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Original Article

Pathology Section

Correlation of p53 Overexpression with the Clinicopathological Prognostic Factors in Colorectal Adenocarcinoma

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ABSTRACT

Introduction: Mutation in p53 gene and accumulation of p53 protein is a common genetic event in colorectal carcinomas. p53 mutation can be detected by various techniques such as DNA sequencing, polymerase chain reaction and immunohistochemistry (IHC). However, IHC is simple and is consistent with other techniques.

Aim: To establish a correlation between overexpression of p53 with the clinical features, tumour histopathology and stage of Colorectal Carcinoma (CRC).

Materials and Methods: This prospective and retrospective study of clinical, histopathological and IHC features of CRC was conducted on colectomy and abdomino-perineal resection specimens received from January 2008 to June 2013. For each case, the clinical features, tumour morphology and p53 status (by IHC) were evaluated.

Results: The most common histologic type of CRC was Non-Specific Type (NST) and grade II tumours were seen predominantly (60%). Overall, 67.5% of CRCs showed p53 positivity on IHC. Intense p53 positivity was observed in 37.5% of CRCs of NST type and 33.3% of mucinous adenocarcinomas showed moderate positivity. Grade III tumours showed variable p53 positivity and those with lymph node metastasis showed moderate (55.6%) or intense positivity (53.8%). But there was no statistically significant correlation of p53 status and various clinicopathological prognostic factors.

Conclusion: As p53 protein overexpression is seen in a relatively high percentage of CRCs, it seems that p53 mutation plays an important role in development of CRC. However, no direct correlation could be established between p53 results and the patients' age, sex, tumour site, size, histological type, grade, lymph node status, or TNM stage. A prolonged follow up is necessary to conclude whether p53 status has any influence on the long, term prognosis and patient survival.

Keywords: Genetic Alterations, Grade, Immunohistochemistry, p53, TNM stage

INTRODUCTION

Globally, nearly 800,000 new colorectal cancer cases are detected each year, which accounts for 10% of all incident cancers. The aetiopathogenesis of colorectal cancer is complex and involves a multistep process characterized by histopathologic precursor lesions and molecular genetic alterations involving APC, KRAS and p53 genes [1]. p53 is a tumour suppressor gene located on the short arm of chromosome 17p13.1 and plays vital role in regulating cell growth [2]. Mutation in p53 gene is the most frequent genetic alteration in human cancers. The prevalence of p53 mutations in colorectal cancer varies from 40 to 60% in various studies. A recent systematic review of 168 reports including 18,766 patients showed that patients with CRC and abnormal p53 were at increased risk of death, due to increased aggressiveness of the disease [1].

p53 protein overexpression is directly related to increased proliferative activity [3], as well as an increased tendency of lympho-vascular invasion, metastasis to lymph node and advanced TNM stage [4], which represents the greater aggressiveness of tumour. p53 gene status can be analysed in laboratory by three methods viz., IHC, Polymerase Chain Reaction (PCR) and detection of serum p53 antibody in peripheral blood samples [1]. In view of pathologists, IHC is cheap, easy and more familiar technique and is used as a standard procedure in the routine diagnosis with comparison to DNA sequencing. The present study was undertaken to estimate the frequency of p53 protein overexpression by IHC in CRCs and to evaluate its association with various prognostic factors like clinical features, tumour histopathology, tumour grade, lymph node status, and stage of the tumour.

MATERIALS AND METHODS

Study design

This prospective and retrospective study was carried out on a total of 40 cases of colectomy and abdomino-perineal resection specimens in the Department of Pathology, from January 2008 to June 2013. The detailed clinical history and results of relevant investigations were collected from the medical records. Specimens received were fixed in 10% neutral buffered formalin. Multiple sections were taken from representative area and processed routinely, embedded in paraffin and 5 µm thick sections stained with Haematoxylin and Eosin (H&E) were evaluated for the tumour histology, grade, lymph node metastasis and other features. In addition, 4µm sections were cut from a paraffin block of tumour tissue and taken on a glass slide which was coated with adhesive (silane) for IHC to detect p53 overexpression. The study was approved by Institutional Ethical Committee.

