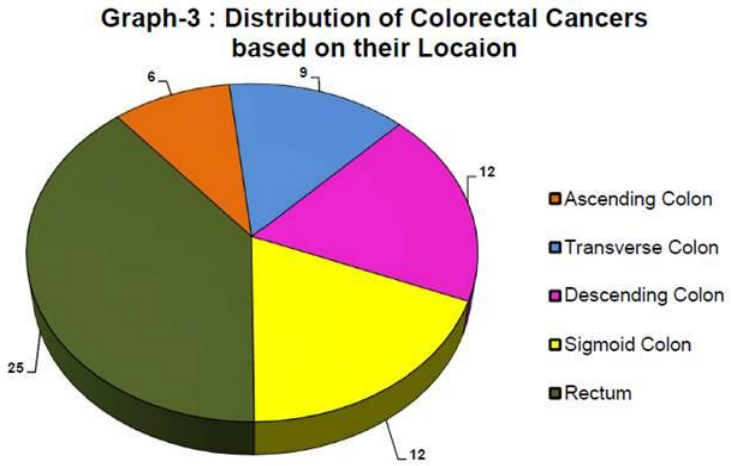
In present study, we have evaluated colorectal resections and few number of biopsies in 64 patients between age groups 20 years to 90 years from 2013 to 2017 (2 years prospective 3 years retrospective) in MGM Hospital, Warangal.

Out of 64 patients of colorectal carcinoma cases male patients were 33(52%) and female patients were 31(48%). Incidence of colorectal carcinoma was more in males when compared to females (**Graph – 1**). Incidence ratio of Male to Female was 1.1:1.

Graph – 1: Sex distribution in colorectal carcinoma.







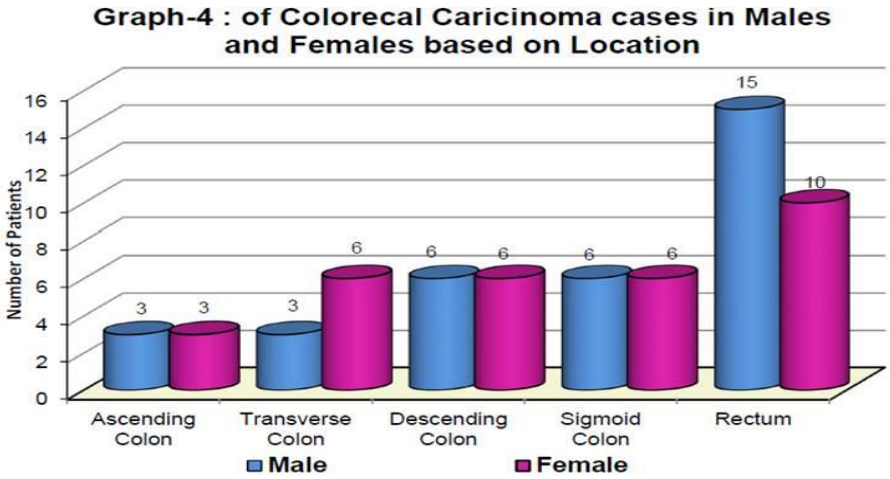


Table - 1: Grading of colorectal carcinoma.

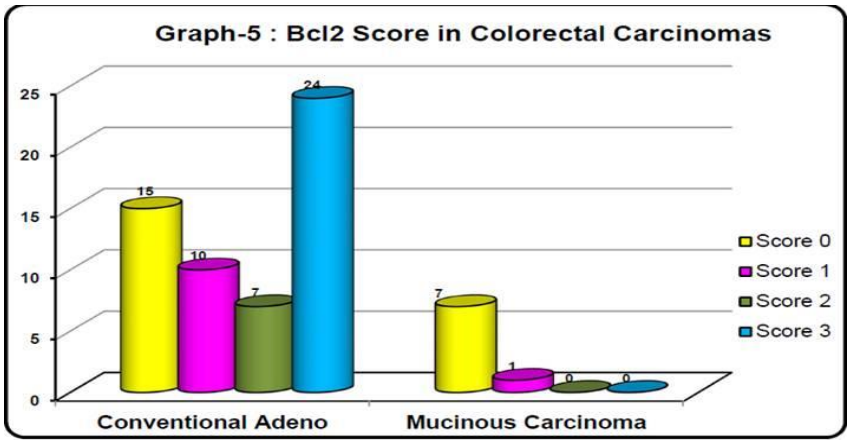
Grade	No. of cases	Male	Female	Total percentage
Well differentiated	26	12	14	40.6%
Moderately differentiated	20	13	07	31.3%
Poorly differentiated	10	05	05	15.6%
Mucinous carcinoma	08	03	05	12.6%

