Evaluation of Immunohistochemical Expression of p53 in Colorectal Carcinoma

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Abstract

Background: The most common gastrointestinal malignancy is colorectal carcinoma and is a major cause of morbidity and mortality. In colorectal carcinoma the most frequently mutated gene is p53 tumor suppressor gene. Mutation of p53 gene gives rise to abnormal protein which can be easily detected by immunohistochemistry. Expression of mutant p53 protein has been associated with poor clinical outcome and increased risk of death due to increased aggressiveness of the disease.

Objective: The aim of the study was to see the clinicopathological correlation of mutant p53 expression in colorectal carcinoma.

Method: Total 50 paraffin embedded tissue blocks of histopathologically diagnosed cases of colorectal cancer were evaluated by immunohistochemical staining for mutant p53 expression. The study was performed in Sir Salimullah Medical College, Dhaka (from March, 2018 to February, 2020).

Results: Out of 50 patients studied, 29 cases (58%) expressed mutant *p53* protein in the nucleus of malignant cells. There was significant association between p53 protein expression and clinicopathologic variables such as age (<40 years vs >40 years, p=0.032), site of tumor (left vs right colon, p=0.028), pathological type (mucinous vs non mucinous, p=0.039), grade (a greater tendency towards poor differentiation, p= 0.039), advanced stage (both TNM and Dukes), whereas no significant association was found between mutant p53 protien expression and other parameters like gender and morphological types.

Conclusion: The results of this current study revealed that mutant p53 positive colorectal cancer tended to be related to a higher grade of malignancy, advanced tumor stage and mucinous morphology. The results of this current study revealed that mutant p53 positive colorectal cancer tended to be related to a higher grade of malignancy, advanced tumor stage and mucinous morphology. So, p53 is an important immunohistochemical marker for colorectal cancer patients.

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Keywords: Colorectal cancer, p53, Immunohistochemistry

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Introduction

Colorectal cancer (CRC) is the most common malignancy of gastrointestinal tract and it is one of the leading causes of cancer related morbidity and mortality in the world. Over 1.9 million new CRC cases and 930 000 deaths were estimated in 2020 and the burden of CRC is projected to increase to 3.2 million new cases and 1.6 million deaths by 2040.[1] Adenocarcinoma is the most common form of colorectal cancer (>95%).[2] The essential elements of the pathological assessment of colorectal cancer resection specimens include the pathologic determination of TNM stage, tumor type, histologic grade, status of resection margins, and vascular invasion.[3] Despite the numerous prognostic indicators currently in use or under investigation, it is impossible to predict the outcome in an individual patient. For this reason there is continuous search for better or more refined biological markers for prognosis and effective treatment modalities. 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.[4] The occurrence of CRC is involving many biomarkers, of which p53 abnormality is one of most important and familiar events. Studies have demonstrated that p53 abnormality was detected among more than 50% of the CRC patients and was closely connected to the development, infiltration, and metastasis of CRC.[5] p53 has been described as the guardian of genome because of its role in conserving stability by preventing gene mutation and it regulates cell cycle progression.[6] The p53 overexpression confers cancer cell immortalization by inhibiting the apoptotic cell death machinery.[7] Mutation of p53 gene gives rise to abnormal protein with a long half life rendering it to be easily detected by immunohistochemistry (IHC).[8] Moreover, IHC is known for its simplicity and for its compatibility with other techniques like DNA sequencing. Several studies have shown that tumor cells with impaired p53 function have poor response to adjuvant or neoadjuvant therapy.[9,10,11] Detection of mutant p53 protien in malignant cells has been associated with poor clinical outcome and reduced survival in several tumor type including colorectal cancer.[12,13] The present study was undertaken to see the clinicopathological correlation of mutant p53 expression in colorectal carcinoma.

