Original Article

# p53 Expression in Colorectal Carcinomas and Its Correlation with Clinicopathological Parameters

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## Abstract

**Background:** p53 over expression is based on the fact that the mutant form of p53 protein having prolonged half-life can accumulate and be over expressed in nuclei which can easily detected by immunohistochemistry. **Objective:** To estimate the frequency of p53 protein overexpression in colorectal carcinoma and its correlation with some clinicopathologic parameters. **Methods:** The overexpression of p53 protein was studied in sixty paraffin-preserved colorectal carcinoma samples using a monoclonal antibody (clone DO-7). The number of cells stained were scored semiquantitatively as (score 0): <5% positive cells, (score 1+): 5 – 25% positive cells,(score 2++): 25 – 75% positive cells, and (score 3+++): >75% positive cells..The correlation between p53 protein over expression and clinicopathological parameters were evaluated using Chi-square analysis. **Results:** p53 staining was positive in 42 of 60 specimens. Out of these , 10 were weekly (score1+), 16 moderately (score 2++), and 16 intensely(score 3+++) positive for P53 protein overexpression. There was statistically significant correlation between p53 staining and age( < 40 years vs >= 40 years; P- 0.006). Statistically significant correlation was also found between p53 staining and pathological type( mucinous vs non mucinous; p- 0.007) There were no significant correlations between p53 staining and gender (P =0.86 ), site of tumor (right colon vs. left colon and rectum; P=0.69) and stage of the disease (P =0.34 ). **Conclusion:** As p53 protein overexpression is seen in relatively high percentage of patients of colorectal carcinoma., it seems that p53 mutation plays an important role colorectal carcinogenesis. There was significant association between p53 protein expression and some common clinicopathologic variables such as age and pathologic type ( mucinous/nonmucinous), whereas no significant association was found between p53 expression and other parameters like gender, site of tumor, tumor grade and stage of the disease.

**Keywords:** *Colorectal carcinoma, immunohistochemistry, p53 gene, protein expression*

## Introduction

Colorectal carcinoma is one of the leading causes of cancer-related morbidity and mortality in the world. Globally, nearly 1.4 million new colorectal cancer (CRC) cases are believed to occur each year, which account for over 9% of all incident cancers.[1]

The etiology of CRC is complex, involving interplay of environmental and genetic factors. Colorectal carcinoma develops through a multistep process and progression from benign colorectal adenoma to malignant carcinoma arises from accumulation of several events, including chromosomal abnormalities, genetic mutations, and epigenetic changes.[2-4] These changes result in inactivation of tumor suppressor genes and DNA mismatch repair genes or activation of oncogenes. Accumulation of molecular alterations including mutations in Kirsten-ras, p53, and adenomatous polyposis coli contributes to colorectal carcinogenesis.[5]

p53 has been described as the guardian of genome because of its role in conserving stability by preventing gene mutation. The p53 gene (also known as TP53) is a tumor suppressor gene located on short arm of chromosome 17p13.1,[6] has a molecular weight of 53 KDa, and plays an important role in controlling cell growth.

p53 mutations is the most frequent genetic event in human cancers and accounts for more than 50% cases.[7] Among the various genes implicated in colorectal carcinogenesis, much work has been done to know p53 expression as a diagnostic and prognostic marker in colorectal carcinoma. The increasing number of studies about the molecular biology aspects of CRC has raised great interest about p53 gene and respective protein function.[8-17]



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With the recent trends of increasing incidence of colorectal carcinoma worldwide, the present study was done to know the incidence and pattern of colorectal carcinoma in Indira Gandhi Medical College as it is the major referral institute in Himachal Pradesh. We studied the p53 expression in these tumors and its utility as a diagnostic and prognostic marker by correlating p53 expression with various clinicopathological parameters.

## Materials and Methods

Sixty cases of biopsy-proven colorectal carcinoma who underwent tumor resection in the Department of Surgery, Indira Gandhi Medical College, Shimla, for 1 year were selected for this study. Detailed clinicopathologic information of all these patients including gender, age, site of tumor (right and left colon using middle of the transverse colon as partition), histologic type (mucinous vs. nonmucinous), grade, and stage of the disease were obtained from the files maintained at the Department of Surgery and Pathology. The specimens were grossed and the sections were fixed in 10% formalin and paraffin embedded. Consecutive 4 μm sections were cut from the paraffin blocks. Sections were subjected to histologic evaluation to select blocks without necrotic and hemorrhagic areas. Histopathological diagnosis was established on routine hematoxylin and eosin staining of the sections. Immunohistochemistry for p53 was carried out on BioGenex Xmatrix fully automated front-end processing system using monoclonal antibody against p53 protein (clone DO-7; BioGenex, Fremont, California, USA). Immunoreactivity for p53 was evaluated semiquantitatively by two observers under ×40 magnification. All tumors showing p53 immunoreactivity (in at least 5% of nuclei) were considered to be positive.

The pathologists were not aware of the report of each other. According to the percentage of positive tumor nuclei, immunoreactivity for p53 in various colorectal carcinomas was scored as follows:

- None (<5%, Score 0)
- Weak (+, 5%–25%, Score 1)
- Moderate (++, 25%–75%, Score 2)
- Intense (+++, >75%, Score 3).