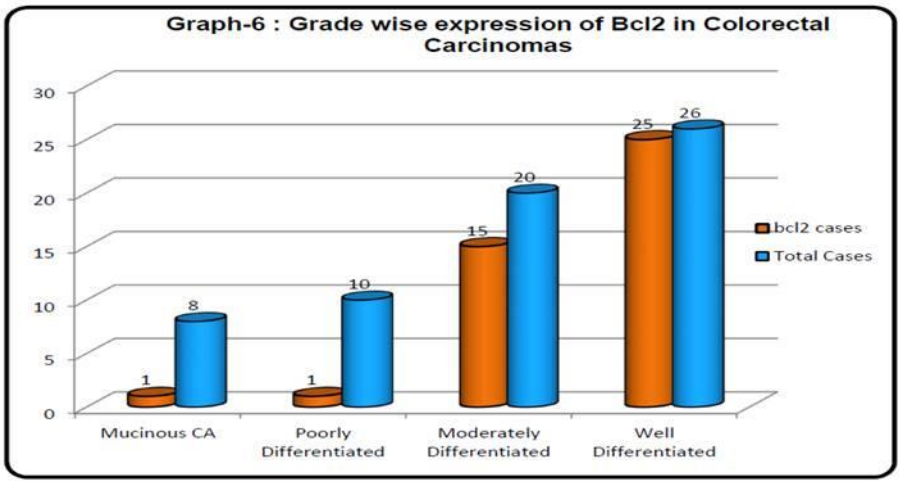
Table - 2: BCL-2 expression in colorectal adenocarcinoma.

BCL-2 expression	Positive	Negative	Total
Conventional	41(97.6%)	15	56
Mucinous	1(2.4%)	07	08
Total	42	22	64

Table - 3: BCL-2 score in relation to histological grade.

Histological grade	BCL-2 score				Total	Total positive
	0	1+	2+	3+		
Well differentiated	1	3	3	19	26	25(96.2%)
Moderately differentiated	5	6	4	5	20	15(75%)
Poorly differentiated	9	1	0	0	10	1(10%)
Mucinous carcinoma	7	1	0	0	8	1(12.5%)
Total	22	11	7	24	64	42(65.6%)





Highest incidence of colorectal carcinoma in this study was found in the 7th decade which constitutes 34%, of which majority (13 cases) were female patients. In the age group of 20-29 years, only 3 cases were seen and all of them were male patients. In the 4th and 5th decades majority were female patients. In the age group of <30 and >70 years male patients were predominantly affected (**Graph – 2**).

In present study, most frequent site was rectum consisting of 25 cases (39%). Similar number of cases (12) was found in each of sigmoid colon and descending colon. Both of them constituted around 18.7%. Least number of cases (6) was found in ascending colon. It constitutes around 9.3% (**Graph – 3**).

Most common site in present study was rectum 25 cases (39%). In both sex, rectum was the most common site followed by sigmoid and descending colon. In rectum, male patients (15) outnumbered female patients (10). Equivalent number of cases was found in each of sigmoid colon and descending colon in both sexes. In transverse colon male patients involved in 3 cases female patients in 6 cases. Male patients comprised large number of cases in rectum and transverse colon, where as in transverse colon female patients were more commonly involved (**Graph – 4**).

Colorectal carcinomas - microscopic findings

Out of 64 cases, 26 cases (40.6%) were well differentiated adenocarcinomas. 20 cases (31.3%) were moderately differentiated adenocarcinomas, 10 cases (15.6%) were poorly differentiated adenocarcinomas and 8 cases (12.6%) were mucinous carcinomas (**Table – 1**).

BCL-2 immunostaining results on colorectal carcinomas

BCL-2 IHC was done on all the cases. Out of 64 casesm 42 were positive for BCL-2 in which 56 were colorectal adenocarcinomas and 8 were mucinous carcinomas. 42(65.6%) cases of colorectal carcinoma showed positive immuno staining for BCL-2 and 22 cases were negative (**Table – 2**).

