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Original Research Article

IMMUNOHISTOCHEMICAL EXPRESSION OF P_{53} AND BCL_2 IN COLORECTAL CARCINOMA

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ABSTRACT

Background: Colorectal carcinoma arises from genetic alterations that affect cell growth, with p53 and Bcl2 playing key roles. P53 overexpression occurs late in tumour progression, whereas Bcl2 inhibits apoptosis early on, contributing to tumour growth. This study aimed to analyse the immunohistochemical expression of p53 and Bcl2 in colorectal adenomas and adenocarcinomas, and to correlate their expression with clinicopathological parameters. **Materials and Methods:** This retrospective and prospective study (descriptive study) included 50 patients, including 8 with adenomatous polyps and 42 with colorectal adenocarcinoma, at Meenakshi Medical College Hospital and Research Institute, Kancheepuram, between January 2016 and April 2019. Histopathological features were studied using haematoxylin and eosin staining, and immunohistochemical assays were performed for p53 and Bcl2 expression. **Results:** p53 was detected in 25% of colorectal polyps, 65% of well-differentiated carcinomas, and 100% of moderately and poorly differentiated carcinomas, showing a significant difference in adenocarcinomas (p=0.01). p53 expression increased with tumours stages I–III (p=0.006). Bcl2 was found in 100% of adenomatous polyps and 96% of well-differentiated adenocarcinomas. Bcl2 expression was significantly higher in adenomas than in adenocarcinomas (p=0.03). Positive p53 expression was more prevalent in patients over 60 years of age (p=0.24), whereas Bcl2 expression was increased in tumours < 1 cm (p=0.02). No significant differences were found in sex, tumour site, or size for protein expression. **Conclusion:** P53 overexpression occurs late in colorectal carcinogenesis and serves as a prognostic marker, while Bcl2 overexpression is an early event that promotes tumour progression. Assessing both markers helps predict therapeutic response and prognosis in patients.

INTRODUCTION

Colorectal carcinoma is the fourth most common cause of cancer-related morbidity and mortality, with approximately 14 million new patients and 8.2 million cancer-related deaths reported worldwide.[1] It can be subdivided into colon cancer, which ranges from the caecum to the sigmoid colon, and rectal cancer, which ranges from the rectosigmoid to the anus. It begins initially as a noncancerous growth that grows on the inner lining of the colorectum and propagates slowly, throughout 10 to 20 years.[2] Adenocarcinoma accounts for approximately 96% of all colorectal carcinomas (CRCs).[3] Males have higher incidence and mortality rates than females. The most common clinical presentation includes abdominal pain, rectal bleeding, altered bowel habits, and unexplained weight loss.

CRC carcinogenesis arises from the substantial accumulation of genetic and epigenetic alterations. The process is also influenced by environmental risk factors such as obesity, poor diet, smoking, and heavy alcohol consumption which accounts for approximately 80% of all colorectal cancer patients.[4] Genetic susceptibility is associated with familial adenomatous polyposis (FAP) and Lynch Syndrome (hereditary non-polyposis colorectal cancer or HNPCC), which account for 10% of all colorectal cancer cases. Individuals who have these diseases have an increased lifetime risk of CRC of up to 80%.[5]

Colorectal carcinoma develops from a multistep process characterised by the accumulation of molecular genetic alterations that cause disorders in cell growth, differentiation, and apoptosis.[6] Carcinogenesis and tumour progression seem to

depend upon a controlled balance between cell proliferation and cell death in the normal colorectal mucosa. Two genes implicated in this process are p53 and Bcl2, which appear to play important roles in apoptosis. p53 is encoded by Tp53 gene which is located on chromosome 17p13.1. p53 expression is abnormal in more than 50% of human tumours.[7] The expression of p53 protein expression is maintained at an extremely low level under normal conditions. However, p53 rapidly accumulates in the nucleus in response to multiple cellular stressors. p53 exerts its pro-apoptotic function when cellular DNA damage is severe and repair is impossible. On the other hand, p53 promotes G1 cell cycle arrest in the early stage of DNA damage response.[8]

A p53 mutation is the final step in converting adenoma to carcinoma, which results in an abnormal protein that accumulates in the nucleus, thus allowing its detection by immunohistochemistry.[9] This mutation reduces the capacity to undergo apoptotic cell death, and it could be an important step in the development of neoplasia.