Processing for IHC

IHC procedure was carried out by antigen retrieval in citrate buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibody against p53 protein (Biogenex), linking with rabbit anti mouse secondary antibody (Novocastra, UK), enzyme labeling with streptavidin-horseradish peroxidase (Novacastra, UK), developing chromogen with 3, 3'-diaminobenzidine (DAB) and counterstaining

with haematoxylin. Both positive and negative controls were run with each batch of slides. The immunostained slides were examined for nuclear staining with anti-p53 antibody and graded [Table/Fig-1] [1]. The relationship between various parameters such as age, sex, anatomic site of tumour, size, histologic type and grade, lymph node status, and staging of the tumour with overexpression of p53 were studied.

STATISTICAL ANALYSIS

Statistical analysis was done using Chi-square test and p-value of less than 0.05 was considered as statistically significant. All the statistical analysis was performed using IBM SPSS Statistics for Windows (version 20.0. Armonk, New York: IBM Corporation).

RESULTS

The age range of study population was from 27 to 75 years and peak was from 60 to 69 years. In our study, females outnumbered males with ratio being 1.11: 1. The frequent presenting complaint was weight loss (20/40, 50%) followed by per-rectal bleeding (17/40, 42.5%) and altered bowel habits (14/40, 35%).

The most common site for CRC was rectum (10, 25%) followed by caecum (9, 22.5%), sigmoid colon (5, 12.5%), recto-sigmoid (4, 10%), colon (3, 7.5%), hepatic flexure (3, 7.5%) and rectum with anal canal (2, 5%). Caecum with ascending colon, ileo-caecum, sigmoid colon with rectum and splenic flexure constitute each one case. The tumour size was more than 4 cm in 32 (80%) cases. Adenocarcinoma, Non-Specific Type (NST) (24, 60%) was the predominant WHO histologic type followed by mucinous (12, 30%), papillary (2, 5%) and signet ring cell (2, 5%) carcinoma. Majority of patients with CRC presented with grade II tumours (24, 60%) [Table/Fig-2].

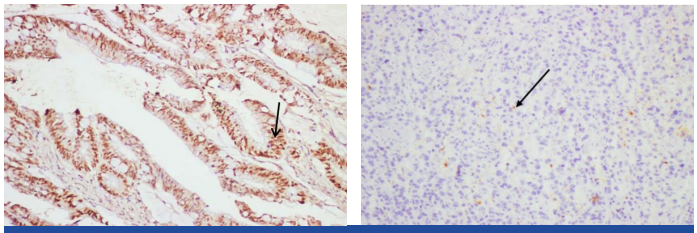
Out of 40 CRC, 19 (47.5%) showed metastasis to lymphnodes and majority were in age group of 60-69 years. Majority of them were in the stage IIIB (11, 27.5%) or IIIC (9, 22.5%) [Table/Fig-2].

On p53 staining, 67.5% of CRCs showed p53 positivity and most (15, 37.5%) of them were 3+ positive. Majority (15, 37.5%) of tumours showed p53 positivity in 26%-75% of tumour cells [Table/Fig-3] followed by positivity in <5% cells (13, 32.5%) [Table/Fig-4], 5%-25% tumour cells in 8 (20%) tumours [Table/Fig-5] and >75% of cells were p53 positive in 4 (10%) cases [Table/Fig-6]. Among histological types, adenocarcinoma, NST (37.5%) showed intense and 33.3% of mucinous adenocarcinomas showed moderate p53 positivity. Lympho-vascular invasion was noted in 13 (32.5%) cases. However, none of the cases showed distant metastasis.

We attempted to correlate the p53 status and age, sex, anatomic site of tumour, size, histological type and grade. However, none of these clinicopathological prognostic parameters showed statistically significant correlation with p53 {age (p=0.930), sex (p=0.324), tumour site (p=0.234), tumour size (p=0.219), histological type (p=0.869), grade (p=0.506)}. Large number of cases of CRCs with

Percentage	Intensity	Result
<5%	0/1+	Negative
5-25%	1+/2+	Weak positive
26-75%	2+/3+	Moderately positive
>75%	3+/4+	Intense positive

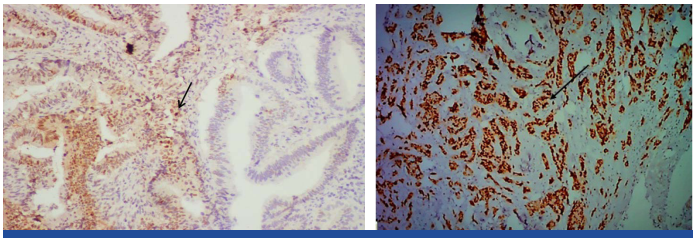
[Table/Fig-1]: Method of reporting for p53 staining.



