Original Article

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Evaluation of Immunoreactivity of p53 in Colorectal Adenocarcinomas

Pathology Section

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ABSTRACT

Introduction: Colorectal Cancer (CRC) is regarded as one of the most widespread malignant tumour in the world. The p53 tumour suppressor gene is the most commonly mutated gene in human cancers and is a frequent abnormality in CRC. Patients with abnormal p53 might be at increased risk of death, due to increased aggressiveness of the disease.

Aim: To study the immunohistochemical expression patterns of p53 in colorectal adenocarcinomas and to evaluate the relation between p53 status and various clinico-pathologic parameters.

Materials and Methods: Fifty paraffin-embedded tissue blocks of colectomy specimens were used in this study (prospective and retrospective). Data like age at diagnosis, gender, type of surgery, tumour location, tumour size, histological type, tumour grade, depth of invasion, number of lymph nodes resected and number of lymph nodes with metastases were analysed. Two sections of 4 micrometer thickness were taken from each paraffin embedded tissue block. First section was taken for Haematoxylin and Eosin (H&E) stain and other one for immunohistochemistry (anti-p53 monoclonal antibody) by using Novolink Polymer Detection system.

Statistical Analysis: The correlation between p53 protein overexpression and each variable was evaluated using Chi-square analysis.

Results: The p53 staining was positive in 88.0% (44/50) of the cases. Out of the 50 cases, 06 cases were negative, 12 were weekly (+), 12 moderately (++), and 20 intensely (+++) positive for p53 protein overexpression. The p53 expression was correlated with histological grade 2 of the tumour, with majority of the tumours showing intense positivity for p53 protein (p= 0.001). However, there was no significant association between p53 protein expression and other clinicopathologic variables such as age, gender, site of tumour, pathologic type, pT stage and pN stage of the disease.

Conclusion: The p53 mutation plays an important role in development of colorectal carcinoma as p53 protein over expression is noted in majority of the patients. Assessment of p53 protein expression could be a useful tool to identify patients who might benefit from adjuvant therapy.

Keywords: Carcinoma colon, Mutated p53, p53 overexpression

INTRODUCTION

One of the major causes of morbidity and mortality throughout the world is colorectal carcinoma. It accounts for over 9% of all cancer incidence [1]. It is also the third most common cancer worldwide and the fourth most common cause of death. The p53 abnormalities are frequent, occurring in more than half of the cases of CRC [2-4]. Immunohistochemical detection of mutated p53 protein, which is metabolically more stable than its wild-type, stands at the top of the examined parameters in the search context for carcinogenesis of CRC and other cancers. The p53 protein overexpression confers cancer cell immortalization by inhibiting the apoptotic cell death machinery [5].

MATERIALS AND METHODS

This is a prospective and retrospective study of 50 samples of adenocarcinoma of the colon, conducted for three years from July 2012 to August 2015 in the Department of Pathology, JSS Medical College, Mysore,India.

Inclusion criterion was all cases of adenocarcinoma and exclusion criterion was cancers other than adenocarcinomas. There was no follow-up of the patients. The patient's age and sex were retrieved from the biopsy request forms. Haematoxylin and eosin stained sections were reviewed for the cases to assess their types and grades. Tumours were classified according to World Health Organisation (WHO) classification. Tumour (T), Node (N) and Metastasis (M) cancer staging system of the American Joint Committee Of Cancer (AJCC), seventh edition, was followed for staging of the colectomy specimens. The size of the tumour was taken as the maximum diameter of the tumour in centimeters.

The surgical pathology reports indicated that 05 tumours were located in the caecum, 08 in the ascending colon, 03 in hepatic flexure, 02 in transverse colon, 1 in splenic flexure, 2

in descending colon, 12 in sigmoid colon, 03 in rectosigmoid junction and 14 in the rectum. For further analysis these anatomic locations were grouped into right (beginning from the caecum extending to the hepatic flexure and transverse colon) and left (starting from splenic flexure extending to the sigmoid colon and rectum). Paraffin blocks best representing the tumour in each patient were selected after reviewing the H&E slides. 3-4 µm thick sections were taken on poly-L-Lysine coated slides and air dried. The slides were baked at 60°C for 1 hour in hot air oven. Slides were deparaffinised, rehydrated and heated in a pressure cooker containing antigen retrieval solution, sodium citrate buffer at pH 6.