In present study, out of 64 cases 42 cases showed BCL-2 cytoplasmic positivity which constituted 65.6% cases. Out of 42 cases of conventional adenocarcinoma 41(97.6%) were BCL-2 positive, 15 were negative. Out of 8 mucinous carcinoma 1(2.4%) was BCL-2 positive, 7 were negative (**Graph – 5**).

In conventional adenocarcinoma, 15 cases showed score 0 (negative), 10 cases (24.4%) showed score 1, 7 cases (17.1%) showed score 2 and 24 cases (58.5%) showed score 3. Majority of cases showed score 3 in conventional adenocarcinoma. In mucinous carcinoma, 7 cases showed score 0 (negative) and 1 case showed score 1 (100%) as per **Table - 3**.

BCL-2 score of at least 1+ was considered positive. Higher scores were seen in well to moderately differentiated carcinomas than poorly differentiated carcinomas. In well differentiated carcinoma, 19 cases showed score 3, 3 cases showed score 2 and 3 cases showed score 1. In moderately differentiated carcinoma, 5 cases showed score 3, 4 cases showed score 2 and 6 cases showed score 1. In poorly differentiated carcinoma, 1 case showed score of only 1. Majority (9 cases) were score 0 (negative). In mucinous carcinoma, 1 case showed score of only 1. Majority (7cases) were score 0 (negative) as per **Graph - 6**.

In present study, total of 64 cases of colorectal carcinoma cases were subjected to BCL-2 staining. Out of these 42 cases showed positive staining these included 29 (69.1%) cases, in <60 years of age and 13(30.9%) cases in >60 years of age. 22 cases showed negative staining, of these 19(86.3%) cases were <60 years of age and 3 (13.6%) cases were >60 years of age (**Graph – 7**). In this study, BCL-2 expression was higher in males compared to females. Out of 33 cases males showed 22 positive cases and 11 negative cases. Out of 31 cases females showed 20 positive and 11 negative cases (**Table – 4**).

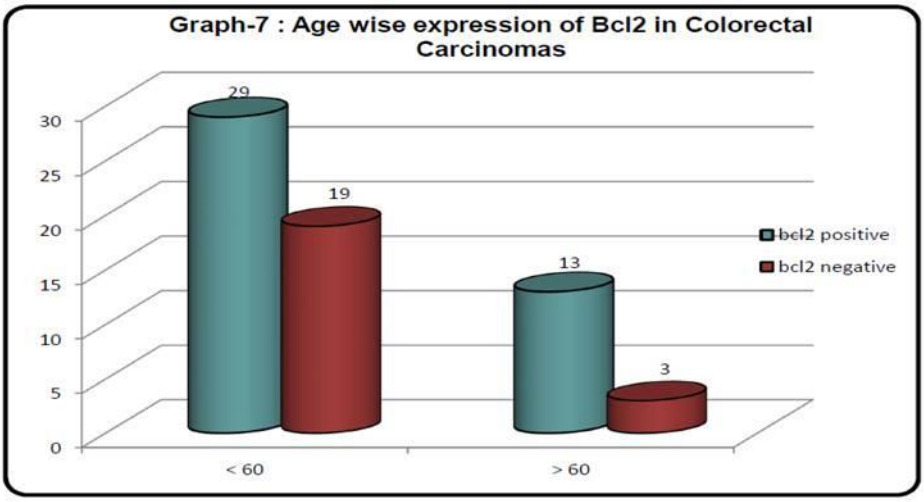


Table - 4: Clinicopathologic features and BCL-2 over expression of colorectal carcinoma.

Variables		Positive	Negative	Total	P – value
Gender	Male	23	10	33	<0.750
	Female	20	11	31	
Age	<65 years	29	19	48	<0.250
	>65 YRS	13	3	16	
Location	Rectum	19	6	25	< 0.2275
	Sigmoid	05	7	12	
	Descending colon	11	5	16	
	Proximal colon	07	4	11	
Differentiation	Well	25	1	26	<0.00001
	Moderately	15	5	20	
	Poor	01	9	10	
	Signet ring	01	7	08	

BCL-2 cytoplasmic positivity was higher in left sided tumors. Rectum showed 47% of positive cases followed by 36% in left colon and 17% in right colon (**Table – 4**).