[Table/Fig-3]: 3+ p53 positivity (arrow), 26-75% (moderate) in a colonic mucinous adenocarcinoma (anti-p53-polyhorse radish peroxidase-DAB chromogen, x100).
[Table/Fig-4]: 1+ p53 positivity (arrow), <5% (negative) in a colorectal adenocarcinoma, (anti-p53-polyhorse radish peroxidase-DAB chromogen, x100).

Pathological Parameters		No. (%)	p53 staining				α^2 value	p-value
			Negative (%)	Weak (%)	Moderate (%)	Intense (%)		
Histological type	Adenocarcinoma NST	24 (60)	8 (33.3)	3(12.5)	4(16.7)	9(37.5)	4.598	0.869
	Mucinous	12 (30)	3(25)	2(16.7)	4(33.3)	3(25)		
	Papillary	02 (5)	1(50)	0 (00)	1 (50)	0 (00)		
	Signet Ring Cell	02 (5)	1 (50)	0 (00)	0 (00)	1 (50)		
	Total	40(100)	13(32.5)	5(12.5)	9(22.5)	13(32.5)		
Grade	I	07 (17.5)	02 (28.6)	01(14.3)	00(00)	04(57.1)	5.3	0.506
	II	24 (60)	09(37.5)	3(12.5)	07(29.2)	05(20.8)		
	III	09 (22.5)	02(22.2)	01(11.1)	02(22.2)	04(44.5)		
	Total	40(100)	13(32.5)	5(12.5)	9(22.5)	13(32.5)		
TNM stage	I	08 (20)	2(25)	0(00)	2(25)	4(50)	7.885	0.928
	IIA	09 (22.5)	3(33.33)	2(22.22)	2(22.22)	2(22.22)		
	IIB	02 (5)	1(50)	0(00)	0(00)	1(50)		
	IIC	01 (2.5)	1(100)	0(00)	0(00)	0(00)		
	IIIA	00 (00)	0(00)	0(00)	0(00)	0(00)		
	IIIB	11 (27.5)	4(36.36)	2(18.18)	3(27.27)	2(18.18)		
	IIIC	09 (22.5)	2(22.22)	1(11.11)	2(22.22)	4(44.44)		
	Total	40(100)	13(32.5)	5(12.5)	9(22.5)	13(32.5)		
Lymph node status	Metastasis present	19(47.5)	5(26.32)	2(10.53)	5(26.32)	7(36.84)	0.983	0.805
	Metastasis absent	21(52.5)	8(38.1)	3(14.29)	4(19.05)	6(28.57)		
	Total	40(100)	13(32.5)	5(12.5)	9(22.5)	13(32.5)		

[Table/Fig-2]: Correlation of p53 expression with pathological parameters.



[Table/Fig-5]: 2+ p53 positivity (arrow) in 5-25% (weak) in a colorectal mucinous adenocarcinoma (anti-p53-polyhorse radish peroxidase-DAB chromogen, x100).
[Table/Fig-6]: 4+ p53 positivity (arrow) in >75% (intense) in a rectal adenocarcinoma NST (anti-p53-polyhorse radish peroxidase-DAB chromogen, x100).

lymph node metastases showed moderate (5, 26.32%) or intense (7, 36.84%) positivity, but was not statistically significant. There was no correlation found between TNM stage and p53 status [Table/Fig-2].

DISCUSSION

p53, also known as TP53 or tumour protein is a gene coding for a protein that regulates cell cycle and hence functions as a tumour suppressor. It has been stated by various authors that there is a high frequency of p53 mutations in various human cancers showing that the loss of p53 function plays a crucial part in the development of tumours [5]. Mutation and overexpression of p53 in CRC occurs 1.5-3 times more frequently in distal as compared to proximal tumours and are common in tumours that are aneuploid, non-mucinous, and do not show either the Microsatellite Instability (MSI) or methylator (CIMP-) molecular phenotypes. No consistent associations have been shown with other clinicopathological features including tumour stage, grade, sex or age, or with KRAS gene mutations [6].