The Bcl2 gene is a known inhibitor of apoptosis that may allow the accumulation and propagation of cells containing genetic alterations. It also plays a role in cell division progression. All these actions will potentiate tumour growth.[10] Bcl2 protein has been localised at the base of crypts over the epithelial cells in the large intestine where stem cell proliferation takes place and plays an important role in colorectal carcinogenesis.[11] Both p53 and Bcl2 protein overexpression confers cancer cell immortalisation by inhibiting the apoptotic cell death machinery.[12]

Aim

This study aimed to analyse and compare the immunoexpression of p53 and Bcl-2 in colorectal adenomas and adenocarcinomas. We will evaluate their association with clinicopathological parameters, including age, sex, tumour location, size, histological type, degree of differentiation, and TNM (AJCC) stage. Additionally, we investigated the combined expression of p53 and Bcl-2 in colorectal lesions.

MATERIALS AND METHODS

This retrospective and prospective study (descriptive study) included 50 patients, including 8 with adenomatous polyps and 42 with colorectal adenocarcinoma, in the Department of Pathology at Meenakshi Medical College Hospital and Research Institute, Kancheepuram, between January 2016 and April 2019. This study was approved by the Institutional Ethics Committee before initiation, and informed consent was obtained from all patients.

Inclusion Criteria

Patients with colorectal polyps and adenocarcinomas were included.

Exclusion Criteria

Chemotherapy and/or radiotherapy before sampling, recurrent adenocarcinomas and cancer types other than adenocarcinomas were excluded.

Methods

The histopathological features of all the patients were studied using Haematoxylin and Eosin (H&E) staining. All 50 patients were assessed for Bcl2 and p53 expression using immunohistochemical assays.

Scoring criteria for p53 and Bcl2

Positive tumour cells were expressed as a percentage of the total number of tumour cells and assigned to one of five categories. The expression was positive in more than 5% of the tumour cells. The scoring system for the proportion of positive cells was as follows: 0 indicated that less than 5% of the cells were positive. A score of 1 corresponds to 5-25% of the cells being positive, whereas a score of 2 represents 25-50% positivity. A score of 3 indicates that 50-75% of the cells were positive, and a score of 4 was assigned when 75-100% of the cells were positive.

Haematoxylin and eosin (H&E) staining was performed by deparaffinizing the tissue sections in xylene for 10-20 minutes, followed by rehydration with 100% alcohol for 1-2 minutes and 95% alcohol for 1-2 minutes. After rinsing in tap and distilled water, the sections were stained with haematoxylin for 3-5 minutes, washed in tap water, differentiated in 1% HCl in 70% alcohol for 1-2 dips, and examined under a microscope. The slides were then washed under running tap water for 15 min, stained with eosin for 1-4 minutes, dehydrated with alcohol, cleared in xylene, and mounted using DPX mounting media.

Immunohistochemistry using the Avidin-Biotin Complex (ABC) detection system involves processing formalin-fixed paraffin-embedded tissue sections. After preparing 3–4-micron sections on positively charged slides, the sections were deparaffinized and rehydrated, followed by antigen retrieval using EDTA buffer (pH 9.0) with autoclaving. Endogenous peroxidase was blocked using H2O2 and primary antibodies (Mouse Monoclonal p53 and Rabbit Monoclonal Bcl-2) were applied. After incubation, secondary biotinylated antibody and streptavidin-peroxidase reagent were added. DAB chromogen was used for staining, followed by counterstaining with Mayer's haematoxylin. The slides were dehydrated, cleared in xylene, and mounted using DPX. Positive controls included lymph node sections for Bcl-2 and colorectal adenocarcinoma for p53, with brown nuclear staining indicating p53 positivity, and brown cytoplasmic staining indicating Bcl-2 positivity.

Statistical Analysis

The data are presented as frequencies and percentages. Categorical variables were compared using Pearson’s chi-square test. Significance was defined as $p < 0.05$, using a two-tailed test. Data analysis was performed using IBM-SPSS version 21.0 (IBM-SPSS Science Inc., Chicago, IL).