BCL-2 expression was compared with clinicopathologic variables like age, gender, location and histological type of tumor. Statistical analysis revealed a significant correlation between histological type and BCL-2 expression. There was no statistically significant correlation between bcl2 expression and age and gender, tumor location.

Discussion

Colorectal carcinoma is by far most common and most curable cancer of GIT. More than 90% of cancers in this region are adenocarcinomas. In this study, age group ranges from 20-90 years and highest incidence found between 60-69 years. This is consistent with studies done by Contu, et al. [15] and Neagoes, et al. [16]. Most of colorectal carcinomas exhibited ulceroproliferative growth grossly. In this study there is male preponderance 33 (52%) and females 31 (48%). M: F ratio is 1.1:1. In the present study no significant relationship between Bcl2 expression and age or sex, site of colorectal carcinoma is consistent with Al-Temimi [17] who found no relationship between these variables. In present study, adenocarcinoma accounts for 88% of cases and mucinous carcinoma accounts for 12% of cases. This is consistent with study done by Nicholas FS

Watson, et al. [18], they reported in their study 85% are adenocarcinomas, 11% mucinous and 1% signet ring, 1% columnar, 2% unknown carcinomas. Rectum was most common site in both sex and in all age groups.

BCL2 expression in relation to histological grade

AJM Watson, et al. [12] studied BCL-2 expression by immune staining in 52 cases of colorectal cancers and correlated the BCL-2 expression with grade and location of tumors. Most of the rectal carcinomas showed BCL-2 over expression. 66.6% of the well differentiated and 26.4% moderately differentiated tumors were positive for BCL-2 expression. Poorly differentiated as well as signet ring carcinomas showed negative expression. This study didn't mention anything about BCL-2 scoring (**Table – 5**).

Table - 5: Comparison of BCL-2 expression in colorectal Adenocarcinomas in present study with AJM Watson, et al. study.

Variablese		AJM Watson et al Study 1995 yr (36.5% Bcl2 positivity)			Present Study (65.6%)		
		CASES	NO OF Bcl2 POSITIVE	NO OF Bcl2 NEGATIVE	CASES	NO OF Bcl2 POSITIVE	NO OF Bcl2 NEGATIVE
Location	Rectum	18	6(33.3%)	12(66.6%)	25	19(76%)	6(24%)
	Sigmoid	13	6(46.2%)	7(53.8%)	12	5(41.7%)	7(58.3%)
	Descending Colon	5	3(60%)	2(60%)	16	11(68.8%)	5(31.2%)
	Proximal Colon	15	4(26.7%)	11(73.3%)	11	7(63.6%)	4(36.4%)
Differentiation	Well	12	8(66.4%)	4(33.3%)	26	25(96.2%)	1(3.8%)
	Moderate	34	9(26.4%)	25(73.6%)	20	15(75%)	5(25%)
	Poor	2	0(0%)	2(100%)	10	1(10%)	9(90%)
	Signet ring	1	0(0%)	1(100%)	8	1(12.5%)	7(87.5%)

Of all the BCL-2 positive cases 15.8% of cases had a diffuse pattern of staining whereas 84.2% had a focal immune reactivity.

In present study 25(96.5%) of well differentiated, 15(75%) of moderately differentiated and 1 (10%) poorly differentiated adenocarcinomas were positive for BCL-2 over expression, results are comparable with AJM Watson, et al. [12] study results.

The present study showed significant association ($P< 0.00001$) of increasing grade of tumor with bcl2 expression which correlates well with AJM Watson, et al. [12] and Al-Temimi [17].

BCL-2 immunoreactivity was more frequent in well differentiated than moderately or poorly differentiated adenocarcinomas but no relationship was found between tumor sites within the colon. This is in concordance with study by Al-Temimi [17] who found BCL-2 over expression in 85.7% of well to 35.3% moderate versus 7.7% of poorly differentiated tumors. BCL-2 gene activation, which has been shown to occur earlier in the adenoma-carcinoma sequence, might represent a marker involved in tumor initiation. The localization of the BCL-2 to the dysplastic cells in colorectal adenomas confirms its role in initiation of tumor growth by

inhibiting apoptosis and consequently the accumulation of genetic alterations [19].