After primary evaluation of the results, cases with major incompatibility in reports were rechecked by both pathologists and after reaching an agreement, a single report was submitted. Then, p53 scoring was correlated with clinicopathological parameters. The frequency of p53 positive tumors with each variable was compared using $\chi^2$ analysis. $P < 0.05$ was considered statistically significant.

## Results

Among the 60 patients, 29 patients were male and 31 were female. The mean age of patients was 55.23 (range: 26–80) years. The tumor was located in the right colon in 27 cases and left colon in 33.31 cases (51.6%) were well-differentiated adenocarcinomas, 24 cases (40%) were moderately differentiated, and 5 cases (8.4%) were poorly differentiated adenocarcinomas. Among these, 16 cases (26.6%) were mucinous and 44 cases (73.4%) were nonmucinous. Lymphovascular invasion was detected in 18 cases (30%) and lymph node metastasis was found in 22 cases (36.6%). The stage was Duke B1 in 18, B2 in 15, C1 in 14, C2 in 8, and D in 5 patients. We did not have any case with stage Duke A [Table 1].

p53 staining was positive in 42 of 60 cases. Of these, 10 cases were weekly positive (Score 1) [Figure 1], 16 were moderately positive (Score 2) [Figure 2], and 16 were intensely positive (Score 3) [Figure 3] for p53 protein overexpression. p53 staining was negative in 18 cases (Score 0). All five cases of poorly differentiated

**Table 1: Clinicopathological correlation with p53 expression**

| Clinicopathological parameters | *n* | p53, *n* (%) | *P* |
|---|---|---|---|
| All cases | 60 | | |
| Gender | | | |
| Male | 29 | 20 (68.9) | 0.86 |
| Female | 31 | 22 (70.9) | |
| Tumor location | | | |
| Left | 31 | 21 (67.7) | 0.69 |
| Right | 29 | 21 (72.4) | |
| Pathologic grade | | | |
| Well differentiated | 31 | 22 (70.9) | 0.48 |
| Moderately differentiated | 24 | 16 (69.5) | |
| Poorly differentiated | 5 | 5 (100) | |
| Pathologic type | | | |
| Nonmucinous | 44 | 35 (79.5) | 0.007 |
| Mucinous | 16 | 7 (43.7) | |
| Stage (Duke) | | | |
| B | 33 | 24 (72.7) | 0.34 |
| C | 22 | 18 (81.8) | |
| D | 5 | 5 (100) | |

