JournalofCardiovascularDiseaseResearch

ISSN:0975-3583,0976-2833 VOL13, ISSUE05,2022

Original research article

A study of expression of CDX2 and BCL2 in colorectal carcinoma with histopathological correlation

[1]Dr. Lanke Yatish Sai, [2]Dr. B Laxminarayana, [3]Dr. Padmini Chinta, [4]Dr. BV Anuradha Devi

[1,3]Senior Resident, Department of Pathology, GMC, Suryapet, Telangana, India

[2]Associate Professor, Department of Pathology, KMC, Warangal, Telangana, India

[4]Assistant Professor, Department of Pathology, GMC, Suryapet, Telangana, India

Corresponding Author:

Dr. B Laxminarayana (drbln69@gmail.com)

Abstract

Background: Colorectal cancer (CRC) is one of the most common malignancies worldwide. Despite the great progress made in clinical treatment, the morbidity and mortality of CRC remains high. Aberrant expression of BCL2 and CDX2 have been implicated in several cancer types including CRC. In this review, we elaborated on the diagnostic utility of CDX2 and BCL2 in Colorectal adenocarcinoma.

Aim & Objectives:

1. Perform and interpret CDX2 and BCL2 staining on all diagnosed cases of colorectal adenocarcinomas by H&E staining.
2. Infer the rate of positivity and negativity of CDX2 and BCL2 expression in all these colorectal lesions, thereby aiding in prognostication.
3. Compare the present study with literature available.

Materials & Methods: This study was conducted in the department of pathology MGM hospital, Warangal, TELANGANA during a period between 2018 to 2021. A total of 62 cases were studied, after routine tissue processing by standard operating procedures, H and E staining was done for histopathological examination. CDX2 and BCL2 (DAKO) immunohistochemical staining was done on all diagnosed cases included.

Results & Discussion: The present study includes 62 surgically resected and biopsy specimens of colorectal cancer at Mahatma Gandhi Memorial (MGM) hospital, Kakatiya Medical college, Warangal. Out of 62 patients of colorectal carcinoma cases male patients were 29 (46.7%) and female patients were 33 (53.3%). Incidence of colorectal carcinoma is more in females when compared to males in our study. Out of the 62 cases 51 were positive for BCL2 and 56 were positive for CDX2. In present study 36 (97.2%) of well differentiated, 13(86.6%) of moderately differentiated and 1(14.2%) poorly differentiated adenocarcinomas were positive for BCL2 over expression, while CDX2 positivity was seen in 36 (97.2%) of well differentiated, 14 (93.3%) of moderately differentiated and 4(57%) poorly differentiated adenocarcinomas. There is a significant correlation between grade and BCL2 and CDX2 expression. There is a significant correlation ($P<0.00001$) between BCL2 and CDX2 expression and grade of colorectal adenocarcinoma. Higher scores are seen in well to moderately differentiated carcinoma than poorly differentiated carcinoma.

Conclusion: In the present study, well and moderately differentiated colorectal adenocarcinomas gave a score of 3+ and 2+ on immunostaining with CDX2 and BCL2 and poor to no staining in poorly differentiated adenocarcinomas. This shows a correlation between the grade of tumors and immunostaining, thus agreeing with previous studies and literature. Recent results suggest that CDX2 and BCL2 are independent prognostic factors associated with favourable outcome, extensive studies are required to validate the same.

Keywords: Colorectal carcinoma (CRC), CDX2, BCL2, IHC

Introduction

Colorectal carcinoma (CRC) is a major cause of mortality and morbidity worldwide. It is a malignant epithelial tumour of the colon or rectum. Only tumours that have penetrated through muscularis mucosae into sub mucosa are considered malignant at this site [1]. In developed countries it is the second most common cancer after Lung cancer. Incidence rates range from 25.3 per 100,000 in Eastern Europe to 45.8 per 100,000 in Australia[2]. Incidence rates in India are quite low about 2 to 8 per 100,0003. Incidence in males-4.3 /1, 00,000, in females-3.4/1,00,000[3]. It is the third most commonly diagnosed cancer in males and the second in females, with more than 1.4 million new cancer cases every year [4].

The age standardized rate (ASR) for CRC in India is low at 7.2 per 100,000 population in males and 5.1

ISSN:0975-3583,0976-2833 VOL13, ISSUE05,2022

per 100,000 population in women [5]. India has a very low 5-year survival rate of less than 40%. The CONCORDE-2 study also revealed falling five year survival rate in India [6]. This points towards shortcomings in diagnostic and treatment pathways in CRC in India.