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RESULTS

Table 1: Demographic details and clinical characteristics

		Frequency (%)
Age (in years)	< 40	7 (14%)
	41-59	25 (50%)
	> 60	18 (36%)
Gender	Male	29 (58%)
	Female	21 (42%)
Symptoms	Lower abdominal pain	32 (64%)
	Bleeding per rectum	36 (72%)
	Abdominal distension	27 (54%)
	Tenesmus	23 (46%)
	Altered bowel habits	19 (38%)
Gross type	Ulcer proliferative	26 (52%)
	Infiltrative	16 (32%)
	Polypoidal	8 (16%)
Site of tumour	Rectum	31 (62%)
	Sigmoid colon	5 (10%)
	Rectosigmoid junction	4 (8%)
	Sigmoid colon + Rectum	3 (6%)
	Right colon	3 (6%)
	Left colon	3 (6%)
	Transverse colon	1 (2%)
Stages of colorectal carcinoma	I	13 (31%)
	II A	7 (16%)
	II B	5 (12%)
	III A	8 (19%)
	III B	8 (19%)
	III C	1 (3%)
Histopathological diagnosis	Well-differentiated adenocarcinoma	26 (52%)
	Moderately differentiated adenocarcinoma	10 (20%)
	Poorly differentiated adenocarcinoma	6 (12%)
	Adenomatous polyps	8 (16%)
Surgical procedure	Anterior perineal resection	10 (20%)
	Right hemicolectomy	4 (8%)
	Left hemicolectomy	3 (6%)
	Low anterior resection	25 (50%)
	Colorectal biopsy	8 (16%)

Most patients were in the age group 41-59 years constituted 50% of the patients; 29 (58%) were males and 21(42%) were females. Most patients presented with complaints of bleeding in the rectum (72%) and lower abdominal pain (64%). Other symptoms included abdominal distension (54%), tenesmus (46%) and altered bowel habits (38%). In the gross type, 52% had ulcer proliferative, 32% had infiltrative growth, and 16% had polypoidal tumour growth. 62% of the tumours were in the rectum, followed by 10% in the sigmoid colon, and 8% at the rectosigmoid junction.

Of the 42 patients with colorectal carcinoma, 13 (31%) had stage I, 8 (19%) had stage IIIa, and stage IIIb. On histopathological examination, 52% of the patients were diagnosed with well-differentiated adenocarcinoma and 20% with moderately differentiated adenocarcinoma. Others included 6 patients with poorly differentiated adenocarcinomas and 8 patients with adenomatous polyps. According to the type of surgical procedure performed, anterior perineal resection was performed in 20% of patients, right hemicolectomy in 8%, left hemicolectomy in 6%, low anterior resection in 50%, and colorectal biopsies in 16%. [Table 1]

Table 2: Comparison of TNM Staging in different grades and diagnosis

Histopathological diagnosis (adenocarcinoma)	TNM staging			Expression	
	I	II	III	p53 Positive	Bcl2 Positive
Well-differentiated	11	6	9	65%	96%
Moderately differentiated	1	2	7	100%	10%
Poorly differentiated	1	4	1	100%	0
Adenomatous polyps	-	-	-	25%	100%

Of the 26 patients with well-differentiated adenocarcinoma, most (11 patients) were TNM stage I. Most of the moderately differentiated adenocarcinomas (seven patients) were stage III, and most of the poorly differentiated carcinomas (four patients) were stage II.

Bcl2 expression was downregulated in adenomatous polyps, well-differentiated adenocarcinomas, moderately differentiated adenocarcinomas, and poorly differentiated adenocarcinomas, whereas the expression of p53 was upregulated in adenomatous polyps, well-differentiated adenocarcinomas,

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moderately differentiated adenocarcinomas, and poorly differentiated adenocarcinomas. [Table 2]

Table 3: Expression of p53 and Bcl2 and co-expression of p53/Bcl2

Expression	p53	Bcl2	p53 and Bcl2
Positive	32 (64%)	34 (68%)	17 (34%)
Negative	18 (36%)	16 (32%)	33 (66%)

p53 was positively expressed in 32(64%) patients and negatively expressed in 18(36%) patients. Bcl2 expression was positive in 34(68%) patients and negative in 16(32%) patients. Combined p53 and Bcl2 expression were positive in 17(34%) patients. Combined p53 and Bcl2 expression was negative in 33 patients (66%). [Table 3]

Table 4: p53, Bcl2, and combined p53/Bcl2 expression in adenoma and adenocarcinoma

		Adenoma	Adenocarcinoma
p53 expression	Positive	2 (25%)	33 (79%)
	Negative	6 (75%)	9 (21%)
Bcl2 expression	Positive	8 (100%)	26 (66%)
	Negative	0%	16 (34%)
Combined p53/Bcl2	Positive	2 (25%)	15 (36%)
	Negative	6 (75%)	27 (64%)

P53 showed positive expression in two patients (25%) and negative expression in six patients (75%). P53 showed positive expression in 33 patients (79%), and negative expression was observed in nine (21%) patients with adenocarcinoma. Bcl2 expression was positive in eight patients (100%), and no negative expression was observed in any of the patients with adenoma. Bcl2 expression was positive in 26 patients (66%) and negative in 16 (34%).