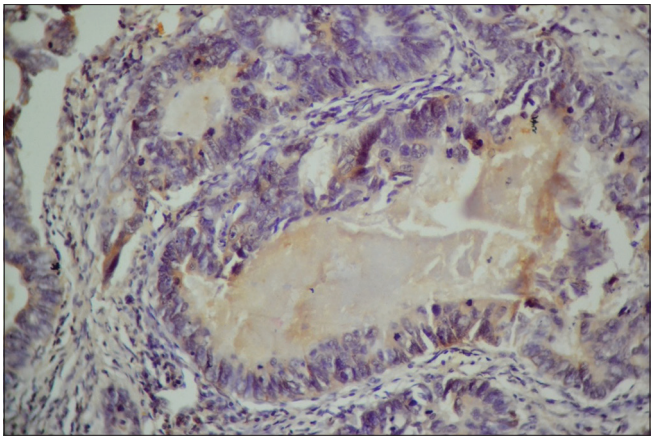

**Figure 1: Score 1 (+ positivity) p53 expression in colorectal carcinoma (immunohistochemistry, ×40)**

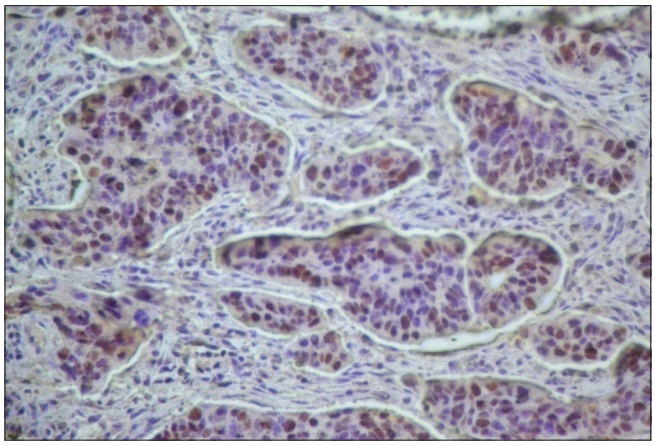

**Figure 2: Score 2 (++ positivity) p53 expression in colorectal carcinoma (immunohistochemistry, ×40)**

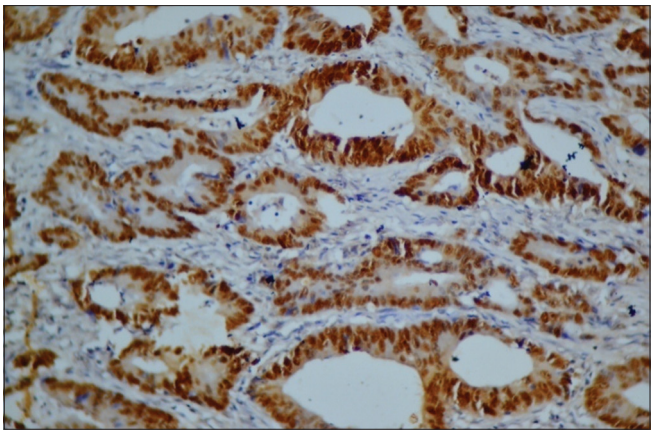

**Figure 3: Score 3 (+++ positivity) p53 expression in colorectal carcinoma (immunohistochemistry, ×40)**

adenocarcinomas showed positivity for p53 staining. The relationship between p53 protein overexpression and several clinicopathologic variables is summarized in Table 1. There was statistically significant correlation between p53 staining and age (<40 years vs. ≥40 years; $P = 0.006$) and pathological type (mucinous vs. nonmucinous; $P = 0.007$). There were no significant correlations between p53 staining and gender ($P = 0.86$), site of tumor (right colon vs. left colon and rectum; $P = 0.69$), grade ($P = 0.48$), and stage of the disease ($P = 0.34$). Although we found higher percentage of p53 positive cases in advanced stages of disease, the difference between various stages was not statistically significant probably due to less number of cases in stage Duke D.

## Discussion

CRC is one of the most common malignancies with high prevalence and low 5-year survival. CRC is a heterogeneous disease with a complex, genetic, and biochemical background. Tumor suppressor p53 protein is a transcription factor inducing cell cycle arrest, senescence, and apoptosis under cellular stress. It is now generally accepted that p53 signaling is frequently dysregulated in CRC. This study was conducted to correlate the expression of p53 with various clinicopathological parameters so as to assess the utility of p53 expression in diagnosis and prognosis of colorectal carcinoma.

In our study, of 60 cases of colorectal carcinoma samples, 42 cases (70%) displayed p53 protein overexpression. High percentage of p53 expression was also found in study conducted by Yalcinkaya *et al*.[18] (70%), Goh *et al*.[19] (68.1%), Wehmuth *et al*.[12] (67.2%), Theodoropoulos *et al*.[17] (63.4%), and Kruschewski *et al*.[20] Since overexpression of p53 is synonymous with mutation, our result confirms that p53 gene is one of the most common genetic changes in the development of human CRC.

In our study, most of the patients were >40 years of age and 79.5% of these expressed p53 positivity which was similar to the study done by Rambau *et al*.[15] and Nasiri *et al*.[13] This correlation between p53 staining and age (<40 years vs. ≥40 years; $P = 0.006$) was statistically significant.

Our study revealed p53 positivity in 68.9% of all male patients and 71% of all female patients. This was comparable to the study by Wehmuth *et al*.[12] in which p53 positivity was seen in 59.2% of all male patients and 72.2% of all female patients and study done by Nasiri *et al*.[13] in which p53 positivity was found in 54.1% of all male patients and 63.4% of all female patients. There were no significant correlations between p53 staining and gender ($P = 0.86$).

Left-sided tumors were more than right-sided tumors and 67.7% of these revealed p53 positivity. This finding was comparable to the study done by Wehmuth *et al*.[12] and Nasiri *et al*.[13] where the percentage of p53 positivity in left-sided tumors was 73.6% and 55.5%, respectively. However, there was statistically no significant correlations between site of tumor and p53 expression.

Percentage of well-differentiated carcinoma was more (51.6%) and 71% of these revealed p53 positivity. This was similar to the finding by Kang *et al*.[21] where well-differentiated carcinomas were more and 44.4% of them revealed p53 positivity. However, there was no statically significant correlation between pathological grade and p53 expression.

Our study revealed more of nonmucinous type cancers, and of these, p53 positivity was found in 79.5% of the cases. This finding was comparable to the studies done by Wehmuth *et al*.[12] and Nasiri *et al*.[13] where the percentage of nonmucinous tumors was more, and of these, p53 was positive in 66.6% and 60.2% of the cases, respectively. There was statistically significant correlation between p53 staining and pathological type (mucinous vs. nonmucinous; $P = 0.007$).

All patients in stage Duke D revealed p53 positivity. This was similar to the studies done by Nasiri *et al*.,[13]

Theodoropoulos *et al*.,[17] and Starzynska *et al*.[9] in which p53 positivity increased with the tumor stage. Although we found higher percentage of p53-positive test in higher stages, especially in stage Duke D (100% of cases), the difference did not reach statistical significance, probably due to low number of cases with stage Duke D ($P = 0.34$).

## Conclusion

p53 protein mutation seems to play an important role in the carcinogenesis of CRC. Considering the acceptable reliability and feasibility of IHC method for detection of p53 mutation, this technique may be expected to serve as a new genetic marker for predicting recurrence and response to chemotherapy in patients with CRC and p53 can also be considered as an important diagnostic and prognostic marker in colorectal carcinoma.

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### Conflicts of interest

There are no conflicts of interest.

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