Role of CDX2 in colorectal adenocarcinoma

In adults CDX2 is found exclusively in intestinal epithelial cells, most abundantly in the proximal colon. The protein (CDX2) is normally present throughout embryonic and postnatal life within the nuclei of epithelial cells of the intestine from the proximal duodenum to the distal rectum, using molecular techniques, expression of CDX2 mRNA has been found to be highly restricted to intestinal epithelium.

CDX2 expression in colorectal adenocarcinomas has been widely varied in numerous previous studies. Some studies have reported its expression in 98% to 100% of cases, while others have observed loss of CDX2 expression in 14% to 37% of cases [7, 8]. Loss of CDX2 expression in colorectal cancer has been found to correlate with high tumor grade, microsatellite instability or advanced tumor stage [9, 10]. Recently, Dalerba *et al*. showed that lack of CDX2 expression was associated with a worse outcome in stage II and III patients of colon cancer, and that patients with high-risk stage II colon cancer lacking CDX2 appeared to benefit from adjuvant chemotherapy as compared with their CDX2 positive counterpart. Loss of CDX2 is also more frequently encountered in mismatch repair-deficient colorectal cancer [11]. CDX2 loss was associated with high overall mortality among patients with a family history of colorectal cancer [11]. This implies the importance of CDX2 in the suppression of tumorgenesis in a subset of colorectal cancers and its potential for use as a prognostic marker in identifying high risk patients.

Role of BCL2 in colorectal adenocarcinoma

The BCL2 family of proteins play a central role in regulating cell death, of which the BCL2 gene is an important component, and is known gatekeeper to the apoptotic response [14]. The BCL2 gene is located at chromosome 18q21 and is present at the mitochondrial level, the endoplasmic reticulum and the nuclear envelope. It codes for an oncoprotein which serves as a programmed cell death inhibitor (anti-apoptotic), thereby increasing the life span of the cell [12, 13].

In the normal colon, BCL2 has been found to be positive in the cells at the base of the crypts [12]. It has been reported that the expression of BCL2 is both frequent and abnormal, early in the carcinogenesis of colorectal carcinomas [15].

Aims and Objectives

1. To perform and interpret BCL2, CDX2 immunostaining on all diagnosed colorectal carcinomas.
2. To infer the score of positivity and negativity of the BCL2 and CDX2 expression in the colorectal lesions.
3. To correlate the BCL2 and CDX2 expression with the grades of the colorectal carcinomas.
4. To indicate the prognostic value of BCL2 and CDX2 expression in colorectal adenocarcinomas.

Materials & Methods

A present study was done for duration of 3 yrs (2yrs Prospective and 1yr Retrospective) i.e., 2017- 2020 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.

Inclusion criteria

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

Exclusion criteria

1. Non-neoplastic lesions
2. Congenital lesions like Hirschsprungs disease are excluded.
3. 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. 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:

1. One 5-micron section taken for staining with H&E.
2. Two 5-micron sections taken on the polylysine coated slides stained for CDX2 and BCL2 IHC.

Immunostaining using BCL2 and CDX2 antibody (DAKO)

1. Sections underwent histologic evaluation to select blocks without necrotic and hemorrhagic areas.

ISSN:0975-3583,0976-2833 VOL13, ISSUE05,2022

2. Consecutive 3-4µm sections were taken on polylysine coated slides at the next stage, sections were deparaffinized and Antigen-retrieval procedure was performed by trilogy solution using microwave method. Sections are thoroughly washed with wash buffer in between every step.
3. Endogenous peroxidase blocking is done by horse raddish peroxidase. Then, monoclonal antibody against BCL2 and CDX2 protein was applied to the sections and incubated for 30 minutes at room temperature.
4. Then, secondary antibody is added and incubated for 20 minutes.
5. 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 BCL2 and CDX2 was evaluated semiquantitatively according to the percentage of positive tumor nuclei, scored as follows:

1. None (<5%).
2. Weak (+, 5-25%).
3. Moderate (++, 25-50%).
4. Strong (+++, >50%).

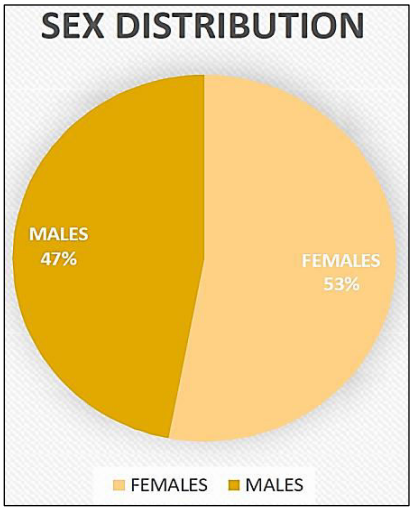
All tumors showing BCL2 and CDX2 immunoreactivity (at least +) were considered to be positive.

