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ORIGINAL PAPER

Immunohistochemical expression of Ki-67 and p53 in colorectal carcinoma

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ABSTRACT

Objectives*: To investigate the expression of Ki-67 and p53 in colorectal carcinomas and to correlate expression patterns of these markers with histopathological grades of colorectal carcinoma.* ***Materials and methods****: We determined immunohistochemically the expression of Ki-67 and p53 antibodies in 62 cases of colorectal adenocarcinomas.* ***Results****: Mean Ki-67 index in our study was 33.9%. Mean Ki-67 increased with dedifferentiation of tumor (Grade I-18.8%, Grade II-33.2%, Grade III-49.1%). The difference in mean Ki-67 index is statistically significant with histopathological grade. (p <0.001). p53 overexpression was seen in 69.3% cases. Percentage of diffuse p53 positivity increased with dedifferentiation of tumor. p53 positivity rate was observed in 22.2% cases of well differentiated carcinoma and increased from 76.2% to 81.8% in moderately to poorly differentiated carcinoma. However, the difference was statistically insignificant with histopathological grade.* ***Conclusion****: As the result of this study it is concluded that in colorectal carcinoma Ki-67 LI correlated with grade and increases with the dedifferentiation of tumor and also p53 over expression increases with dedifferentiation of tumour. Thus, the evaluations of expression of p53 and Ki-67 can be used as a poor prognostic marker allowing the identification of aggressive forms.*

Keywords: Histopathology; immunohistochemistry; Ki-67 labelling index.

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common malignancy with over one million new cases and over 5,00,000 deaths each year worldwide.[1] Incidence of colorectal cancer varies widely, with higher incidence rates in North America, Australia and Europe. Developing countries have lower rates; particularly Africa and Asia.[2] However, with Westernization of lifestyle, the incidence of colorectal cancer is nowadays increasing in many developing countries also. Surgery still remains the primary treatment modality and pathological examination of resected specimen is a powerful tool for assessing the prognosis.[3]

The p53 gene mutation is related to carcinogenesis in most malignant diseases and has been widely studied. More articles reveal that their immunostain could predict the colorectal carcinoma prognosis. The p53 protein is encoded by a gene p53, located on the short arm of chromosome 17, a frequent site of allelic loss in many tumors. The wild p53 maintains the integrity of genes by detecting mutations and preventing the division of cells with damaged DNA. It blocks the cells in G1 phase of cellular cycle. In colorectal carcinoma, the gene p53 may be rearranged and p53 protein may be altered. Therefore, the replication errors and deregulation of cell growth could appear. According to literature, the deletion of p53 gene with the overexpression of p53 protein is correlated with a lower rate of survival, thus being an independent prognostic factor.[4]

Ki-67 is a nuclear antigen, which is expressed in proliferating cells from G1 to M-phase of the cell cycle. Many studies

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have shown a predictive role of Ki-67 in a wide range of human malignancies, including gastrointestinal stromal tumours, gastrointestinal neuroendocrine tumours, and prostate and breast cancer.[5] Quantification of cell proliferation activity in neoplasms has been targeted with the help of Ki-67 antibody.

The present study was conducted to study the combined immunoexpression of Ki-67 and p53 protein in colorectal carcinomas and to correlate expression patterns of these markers with histopathological variables of colorectal carcinoma. The present study evaluated their role in colorectal carcinoma in our set up.

MATERIALS AND METHODS

This is a hospital-based study, done in Department of Pathology, Silchar Medical College and Hospital. We studied 68 consecutive cases of colorectal carcinomas over a period of 2 years (2015 to 2017). But we considered only adenocarcinomas for further study, excluding the mucinous carcinomas and carcinoid. 62 cases of colorectal adenocrcinomas were studied. Blocks were retrieved and H&E staining done. The tumor was then graded as well, moderate and poorly differentiated according to WHO grading criteria. Diagnosis of a colorectal carcinoma as a grade III tumor is done when the poorly differentiated component comprises > 50% of the tumor.[6]

Immunohistochemistry for p53 and Ki-67 were done. Sections (4μ) from formalin fixed paraffin embedded tissue blocks were stained by standard immunohistochemical methods using horseradish peroxidase-linked antibody.

The following primary antibodies were used: antibody to p53 (DO7) (mouse monoclonal antibody, prediluted, CELL MARQUE), antibody to Ki-67 (SP6) (Rabbit monoclonal antibody, prediluted, CELL MARQUE).

The positive controls used were sections of infiltrating duct carcinoma of the breast for both p53 and Ki-67 antigen. **The negative controls** used were sections of the study tissues with no primary antibody incubation.