The prevalence of p53 mutations in colorectal cancer is inconsistent and was estimated to be 40% to 60% [7]. This discrepancy could be due to the choice of methods, sensitivity and specificity of different antibodies used, and various interpretations of the results.

In a study by Mei Wang H et al., 48.4% of CRCs were ≤ to 4 cm, whereas in our study 70% were more than 4 cm [8]. A study by Gurzu S et al., showed lymph node involvement in 47.3% cases which was similar to our study (47.5%) [9]. Adenocarcinoma, NST was the frequently encountered histological type of CRC which was similar to other studies [10]. Most of CRCs were of grade II which was in concordance with study by Nabi U et al., [11].

A study conducted by Ghavam-Nasiri MR et al., on 100 specimens of CRC showed an overall p53 positivity of 63% [1]. There was no significant correlation between p53 positivity and gender (p = 0.34), age (< 40 vs. ≥ 40 year; p = 0.74), site of tumour (right vs. left colon and rectum; p = 0.26), histologic type (mucinous vs. non-mucinous; p = 0.63) and stage of the disease (p = 0.12) [1]. In our study the overall p53 positivity rate in CRC was 67.5% (27/40 cases) and 5/40 cases were weakly positive, 9/40 were moderately positive, and 13/40 were intensely positive. We also found no significant correlation found between p53 staining and age (p = 0.930), sex (p=0.324), site of tumour (p = 0.234), size of tumour (p=0.219), histologic type (p = 0.869), grade (p=0.506) and staging (p = 0.928). Ghavam-Nasiri MR et al., concluded that as p53 protein overexpression was noted in a relatively high percentage of CRCs, it seems that p53 mutation plays an important role in development of CRC [1].

Dan-ping Z et al., observed that 43% of CRCs showed p53 positivity [12]. There was statistical correlation between p53 protein expression and clinico-pathological manifestations (P>0.05) but the survival was significantly worse (p=0.0001) in p53 protein positive cases. p53 expression had no correlation with stage of carcinoma. Lymph node involvement and p53 protein expression were two independent factors that correlated with survival time. The findings of Kressner U et al., were contradictory as they found no significant relationship between p53 overexpression, as determined by IHC and cancer-specific survival [13].

Kaserer K et al., noted that 74% of CRCs showed p53 positivity on IHC and p53 gene mutation in 51% [14]. In 16% patients with p53 gene mutation IHC was negative. They concluded that scattered positive immunohistochemical reactivity of p53 in colorectal cancer cells might therefore represent a functionally active non-mutated p53 gene and should not be considered as a marker of gene mutation and inactivation.

Gurzu S et al., found a correlation between p53 and age of the patients and TNM staging [9], whereas we found no correlation between p53 and clinico-pathological parameters. Our study showed that majority of CRC, NST (37.5%) were having intense p53 positivity followed by mucinous adenocarcinoma (33.3%) showing moderate positivity; but no correlation was found with grading, lymph node status and TNM staging. On the contrary, Huh JW et al., found p53 staining more often in typical adenocarcinoma compared to mucinous adenocarcinoma (49% versus 17%, p=0.007) and in well or moderately differentiated adenocarcinoma compared to poorly differentiated adenocarcinoma (50% versus 32%, p = 0.030) [4]. In their study, the level of expression of p53 protein was related to lymph node metastasis (p < 0.001) and the TNM stage of the CRC (p = 0.006) unlike our study.

The p53 mutation plays a pivotal role in development of CRC. However, no direct correlation could be established between p53 and clinicopathological parameters. A prolonged follow up is necessary to conclude whether p53 status has any influence on the long-term prognosis.

LIMITATION

Further, study on a large number of cases will help to find out whether evaluation of p53 overexpression by a standardized IHC procedure, could be a clinically useful marker for the identification of CRC patients who are likely to benefit from the standard chemotherapy regimen.

CONCLUSION

As p53 protein overexpression was seen in relatively high percentage of patients, it seems that p53 mutation plays an important role in development of CRC. However, no direct correlation could be established between p53 results and the patient's age, sex, tumour site, size, histological type, grade, lymph node status, or TNM staging in case of CRC.

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