Results and Observations

In present study we have evaluated colorectal resections and few number of biopsies in 62 patients between age groups 20 years to 70 years from 2017 to 2020 (2yrs prospective 1yr retrospective) in MGM hospital, Warangal.

Out of 62 patients of colorectal carcinoma cases male patients were 29 (46.7%) and female patients were 33 (53.3%). Incidence of colorectal carcinoma is more in females when compared to males in our study.

Highest incidence of colorectal carcinoma in this study is found in the 7th decade which constitutes 37% of which majority (12 cases) are female patients. In the age group of 20-29 yrs only 2 cases are seen and all of them are male patients. In our study, most patients are in 60 to 69 years age group.



Graph 1: Sex Distribution

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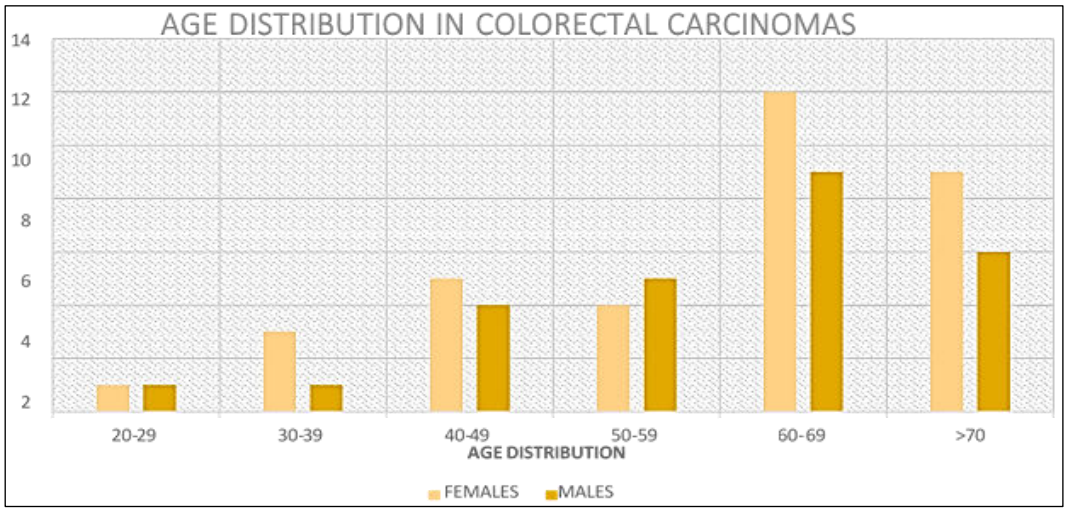
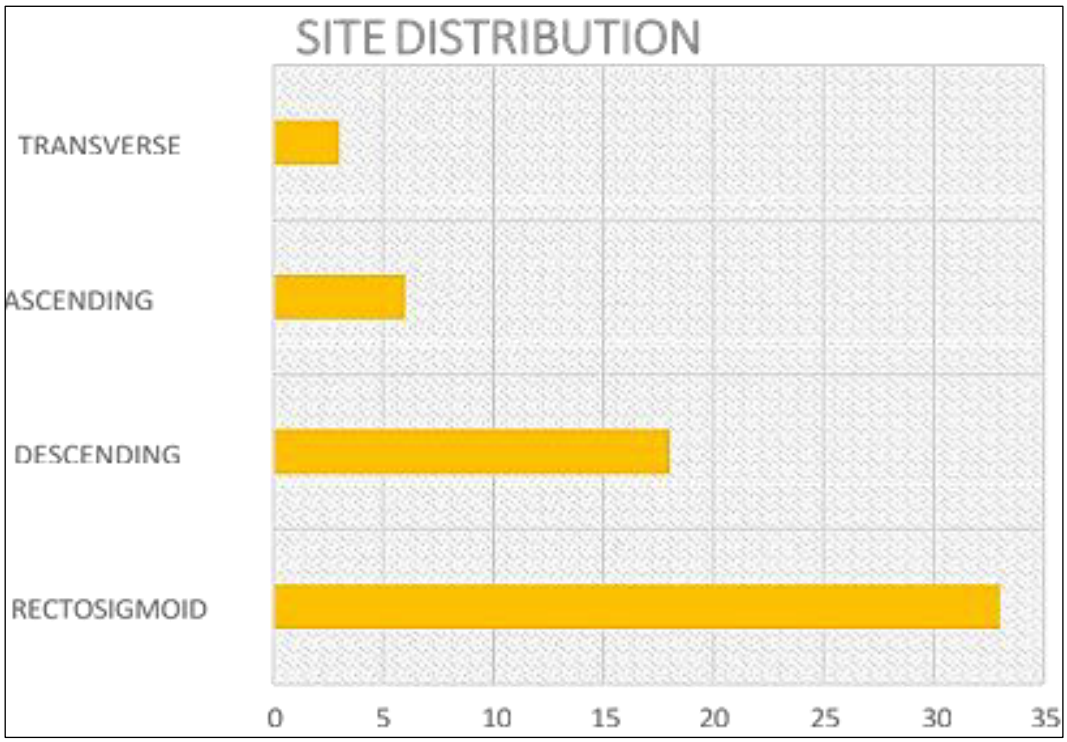