RESULTS

Statistical analysis was done using **Chi square test** and **ANOVA**.

Ki 67 nuclear staining was regarded as positive whereas cytoplasmic staining was considered as artifact. Ki-67 immunostaining index was interpreted as **Labelling index (Ki-67 LI)** = Number of nuclei showing positive staining (brown color)/total number of nuclei × 100 (in 40 X magnification). MIB-1 labelling index (LI) was determined by counting about 500 tumor cells.

A tumor was considered positive with significant proliferating activity only if nuclear Ki-67 accumulation was identified in at least 10% of all malignant cells in a tissue section.

In p53 immunostaining, 10% or more stained malignant nuclei were scored as positive, regardless of the staining intensity. If fewer than 10% of the nuclei were stained, the slide was scored as negative. The staining distribution was either focal or diffuse.

Out of the 68 total cases of colorectal cancer, 62(91.1%) cases were adenocarcinoma, 4(5.9%) cases were mucinous carcinoma, and 2(2.9%) were signet ring carcinomas. Only cases of colorectal adenocarcinoma were studied. Age of the patients ranged from 30 years to 75 years, (mean 57.8 ± 1.2 years). Males (66.1%) were more commonly affected than females (33.9%).

The rectum was the most common affected site contributing to 38 cases (61.2%) followed by ascending colon with 14 cases (22.6%), caecum 6 cases (9.7%) and descending colon 4 cases (6.5%). Most common histopathological type were moderately differentiated adenocarcinoma accounting for 67.7% cases. Well differentiated and poorly differentiated cases were 14.5% and 17.8% cases respectively.

In our study, positive nuclear immunohistochemical staining for Ki-67 antibody was seen in all 62 colorectal carcinomas. Ki-67 LI ranged from 7% to 53%. The mean Ki-67 LI was 33.9%.

Table 1 Expression of Ki-67 in different grades of colorectal adenocarcinoma

GRADE	Ki-67 LI values				
	Minimum	Maximum	Range	Mean	SD
Well differentiated	7	34	27	18.8	±9.03
Moderately differentiated	23	41	18	33.2	±4.08
Poorly differentiated	42	53	11	49.1	±4.08

From **Table1**, it is seen that mean Ki-67 index was 18.8% in grade I, 33.2% in grade II, 49.1% in grade III. The difference in mean Ki-67 index was statistically significant with histopathological grade. $p < 0.0001$, highly significant Anova.

Table 2 Expression of p53 in different grades of colorectal adenocarcinoma

Histopathologic grade	P 53 +	P 53 -
Well differentiated	2(22.2%)	7 (77.8%)
Moderately differentiated	32(76.2%)	10 (23.8%)
Poorly differentiated	9 (81.8%)	2(18.2%)

$p \leq .003$

In our study, it is seen that p53 overexpression was seen in 69.3% of cases. From **Table 2**, it is seen that the positivity increased with increase in the histopathological grade. p53 positivity rate was observed in 22.2% cases of well differentiated carcinoma and increased from 76.2% to 81.8% in moderately and poorly differentiated carcinoma. This difference was statistically significant.

In our study, we found p-53 immunostaining was diffuse (i.e. more than 50% of the cells stained) in 42.8% cases and

focal (i.e., 5-50% cells stained) in 57.2% cases.

Table 3 Percentage of cases with diffuse p53 distribution in different grades of colorectal adenocarcinoma

Grades	Percentage of cases with diffuse distribution of p53
Well Differentiated	31.4%
Moderately Differentiated	40.5%
Poorly Differentiated	59.6%

From **Table 3**, it is seen that diffuse p-53 distribution was seen in 31.4% cases of well differentiated cases, 40.5% of moderately differentiated cases and 59.6% of the poorly differentiated cases.