One litre of retrieval solution was brought to boil in the pressure cooker. Slides were lowered into pressure cooker and completely immersed in the retrieval solution. When operating temperature and pressure were reached, it was timed for 1 minute. The slides were cooled, washed with water and buffer solution. Peroxide block was applied for 10 min and washed with Tris Buffered Saline (TBS). Protein block was applied for 10 min and washed with TBS. The sections were incubated with primary antibody and washed with TBS. Post primary block/enhancer was applied and washed with TBS. The sections were incubated with SS label (polymer) and washed with TBS. The bound antibody was visualised using a DAB-chromogen substrate. The sections were rinsed in running water and counter stained with haematoxylin and again rinsed in water for 5 min. External positive control tissue included sample of breast carcinoma with a diffuse p53 nuclear immunoreactivity. For negative controls, primary antibody was omitted and phosphate buffer saline was substituted.

Immunohistochemical Staining Interpretation: In resected specimens tumour is classified as p53 positive when nuclear staining is observed in 5% or more of the cells counted in 10 high power fields. The percentage positivity was placed in five categories i.e., <5%, 5-25%, 25-50%, 50-75%, >75%. The staining intensity was judged relatively to an intensely stained positive control as 1+, 2+, 3+. Less than 5% of the cells stained was considered negative irrespective of the staining intensity. The positivity was clubbed with intensity and the result was correlated as weakly positive, moderately positive and intensely positive [6].

STATISTICAL ANALYSIS

The relation between p53 expression and the clinicopathological variables was analysed by the Chi-square test. The p-value <0.05 were considered significant. The SSPS software, version 20.0 was used for data analysis.

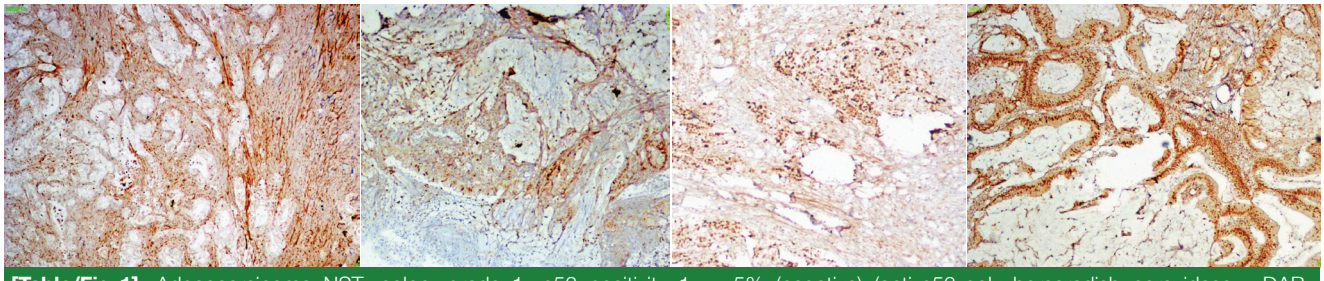
RESULTS

The male to female ratio was 1.63:1 and the patients age ranged from 28-85 years. Out of the 50 cases, 06 cases were negative, 12 were weekly (+), 12 moderately (++), and 20 intensely (+++) positive for p53 protein overexpression. Examples of tumours showing the different staining intensities and percentage positive tumour cells are illustrated in [Table/Fig-1-5]. In majority of the cases 3+ intensity of staining was observed. More than 75% cells were immunostained for p53 in most of the cases [Table/Fig-6].