Graph 2: Age Distribution

Table 1: BCL2 and CDX2 expression in colorectal carcinomas

Type	Total	CDX 2 +	BCL 2+
Conventional	54	52	48
Mucinous	8	4	3
Total	62	56	51

In the present study most frequent site is rectosigmoid, consisting of 33 cases (53.2%), with about 20 cases (32.2%) in descending colon. Least number of cases (3) werefound in transverse colon, It constitutes around 4.8%, while ascending colon has 6 cases (9.6%). Most common site in present study is recto-sigmoid region with 33 cases (53.2%). In both sex, rectosigmoid is the most common site followed by descending & ascending colon.



Graph 3: Site Distribution

Table 2: Grading of Colorectal Carcinoma

Grade	No of Cases	Males	Females	Total
Well-differentiated	37	18	19	59.7%
Moderately-differentiated	15	07	08	24.2%
Poorly-differentiated	07	02	05	11.2%
Undifferentiated	03	01	02	4.8%

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BCL2 and CDX2 immunostaining results on colorectal carcinomas

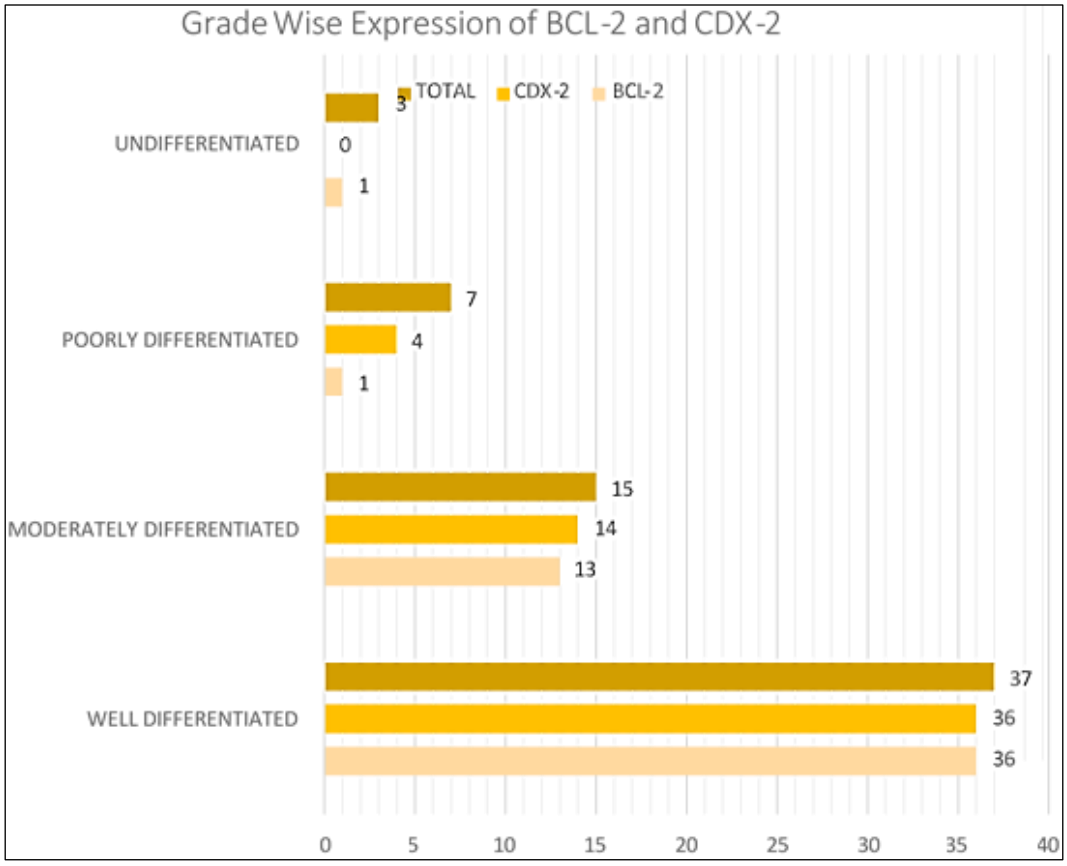
Out of the 62 cases 51 were positive for BCL2 and 56 were positive for CDX2.