In normal colonic tissue (control cases), bcl-2 expression was restricted to the base of colonic glands indicating that basal epithelial cells of the normal colonic crypts uniformly express the bcl-2 protein. Given that the crypt cell population arises from basally located stem cells, the immunolocalization of BCL-2 suggests that it protects stem cells from apoptosis. The finding of a high level of diffuse homogenous BCL-2 expression in dysplastic and malignant cells, in contrast to non-neoplastic cells, suggests that abnormal bcl-2 gene activation is an early event in neoplastic development or progression of colorectal carcinoma [20].

Al Temimi [17] study noticed the strong expression of BCL-2 were seen 4(66.7%) in grade I more than grade II 1(16.7%) while 0(0%) in grade III, all grade III positive cell give weak expression. There was statistically significant relationship between the grade of tumor and the BCL-2 expression and its intensity (p value <0.05) as per **Table - 6**.

Table - 6: The comparison of BCL-2 score in colorectal carcinoma in present study with Al-Temimi study.

	Al-Temimi study (2011yr)				Present Study			
Grades Score	Negative 0	Bcl2 Positive 1	2	3	Negative 0	Bcl2 Positive 1	2	3
Grade-I	1(14.3%)	0	2(33.3%)	**4(66.7%)**	1(3.84%)	3(12%)	3(12%)	**19(76%)**
Grade-II	11(64.7%)	1(16.7%)	4(66.6%)	1(16.7%)	5(25%)	6(40%)	4(26.7%)	5(33.3%)
Grade III	24(92.3%)	2(100%)	0(0%)	**0(0%)**	9(90%)	1(100%)	0(0%)	**0(0%)**

Present study showed 25(96.2%) positive expression in grade I, 15(75%) in grade II and 1(10%) in grade III. The highest score 3 of BCL-2 were seen 19(76%) in grade I more than grade II 5(33.3%) while 0(0%) in grade III, all grade III positive cases give weak expression.

In colorectal carcinoma, BCL-2 expression is more intense in well-differentiated as well as moderately differentiated carcinoma than the expression of BCL-2 in poor differentiated. There is a significant association (P<0.00001) between BCL-2 expression and grade of tumor, hence the role of BCL-2 in colorectal carcinoma is a favorable prognostic factor. These results were in concordance to Miao Ouyang, et al. [21] 2005 where 79.3% of cases with strong BCL-2 positive in grade I, 60.9% of cases with moderate positive in grade II.

Present study was comparable with other studies as per **Table - 7**. There is a significant relation (p value < 0.05) between BCL-2 expression and histological grade of tumor. BCL-2 expression decreases from well differentiated adenocarcinomas (96.2%) to moderately differentiated adenocarcinomas (75%) to poorly differentiated adenocarcinoma (10%). This significant association also found in AJM Watson, et al. [12] and Al Temimi [17] studies.

No significant association between BCL-2 and histological grade of tumour found in Ban Quasim [22] and Nicholas FS Watson, et al. [18] who also found increased cytoplasmic

accumulation of BCL-2 in moderately and poorly differentiated tumors compared to well differentiated tumors. Sinicrope, et al. [20] reported cytoplasmic positivity for BCL-2 in 67% of colorectal adenocarcinomas. Hague, et al. [23] found BCL-2 immunoreactivity in 75% of colorectal carcinoma.

Present study showed BCL-2 positivity in 65.6% cases which is slightly lower than other studies as per **Table - 8**. Diffuse BCL-2 positivity was observed in 24 of 42 (57%) positive cases.

In Sinicrope, et al. [20] study 14 of 21 (67%) cases, BCL-2 staining was detected in carcinoma cells in the malignant glands. Diffuse (>75% of cells), homogenous bcl2 immunostaining was observed in 11 of 14 (78%) positive cases. Localization of staining was predominantly cytoplasmic and in several cases also involved the nuclear membrane. Furthermore, cytoplasmic immunoreactivity was most prominent in the apical portion of the tumor cells. Similar features found in present study as well.

Table - 7: Comparison of BCL-2 overexpression association with grade of colorectal carcinoma in various studies.

Study	BCL-2 association with grade
Nicholas FS Watson, et al. [18]	No significant association
Ban Qasim, et al. [22]	No significant association
AJM Watson, et al. [12]	significant association
AL Temimi [17]	significant association
Present study	significant association

Table - 8: BCL-2 positivity in colorectal carcinomas in various studies.