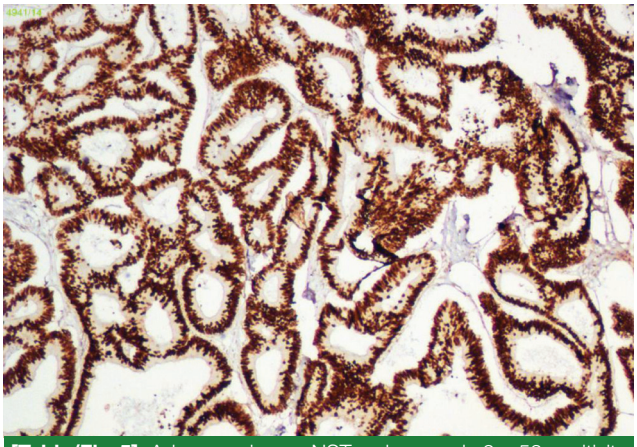
The p53 positivity was seen in 34 (89.5%) out of 38 cases of moderately differentiated tumours with majority of tumours (50.0%) being intensely positive for p53 than poorly differentiated tumours (10.0%) with a significant association between p53 expression and grade 2 tumours (p=0.001). However, there was no significant correlation between p53 staining and gender, age, site of tumour, tumour size, histological type, pT and pN stage of the disease [Table/Fig-7].

DISCUSSION

Colorectal cancer is one of the leading causes of cancer-related deaths worldwide and whilst only surgical resection is still the preferred method of treatment capable of curing colon cancer, adjuvant therapy continues to play an important role in preventing the recurrence and metastasis of colon carcinoma [7,8]. Adjuvant therapy relies on a normal p53 function to trigger apoptosis so that those cells damaged by chemo or radiotherapy can be destroyed for therapeutic purposes [9,10]. Several studies have shown that tumour cells with impaired p53 function have poor response to adjuvant or neo-adjuvant therapy [11-13]. The prognostic role of p53



[Table/Fig-1]: Adenocarcinoma NST- colon, grade 1, p53 positivity 1+, <5% (negative) (anti-p53-poly horseradish peroxidase - DAB-chromogen, 40X). **[Table/Fig-2]:** Mucinous adenocarcinoma colon, p53 positivity 1+, 5-25% (weak) (anti-p53-poly horseradish peroxidase-DAB-chromogen, 40X). **[Table/Fig-3]:** Adenocarcinoma NST- colon, grade 3, p53 positivity 2+, 25-50% (moderate) (anti-p53-poly horseradish peroxidase-DAB-chromogen, 40X). **[Table/Fig-4]:** Mucinous adenocarcinoma colon, p53 positivity 2+, 50-75% (moderate) (anti-p53-poly horseradish peroxidase-DAB-chromogen, x40).



[Table/Fig-5]: Adenocarcinoma NST- colon, grade 2, p53 positivity 3+, >75% (intense) (anti-p53-poly horseradish peroxidase-DAB-chromogen, x40)

Percentage-positive tumour cells	p53 status			Total
	Staining intensity			
	1+	2+	3+	
<5%	6	0	0	6*
5-25%	1	0	0	1
25-50%	0	1	1	2
50-75%	4	2	2	8
>75%	7	9	17	33
Total	18	12	20	50

[Table/Fig-6]: p53 status, percentage positive cells and staining intensity.
*Negative