Table 5: BCL2 Score in Relation to Histological Grade

Histological Grade	BCL2 Score				Total	Total Positive & Percentage
	1+	2+	3+	4+		
Well-differentiated	01	06	14	16	37	97.2% (36)
Moderately-differentiated	02	02	03	08	15	86.6% (13)
Poorly-differentiated	06	01	0	0	07	14.2% (01)
Undifferentiated	02	0	0	0	03	33.3% (01)

Table 6: CDX2Score in Relation to Histological Grade

Histological Grade	CDX2 Score				Total	Total Positive & Percentage
	0	1+	2+	3+		
Well-Differentiated	01	03	06	27	37	97.2% (36)
Moderately-Differentiated	01	03	03	08	15	93.3% (14)
Poorly-Differentiated	02	03	01	0	07	57% (04)
Undifferentiated	03	0	0	0	03	0% (0)



Grade 4: Grade Wise Expression of BCL2 and CDX2

A score of atleast 1+ is considered positive. Higher scores are seen in well to moderately differentiated carcinomas than poorly differentiated carcinomas, while poorly differentiated and undifferentiated carcinomas show low to absent staining.

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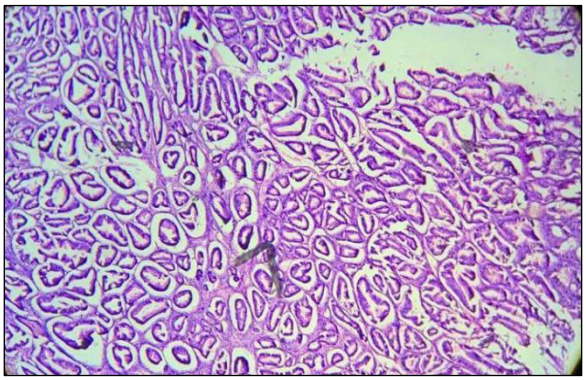
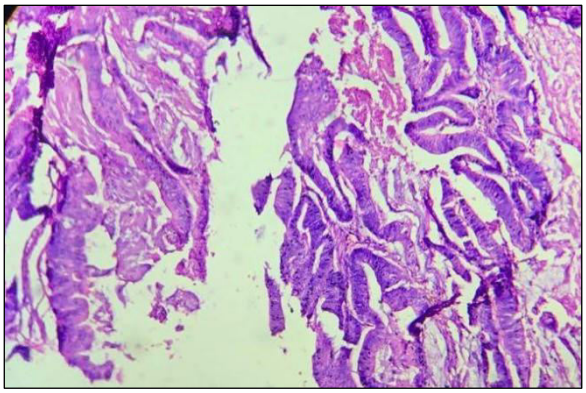
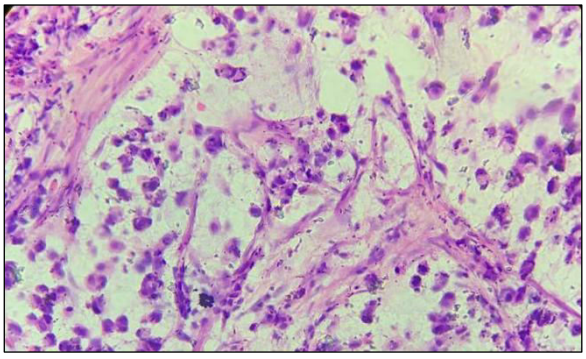
Picture 1: Gross-Descending Colon Grow Growth