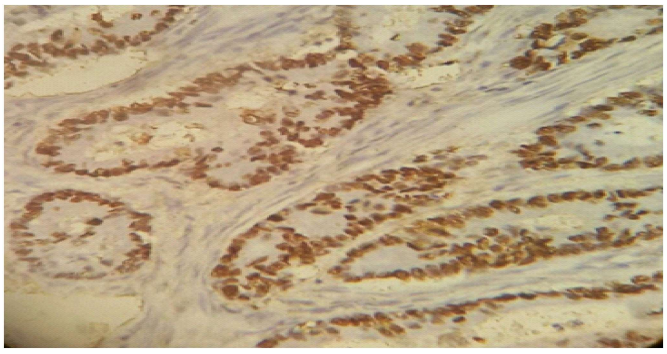

Figure 1 Ki-67 staining in well differentiated adenocarcinoma colon

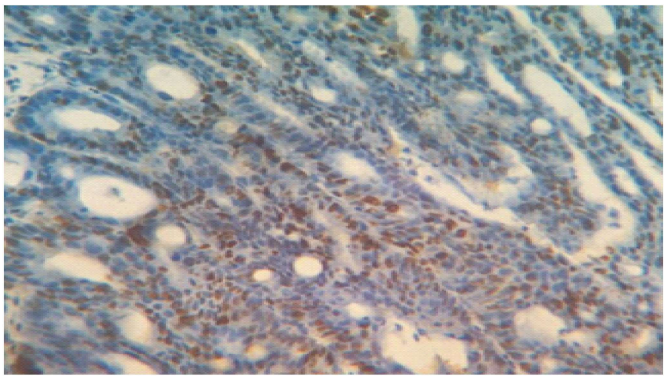

Figure 2 Ki 67 staining in moderately differentiated adenocarcinoma colon

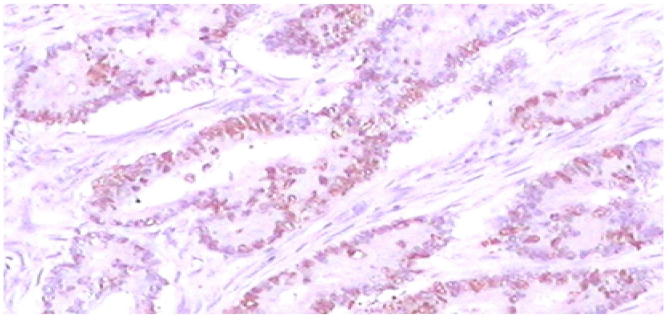

Figure 3 p53 staining of well differentiated adenocarcinoma colon

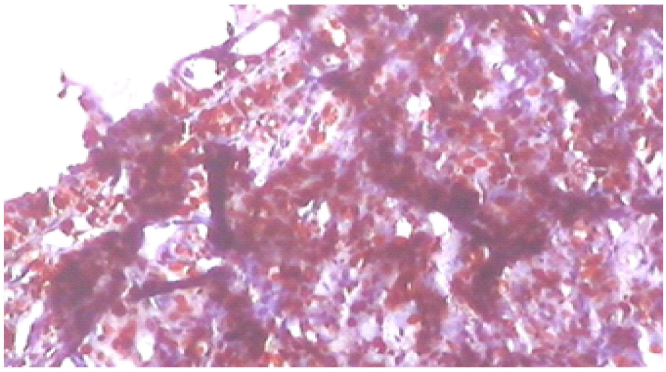

Figure 4 p53 staining in poorly differentiated carcinoma colon

DISCUSSION

In our study, the mean age of colorectal cancer was found to be 57.8 ±1.8 years, which is in accordance with the study done by Nayak et al[7] where mean age was 55.6 ± 1.2 and Peedikayil et al[8] where mean age was 58.4 years. In this study, it was found that rectum was the most common site affected contributing to 61.2% cases, followed by ascending colon 22.6%, caecum 9.7% and descending colon 6.5% cases. Peedikayil et al[8] found 74% of the tumors were located distal to the splenic flexure, Nayak et al[7] reported sigmoid colon to be the commonest site followed by cecum and rectum.

Table 4 Comparison of Mean Ki-67 index in different studies

Study	No. of cases	Mean Ki-67 Index
Ihmann et al.[9]	43	32.8%
Komal Mahendra et al.[10]	47	34.7%
Saleh et al.[11]	52	38.12%
Porschen R et al[12]	61	38.7%
Claudia et al.[13]	41	48%
Palmquist R et al.[14]	56	43.7%
Present study	62	33.9%

In the above studies (**Table 4**), CRCs showed a wide range of Ki-67 LI, ranging from 32-50% indicating a variation in proliferative activity. The mean Ki-67 index in our study was 33.9%. This is in accordance with the study done by Ihmann et al[9] in which Ki-67 LI was 32.8%, Komal Mahendra et al[10] LI was 34.7%, Saleh et al[11] LI was 38.1% and Porschen et al[12] LI was 38.7%. However, Claudia et al[13] and Palmquist et al[14] found a bit higher levels of LI of 48% and 43.7% respectably. Ki-67 has a prognostic and/or predictive value in different tumor types. Some newer studies[15] established the fact that an increased expression of Ki-67 indicates a better survival in rectal and recto sigmoid cancer as these

tumors have better response to radiotherapy.