Clinicopathologic parameters	Overall	p53 Negative	p53 Weakly Positive	p53 Moderately Positive	p53 Intensely Positive	p-value
	N=50	06 (12.0%)	12 (24.0%)	12 (24.0%)	20 (40.0%))	
Age at Diagnosis						
≤55 years	28 (56.0%)	04 (66.7%)	08 (66.7%)	08 (66.7%)	08 (40.0%)	0.326
>55 years	22 (44.0%)	02 (33.3%)	04 (33.3%)	04 (33.3%)	12 (60.0%)	
Sex						
Male	31 (62%)	03 (9.7%)	08 (25.8%)	07 (22.6%)	13 (41.9%)	0.891
Female	19 (38%)	03 (15.8%)	04 (21.1%)	05 (26.3%)	07 (36.8)	
Tumour Location						
Right colon	18 (36%)	01 (5.6%)	07 (38.9%)	05 (27.8%)	05 (27.8)	0.188
Left colon	32 (64.0%)	05 (15.6)	05 (15.6%)	07 (21.9%)	15 (46.9%)	
Tumour Size (in cm)						
≤5	25 (50.0%)	04 (66.7%)	03 (25.0%)	06 (50.0%)	12 (60.0%)	0.215
>5	25 (50.0%)	02 (33.3%)	09 (75.0%)	06 (50.0%)	08 (40.0%)	
Tumour Type						
Adenocarcinoma NST	41 (82%)	06 (14.6%)	07 (17.1%)	09 (22.0)	19 (46.3%)	0.123
Mucinous adenocarcinoma	08 (16%)	0 (0.0%)	04 (50.0%)	03 (37.5%)	01 (12.5%)	
Signet ring cell carcinoma	01 (02%)	0 (0.0%)	01 (100 %)	0 (0.0%)	0 (0.0%)	
Tumour Differentiation						
Grade I	02 (04%)	02 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.001*
Grade II	38 (76%)	04 (66.7%)	07 (58.3%)	08 (66.7%)	19 (95.0%)	
Grade III	10 (20%)	0 (0.0%)	05 (41.7%)	04 (33.3%)	01 (5.0%)	
pT Stage						
pT1	02 (04%)	01 (50.0%)	0 (0.0%)	0 (0.0%)	01 (50.0%)	0.518
pT2	15 (30%)	01 (6.7%)	06 (40.0%)	03 (20.0%)	05 (33.3%)	
pT3	27 (54%)	04 (14.8%)	04 (14.8%)	07 (25.9%)	12 (44.4%)	
pT4	06 (12%)	0 (0.0%)	02 (33.3%)	02 (33.3%)	02 (33.3%)	
pN Stage						
pN0	30 (60%)	05 (83.3%)	07 (58.3%)	07 (58.3%)	11 (55.0%)	0.426
pN1	14 (28%)	01 (16.7%)	03 (25.0%)	02 (16.7%)	08 (40.0%)	
pN2	06 (12%)	0 (0.0%)	02 (16.7%)	03 (25.0%)	01 (5.0%)	

[Table/Fig-7]: Correlation of overexpression of p53 with clinicopathologic variables.
*significant

in CRC is very controversial in the literature. Many studies reveal that the p53 immuno stain is very high in CRC the positivity rate is reported being between 43% and 86.36% [3,14,15]. In our study p53 was expressed in 88.0% of the cases. Other similar studies had shown variable ratios.

The age factor was not significant. This is consistent with the results of other studies [9,12].

While the study done by Slattery et al., [16] detected significant relationship between age and p53 expression

No significant correlation was found with sex. Our result is consistent with other studies and is different from a study that showed a significant correlation with male gender [12].

The correlation with site in this study was not significant similar to few studies [17,18] contrary to others who reported significant correlation [19,20]. The variation in site for p53 expression has been attributed to several factors including right versus left colon, effects of sex hormones, diet, as well as genetic causes. It is likely that there are differences in sensitivity and exposure to carcinogens for the proximal and distal sections of colon [21].

The p53 expression in relation to size is not significant and this result is similar to that of Asaad et al., [20] and differs from that of Demirba S et al.,[22].

The p53 expression in relation to histological type is not significant. Our result is consistent with other studies [4,23] and differs from some studies which had reported significant correlation [23].

The expression of p53 protein in relation to grade 2 was significant and this is consistent with other studies [22] contrary to other studies wherein there was no statistically significant correlation between tumour grade and p53 positivity.

No correlation was found between p53 expression and pT or pN factors in the present study which was in accordance with several studies. A very few studies have found association of p53 with pT stage of the tumour and nodal metastasis.

LIMITATIONS

Follow-up of the patients was not possible in this study.

CONCLUSION

Considering the p53 protein overexpression in a relatively high percentage of patients, it seems that p53 mutation plays an important role in development of colorectal carcinoma. Further, study of p53 immunohistochemical analysis on a large number of cases can help to find out its usefulness as a prognostic marker and identify patients who might benefit from adjuvant therapy.

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