Picture 2: (H&E 40X)-Well Differentiated Adenocarcinoma



Picture 3: (H&E 40X)-Moderately Differentiated Adenocarcinoma



Picture 4: H&E 40x: Poorly Differentiated Adenocarcinoma-Signet Ring Type

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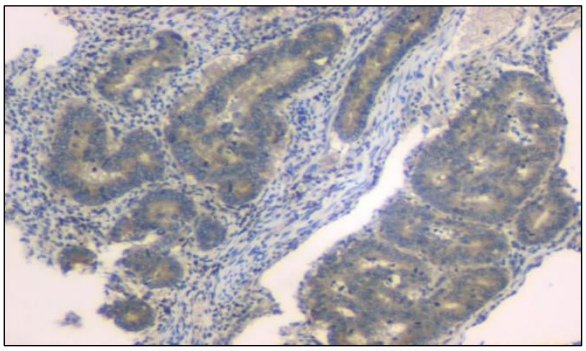
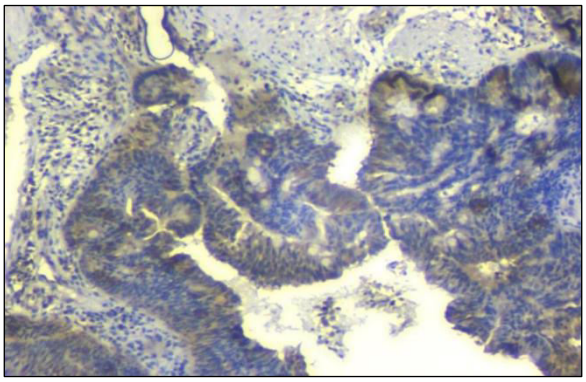
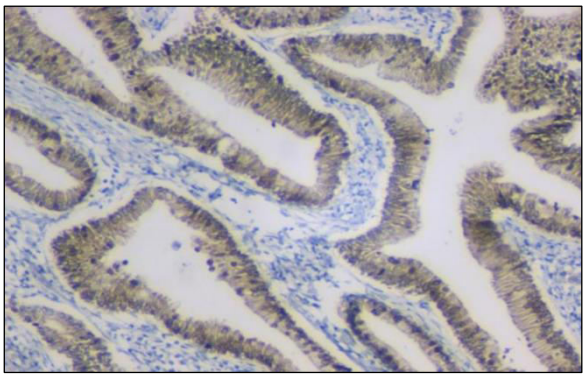


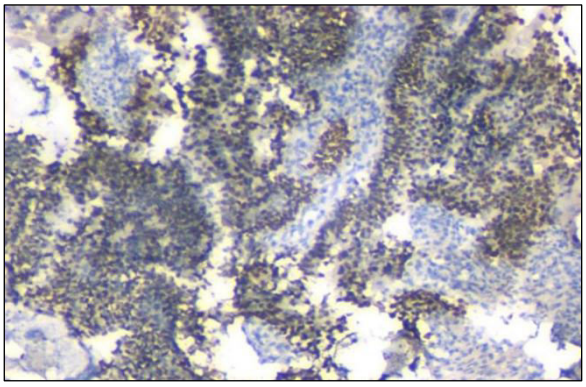
Picture 5: Grade 1 Adenocarcinoma-BCL2 Positive Score 3 IHC: 40X



Picture 6: Grade 1 Adenocarcinoma BCL2 Positive Score 2 IHC: 40X



Picture 7: Grade 1 Adenocarcinoma: CDX2 positive score 3 IHC: 40X



Picture 8: Grade 1 Adenocarcinoma: CDX2 Positive Score 3 IHC: 40X

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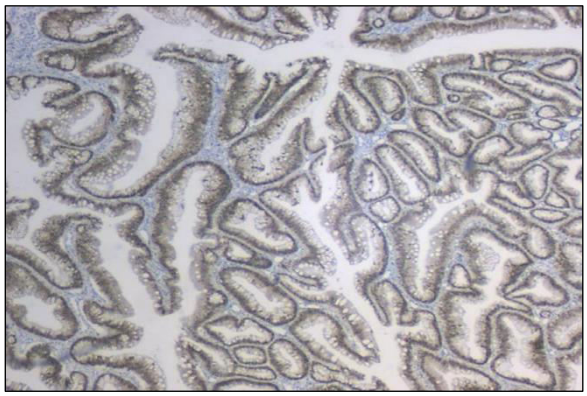

Picture 9: Grade 3 Moderately Differentiated Adenocarcinoma: CDX2 Positive Score 2

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 20-80 years and highest incidence found between 60-69 years. Most of colorectal carcinomas exhibited ulceroproliferative growth grossly.