Colorectal adenomas combined with p53 and Bcl2 showed positive expression in 2 patients (25%) and negative expression in 6 patients (75%). colorectal adenocarcinomas, combined p53 and Bcl2 showed positive expression in 15 patients (36%). Of these, 13 were well-differentiated carcinomas and 2 were moderately differentiated carcinomas. Combined p53 and Bcl2 expression was negative in 27 patients (64%).[Table 4]

Table 5: Comparison of p53 and Bcl2 expression in adenomatous polyps and colorectal adenocarcinoma

		Adenoma	Adenocarcinoma			p-value
		Polyps	Well-differentiated	Moderately differentiated	Poorly differentiated	
p53	Positive	2 (25%)	17 (65%)	10 (100%)	6 (100%)	0.01
	Negative	6 (75%)	9 (35%)	0 (0%)	0 (0%)	
Bcl2	Positive	8 (100%)	25 (96%)	1 (10%)	0 (0%)	0.03
	Negative	0 (0%)	1 (4%)	9 (90%)	6 (100%)	

p53 was positively expressed in 25% of colorectal polyps, 65% of well-differentiated carcinomas, 100% of moderately differentiated carcinomas, and poorly differentiated carcinomas. p53 was significantly more highly expressed in adenocarcinomas (p=0.01). Bcl2 was positively expressed in 100% of adenomatous polyps, 96% of well-differentiated adenocarcinomas, 10% of moderately differentiated carcinomas, and 0% of poorly differentiated carcinomas. Bcl2 expression was significantly higher in adenomas than in adenocarcinomas (p=0.03). [Table 5]

Table 6: Comparison of p53, Bcl2, combined p53/Bcl2 expression with TNM staging

	Stage- I		Stage- II		Stage -III		p-value
	Positive	Negative	Positive	Negative	Positive	Negative	
p53	5 (38%)	8 (62%)	10 (83%)	2(17%)	15 (88%)	2 (12%)	0.006
Bcl2	10 (77%)	3(23%)	6 (50%)	6(50%)	10 (59%)	7 (41%)	0.36
Combined p53/ Bcl2	3 (23%)	10 (77%)	4 (33%)	8 (67%)	8 (47%)	9 (53%)	0.39

p53 expression was observed in 5 patients with Stage I, 10 with Stage II, and 15 with Stage III tumours, with a significant difference (p=0.006). Bcl2 was significantly expressed in 10 patients with Stage I, 6 with Stage II, and 10 with Stage III tumours (p=0.36). Combined p53 and Bcl2 co-expression was observed in three patients in Stage I, four patients in Stage II, and eight patients in Stage III, with a significant difference (p=0.39). [Table 6]

Table 7: Comparison of p53 and Bcl2 expression with demographic details and clinical characteristics

		p53			Bcl2		
		Positive	Negative	P Value	Positive	Negative	P Value
Age (in years)	< 40	4	3	0.01	4	3	0.83
	41-59	11	14		17	8	

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	> 60	16	2		11	7	
Gender	Male	16	13	0.24	19	10	0.66
	Female	15	6		15	6	
Site of tumour	Rectum	17	14	0.24	24	7	0.33
	Sigmoid colon	5	0		3	2	
	Rectosigmoid junction	4	0		1	3	
	Sigmoid colon + Rectum	2	1		2	1	
	Right colon	2	1		2	1	
	Left colon	2	1		2	1	
	Transverse colon	0	1		0	1	
Tumour size (cm)	< 1	16	4	0.83	15	5	0.02
	> 1	17	5		9	13	

Positive expression was found more in the elderly age group > 60 years, which was statistically significant (p=0.24) in p53 expression. There was no significant difference in gender (p=0.24), tumour site (p=0.24), or tumour size (p=0.83) between p53 expression.