In this study, Ki-67 immunostain was positive in all the cases of adenocarcinomas. The mean values for Ki-67 LI for well, moderate and poorly differentiated lesions were 18.8%, 33.2% and 49.1% respectively, and the difference was statistically significant ($p<0.0001$). A strong positive significant correlation between Ki-67 LI and histopathological grade was found. In our study, mean values for Ki-67 LI increased with increase in grades of colorectal carcinoma. This in accordance with studies done by Amway Sen et al[16] who found that the Ki-67 LI increased with the histological grade of adenocarcinomas. There was a strong positive significant correlation between Ki-67 LI and histopathological grade in their study. Georgescu et al[19] found that the Ki-67 LI increased with the histological grade of adenocarcinomas. Also, in studies done by Claudia et al,[13] Saleh et al,[11] Simona et al,[20] Azza et al[15] the mean Ki-67 index increased with grade of tumor or dedifferentiation of the tumor. In contrast to these studies, other studies by Uzma et al[21] and Ishida et al.[22] found that Ki-67 index was lower in cancers with poor differentiation.

Table 5 Comparison of Mean Ki67 index with histological grade of tumor

Mean Ki-67 Index/ Grade	Amway et al.[16]	Claudia et al.[13]	Saleh et al.[11]	Ishida et al.[20]	Present study
Well Differentiated	14.25%	20%	35.7%	57.7%	18.8%
Moderately Differentiated	31.64%	34%		60.9%	33.2%
Poorly Differentiated	43.08%	57%	48.3%	46.6%	49.1%

From **Table 5**, a comparative study of Ki67 LI in different studies is being made. It is seen that in these studies Ki 67 LI increases with increase in grades of colorectal carcinomaexcept in studies done by Ishida et al.

Detection of p53 overexpression by immunohistochemistry is based on the accumulation of p53 protein in cells. In this study, p53 overexpression was seen in 69.3% of cases. The positive cases increased with the increase in the histolopathological grade and results were statistically significant.

Comparison of p53 positivity rate in different studies:

The frequency of p53 over expression ranged from 58% to 70% in different studies. However, this wide range of p53 positivity is due to inter-study variations, including different antibodies, scoring systems, cut-off values, and study populations. The p53 overexpression in our study was found in 42 cases (69.3%). This is in accordance with study done by Saleh et al[11] where p 53 overexpression was seen in 67.3% cases. p53 overexpression was also seen in some previous reported studies such as Yamagiwa H et al[17] 60.6%, Claudia et al[13] (58.5%), Yong Shin et al[18] (60.9%), Komal et al.[10] (72.3%)

Comparison of p53 positivity rate with histological grade

In respect to cell differentiation we found that the p53 positivity increased with the dedifferentiation of tumors. The results were statistically significant. This was in accordance to study by Claudia et al.[13] But Saleh et al[11] found that p53 positivity rate decreased with dedifferentiation though it was not statistically significant.

Comparison of cases with focal and diffuse p53 positivity:

In this study 57.2% cases showed focal p 53 positivity and 42.8% cases with diffuse p 53 positivity. This is in accordance with study done by Claudia et al[13] where 58.3% cases had focal p53 positivity and 41.6% cases had diffuse p53 positivity. However, Komal et al[10] found that 17.65% cases had focal p 53 positivity and 82.35% cases had diffuse p53 positivity.

Comparison of percentage of cases with diffuse p53 positivity with histological grade of tumor:

In this study, diffuse p53 positivity was found in 31.4% cases of well differentiated, 40.5% and 59.6% in moderate and poorly differentiated colorectal adenocarcinoma respectively. The high p53 positive rate in high grade adenocarcinomas suggest that p53 is involved in cell dedifferentiation in colorectal adenocarcinomas. This is in accordance with study by Claudia et al[13] and Komal et al.[10]

CONCLUSION

As the result of this study it is concluded that in colorectal carcinoma Ki-67 LI correlated with grade and increases with the dedifferentiation of tumor and also p53 positivity rate increases with dedifferentiation of tumour. Thus, the evaluations of markers expression p53 and Ki-67 can be used as a poor prognostic marker allowing the identification of aggressive forms. This can also help in selecting patients for targeted therapies in near future.

Conflict of Interest: None.

Ethical Clearance: Taken.

Contribution of Authors: We declare that this work was

done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The study was conceived, designed by Dr. Mili Das and co-authors along with data collection. Statistical analysis was carried out by Dr. Mili Das.

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