In our study, BCL2 immunoreactivity was more frequent in well differentiated than moderately or poorly differentiated adenocarcinomas but no relationship was found between tumor site within the colon.

There is a significant association (P<0.00001) between bcl2 expression and grade of tumor, hence the role of bcl2 in colorectal carcinoma is a favorable prognostic factor.

CDX2 immunoreactivity was more frequent in well differentiated than moderately or poorly differentiated adenocarcinomas, corresponding to the presence of CDX2 marker in mature gastrointestinal (especially colonic) epithelium, and thus Presence of CDX2 is considered generally as a good prognostic factor. But, no relationship was found between tumor site within the colon as is the case with BCL2 expression in the present study.

Table 7: Comparision of BCL2 Expression in Colorectal Adenocarcinomas in Present Study with AJM Watson *et al.* Study

Variables	AJM Watson *et al.* Study			Present study (P value <0.0001)		
	Cases	BCL2 Positive	BCL2 Negative	Cases	BCL2 Positive	BCL2 Negative
Well differentiated	12	8(66.4%)	4 (33.3%)	37	36(97.2%)	1(2.8)
Moderately differentiated	34	9(26.4%)	25(73.5%)	15	13(86.6%)	2(13.4%)
Poorly differentiated	2	0 (0%)	2 (100%)	7	1(14.2%)	6(85.8%)
undifferentiated	1	0(0%)	1(100%)	3	1(33.3%)	2(66.6%)

Table 8: Comparision of CDX-2 Expression in Colorectal Adenocarcinomas in Present Study with Reyhan Bayrak *et al*. Study

Variables	Rehyan Bayarak *et al.* Study			Present study (P value <0.00001)		
	Cases	CDX2 Positive	CDX2 Negative	Cases	CDX2 Positive	CDX2 Negative
Well-differentiated	48	47(97.9%)	1(2.1%)	37	36(97.2%)	1(2.8%)
Moderately differentiated	91	87(95.6%)	4(4.4%)	15	14(93.3%)	1(6.7%)
Poorly differentiated	5	2(40%)	3(60%)	7	4(57%)	3(43%)
undifferentiated	3	1(33.3%)	1(66.6%)	3	0(0%)	3(100%)

Table 9: Comparision of BCL. 2 Expression and the Association with Grade of Adenocarcinoma among Various Studies

Previous studies on BCL2 Expression	Results	BCL2 Expression Association with grade
AJM Watson *et al.* study	Showed BCL2 over expression in 66% of well differentiated and 24% moderately differentiated colorectal lesions	Showed significant association with grade of adenocarcinoma
AL Temini *et al.*	Showed high positivity for BCL2 for grade-1 when compared to grade-3	Showed significant association
MIA Ouyang *et al.*	Showed 79.3% cases with strong BCL2 positivity in grade-1 and grade-2 lesions.	Showed significant association
Ban Qasim *et al.*	Showes BCL2 cytoplasmic positivity in 67% cases of colorectal carcinomas.	Showed no significant association.
Present study	Showed high BCL2 positivity in grade 1 and 2 adenocarcinoma and low positivity in grade 3 and 4.	Showed significant association

ISSN:0975-3583,0976-2833 VOL13, ISSUE05,2022

Table 10: Comparision of CDX2 Expression and the Association with Grade of Adenocarcinoma among Various Studies

Previous Studies on CDX.2 Expression	Results	CDX2 expression association with grade
Bayak *et al.*	CDX2 was positive in 97% cases of colorectal carcinomas, most of them were well differentiated lesions	Showed significant association with grade of tumor
Werling *et al.*	CDX2 shows a positivity in about 95% cases, i which about 60% cases are either 2+ or 3+	Showed significant association
Tanaka and Saito *et al.*	CDX2 showed 80% positivity with well differentiated lesions of colorectal adenocarcinomas.	Showed significant association
Mao Be and Hun Lee *et al.*	loss of CDX2 is associated with poor prognosis in colorectal cancer patients.	Showed significant association
Present study	Grade 1 and 2 adenocarcinoma showed higher positivity with CDX2, while grade 4 showed no positivity	Showed very significant association like other previous studies

There was no significant association between BCL2 and CDX2 positivity and sex in this study. Al Temini *et al*. and Reyhan Bayrak *et al*. also found no significant association between BCL2 and CDX2 positivity and sex in their respective studies.