DISCUSSION

In our study, the ages of the patients ranged from 33 to 85 years old. The mean patient age was 55 years, with 29 (58%) were males and 21 (42%) were females. Chu et al. and Gendi et al. found that the median age at diagnosis was 58 and 55 years respectively.[13,14] El-Bolkainy et al. observed that the mean age was 51 years, which is like the present study.[15] In the study by Gill et al. and Ghaffarzadegan et al,[24] (60%) were male and,[16] (40%) were female.[16,17] However, Cressy et al. reported a higher incidence in female patients (63%).[18]

In our study, 62% of the tumours were located in the rectum, 10% in the sigmoid colon, and 8% at the rectosigmoid junction. 6% were in the right and left colons. Similar results were observed in a study conducted by Laishram et al., who reported that the rectum was the most common site involved in CRC.[19] Bhagyalakshmi et al. also observed that the rectum was the most affected site (45.1%), followed by the right and left colons.[20] Missaouia et al. observed that the colon was more commonly affected than the rectum.[21] In two other studies by Chalya et al. and Ojo et al., the rectosigmoid junction was the most common site affected by CRC.[22,23]

In our study, 84% of the patients had adenocarcinomas, 52% were diagnosed as well differentiated, 20% were moderately differentiated, and 12% were poorly differentiated. This observation was similar to that of a study by Lanza et al., who reported that 85% were adenocarcinomas.[24] Bhagyalakshmi et al. observed that 51% were diagnosed as well-differentiated, 26% as moderately differentiated, and 23% as poorly differentiated, which was in concordance with our study,[20] However, this was contrary to the study conducted by Sen et al., who observed that moderately differentiated (69.1%) constituted the most common type, followed by well-differentiated (11.8%), and poorly differentiated (19.1%).[25]

In the present study, p53 was detected in 32 patients (64%). Bcl2 expression was observed in 34 patients (68%). Combined p53/Bcl2 expression was observed in 17 (34%) patients. This corresponds to the findings of Kressner et al., who observed that p53 was detected in 62% of patients.[26] Another study by Tanigawa et al. stated that the incidence of p53 was 42%.[27] A study by Sinicrope et al., who observed that Bcl2 staining was detected in 67% of patients and combined p53/Bcl2 in 38% of patients.[28]

In our study, p53 staining showed significant differences according to age ($p<0.05$). This was in concordance with a study conducted by Gurzu et al., in which the age of the patients was strongly correlated with p53 expression ($p<0.0001$).[29]

Our study showed that p53 expression was significantly higher in colorectal carcinoma than in colorectal polyps ($p<0.01$), and its expression was directly proportional to the tumour stage. Similar results were observed in the study done by Moran et al. and Lashner et al. where p53 abnormalities in colorectal carcinogenesis increased with the progression of the lesion.[7,30] Similar positive correlations were observed where colorectal carcinoma was higher than in colorectal polyps in the studies conducted by Nussrat et al. and Al-Jeboori et al.[31,32]

In our study, Bcl2 staining showed significant differences in tumour size and histopathological diagnosis (p=0.02). Similar results were observed in studies done by Saneei et al, Yan-fang et al., and Saleh et al., where bcl-2 was significantly higher in colorectal polyps when compared to colorectal carcinoma with a $p<0.05$.[33-35]

In our study, among the 42 patients with colorectal carcinoma, Bcl2 expression was significantly correlated with tumour size ($p<0.05$). Similar results were observed in a study by Qasim et al., who observed a significant correlation between bcl-2 expression and decreasing tumour size.[36]

CONCLUSION

P53 overexpression is a late event in colorectal carcinogenesis, and the frequency of p53 abnormalities increases with lesion progression. Thus, it can be used as an ancillary marker for

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predicting the risk of malignant transformation and as a good prognostic factor in the follow-up of patients with colorectal tumours. Bcl2 expression is significantly higher in adenomas than in carcinomas, indicating that abnormal activation of Bcl2 is an early event in colorectal tumorigenesis, which can inhibit apoptosis in vivo, thereby facilitating tumour progression. Bcl2 protein expression plays an important role in the early stages of the adenoma-carcinoma sequence. Hence, assessment of both Bcl2 and p53 status may be valuable in predicting the therapeutic response and prognosis of patients.

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