Study	No. of cases	BCL-2 positivity
AJM Watson, et al. [12]	52	19(36.5%)
Al Temimi [17]	50	14(35.7%)
Nicholas FS, et al. [18]	462	199(43.1%)
Sinicrope, et al. [20]	21	14(67%)
Bronner, et al. [24]	26	11(42.3%)
Ofner, et al. [13]	100	36(36%)
Hague, et al. [23]	100	75(75%)
Zhao, et al. [25]	93	53(57%)
Present study	64	42(65.6%)

BCL-2 expression in relation to sex

In this study BCL-2 expression is higher in males compared to females. Out of 33 cases males show 22 positive cases and 11 negative cases. Out of 31 cases females show 20 positive and 11 negative cases. There was no significant association between BCL-2 positivity and sex. Ban Qasim, et al. [22] and Al Temimi [17] found no significant association between BCL-2 positivity and sex.

BCL-2 expression in relation to tumor location

In this study BCL-2 positive cases favored commonly in left side colon with most of the cases involving rectum (47%) followed by descending colon (36%) and ascending colon (17%) but no significant association between bcl2 expression and site of tumor. Similar results found in Al Temimi [17] study in which rectum involved in 38.5% cases which showed positive expression more than in colonic carcinoma (16.7%) but no statistically significant between site of tumor and BCL-2 expression, these result concordance with Petrisor O, et al. [26] 2008, in their study the positive expression of BCL-2 in

rectal was (60%) more than colonic (46.6%) from the 15 cases of colonic, and 15 rectal carcinoma with no significant association with any of two locations of rectal and colonic carcinoma.

Follow up

Out of 64 cases, 16 patients were expired due to metastasis and 3 patients were expired due to non-metastatic causes. 36 patients were survived until this year and lost follow up of 9 cases. Overall survival rate 65.4% after 3years.

In a total of 42 positive patients 24 cases were BCL-2 score 3, 7 cases were BCL-2 score 2, 11 cases were score 1, 22 cases were BCL-2 score 0, Cases with BCL-2 score 3 were found to have high survival rate whereas cases with BCL-2 Score 0 found to have high mortality with worst prognosis.

In view of the above results, cases with BCL-2 score 3 is considered as low grade. Cases with BCL-2 score 0 is considered as high grade and have worst prognosis. Hence BCL-2 scoring is an important in assessing the prognosis and outcome in patients with colorectal carcinoma. This analysis suggests that expression of BCL-2 protein is associated with favorable prognosis in patients with colorectal carcinoma. Present study showed immunohistochemical results on tumors from patients with colorectal carcinoma revealed a statistically significant association of bcl2 expression with favorable clinical outcome.

BCL-2 was detectable in colorectal carcinoma cases with uneventful clinical course, whereas the cases with the development of metastasis and local recurrence completely lacked immunohistochemically detectable BCL-2 protein. These findings support the concept that BCL-2 expression is related to slower local tumor growth

Conclusion

In present five year study we have 64 cases of colorectal carcinoma of which 42 cases are BCL-2 positive and 22 cases are BCL-2 negative. Majority of cases in which there is Bcl2 expression belongs to conventional adenocarcinoma (41 out of 42) and one is mucinous type of carcinoma. There is significant over expression of BCL-2 in as well as moderately differentiated carcinomas, when compared to poorly differentiated carcinomas. Hence BCL-2 has a definite role in colorectal cancer differentiation. Therefore there is significant correlation between BCL-2 expression and grade of tumor. Since grade is a proven prognostic marker, along with the grade, BCL-2 over expression and scoring will also be another good prognostic marker in colorectal carcinomas. But there is no association between BCL-2 expression and other variables like Age, gender, site of tumor. Several studies have shown tumors which lose this inhibition of apoptosis actually have a worse prognosis than which retain BCL-2 expression. BCL-2 regulates radiation induced apoptosis in colorectal epithelium and it provides information predicting the response of colorectal tumors to radio and chemotherapy and also patient survival. Recent results suggest that bCL-2 expression is an independent prognostic factor associated with favorable clinical outcome.

BCL-2 oncoprotein expression in colorectal carcinoma has been demonstrated as a being a favorable prognostic factor and associated with less aggressive tumor behavior. Analysis of colorectal tumors for BCL-2 expression of value in predict therapeutic response, prognosis and potential targets of new pharmacological agent

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