Conclusion

In present study we have 62 cases of colorectal carcinoma of which 51 cases are BCL2 positive and 11 cases are BCL2 negative, and 56 cases are CDX2 positive while 6 cases are CDX2 negative. Majority of well-differentiated and moderately-differentiated cases show BCL2 and CDX2 expression.

There is significant expression of BCL2 and CDX2 in well differentiated and moderately differentiated carcinomas, when compared to poorly-differentiated colorectal adenocarcinomas and undifferentiated carcinomas. Hence BCL2 and CDX2 markers have a definite role in colorectal cancer differentiation. Therefore, there is significant correlation between BCL2 and CDX2 expression and grade of tumour (P value <0.00001), since grade is a proven prognostic marker, BCL2 and CDX2 expression and scoring will also be another good prognostic marker in colorectal carcinomas.

But there is no association between BCL2 and CDX2 expression and other variables like Age, gender, site of tumour.

Recent results suggest that BCL2 and CDX2 expression could both be considered as independent prognostic factor associated with favorable clinical outcome in colorectal adenocarcinomas and that their expression is higher in low grade variants in comparison with high grade.

References

1. Hamilton SR, Aaltonen LA. editors: WHO classification of tumours. Pathology and genetics of tumours of the digestive system. IARC press Lyon, 2000, 103-142.
2. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol. 2000;2:533-543.
3. Peter Boyle, Langman JS. ABC of colorectal cancer Epidemiology, Clinical review. BMJ, 2000, 321.
4. Ahmedin Jemal, Freddie Bray, Melissa M Centre, Jacques Ferlay, Elizabeth Ward, David Forman. Global Cancer Statistics. Ca Cancer J Clin. 2011;61:69-90.
5. Suh E, Chen L, Taylor J, Traber PG. A homeodomain protein related to caudal regulates intestine-specific gene transcription. Mol Cell Biol. 1994;14:7340-7351.
6. Bai YQ, Miyake S, Iwai T, Yuasa Y. CDX2, a homeobox transcription factor, upregulates transcription of the p21/WAF1/CIP1 gene. Oncogene. 2003;22:7942-7949.
7. Moskaluk CA, Zhang H, Powell SM, Cerilli LA, Hampton GM, Frierson HF. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. Mod Pathol. 2003;16:913-919.
8. Lugli A, Tzankov A, Zlobec I, Terracciano LM. Differential diagnostic and functional role of the multi-marker phenotype CDX2/CK20/CK7 in colorectal cancer stratified by mismatch repair status. Mod Pathol. 2008;21:1403-1412.
9. Bakaris S, Cetinkaya A, Ezberci F, Ekerbicer H. Expression of homeodomain protein CDX2 in colorectal adenoma and adenocarcinoma. Histol Histopathol. 2008;23:1043-1047.
10. Bakaris S, Cetinkaya A, Ezberci F, Ekerbicer H. Expression of homeodomain protein CDX2 in colorectal adenoma and adenocarcinoma. Histol Histopathol. 2008;23:1043-1047.
11. Baba Y, Nosho K, Shima K, Freed E, Irahara N, Philips J, *et al*. Relationship of CDX2 loss with molecular features and prognosis in colorectal cancer. Clin Cancer Res. 2009;15:4665-4673.
12. Poincloux L, Durando X, Seitz JF, *et al*. Loss of Bcl-2 expression in colon cancer: A prognostic factor for recurrence in stage II colon cancer; Surgical Oncology. 2009;18:357-65.
13. Saleh HA, Jackson H, Khatib G, *et al*., Correlation of bcl-2 oncoprotein immunohistochemical expression with proliferation index and histopathologic parameters in colorectal neoplasia, Pathol Oncol Res. 1999;5:273-9.

ISSN:0975-3583,0976-2833 VOL13, ISSUE05,2022

14. Yip KW, Reed JC. Bcl-2 family proteins and cancer. Oncogene Journal. 2008;27:6398-406.
15. M.P. Bronner MP, Culin C, Reed JC, Furtht EE. TheBcl-2 Proto-Oncogene and the Gastrointestinal Epithelial Tumor Progression Model. American Journal of Pathology. 1995;146:20-6.