Methods

This cross sectional study was conducted in Department of Pathology, Sir Salimullah Medical College, Dhaka during the period from March 2018 to February, 2020. After approval from the institutional ethics committee and obtaining informed written consent, 50 adult patients with colorectal carcinoma diagnosed histopathologically were included. Tissue blocks with extensive hemorrhage or necrosis, previously exposed patients to chemotherapy or radiotherapy, or with metastatic carcinoma were excluded. Histopathology was done by H&E followed by immunohistochemistry for p53 and for every batch of slides, both positive and negative controls were included and analyzed. Immunohistochemical staining for p53 was evaluated by the number (%) of p53 positive cells and classified on a four point scale as – (<5%), 1+ (5-25%), 2+(26-75%), 3+(>75%). 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 (Dukes stage) with overexpression of p53 were studied. Statistical analyses were carried out by using SPSS version 22 for Windows. A descriptive analysis was performed for all data. Observations were indicated by frequencies and percentages. Statistical significance was set at "p" value <0.05.

Results

The age range of study population was from 32 to 75 years and more than one third (42.0%) of patients belonged to age 50-59 years. Among the patients 64% were male.

The most common site for CRC was sigmoid colon (18, 36%) followed by rectum (14, 28%), caecum (12, 24%), and transverse colon (6, 12%). It was observed that more than one third (34.0%) of patients had annular growth followed by polypoid growth (15, 30.0%), ulcerative growth (15, 30.0%) and infiltrating growth (3, 6.0%). More than one third (36.0%) of malignant tumors were mucinous carcinoma and 32(64.0%) were non-mucinous carcinoma.

It was observed that almost half- 24(48.0%) of patients had moderately differentiated carcinoma followed by poorly differentiated carcinoma 15(30.0%), and well differentiated carcinoma 11(22.0%).Out of 50 CRC, 26 (52%) showed metastasis to lymphnodes.

On p53 staining more than one third of tumors showed P53 expression negative (21, 42.0%) followed by weakly positive (11, 22%), moderately positive (9,18%) and strongly positive (9,18%) (Table I).

There was positive significant correlation (p=0.032) between advanced age of patients and p53 expression. According to site, the mean P53 expression was more in left colon (26.46±8.25) than in right colon (20.57±9.73) which was statistically significant ($p<0.05$). The association between grading and P53 expression were statistically significant ($p<0.05$) (Table II). The mean P53 expression was 26.35±10.91 in mucinous tumor and 20.13±9.5 in non-mucinous tumor. The difference was statistically significant ($p<0.05$) between two groups. The association between staging pT, pN, Dukes staging and P53 expression were statistically significant ($p<0.05$))(Table III,IV,V)..

Furthermore, we attempted to correlate the p53 status with sex, and morphologic type. However, none of these clinicopathological parameters showed statistically significant correlation with p53 {sex (p=0.108), morphological type (p=0.108)}.

Table I: Distribution of the study cases by P53 Expression (n=50)

P53 Expression	Number of cases	Percentage (%) of cases
<5% (Negative)	21	42.0
5-25% (Weakly positive)	11	22.0
26-75% (Moderately positive)	9	18.0
>75% (Strongly positive)	9	18.0

Table II: Association between grading and P53 expression (n=50)

Grading	P53 Expression N	Mean±SD	Min-max	P value
Well-differentiated (A)	11	18.55±15.52	0-90	
Moderately differentiated (B)	24	20.1±14.7	0-80	
Poorly differentiated (C)	15	31.53±14.21	0-80	
Statistical analysis				
A vs B vs C				0.039[s]
A vs B				0.777[ns]
A vs C				0.035[s]
B vs C				0.021[s]

s =significant
ns= not significant
ANOVA followed by Bonferroni test was performed to compare between groups.

Table III: Association between staging pT with P53 expression (n=50)

Staging T	P53 Expression n	Mean±SD	Min-max	P value
T1 (A)	7	13.71±23.71	0-90	0.003[s]
T2 (B)	26	16.27±14.25	0-90	
T3 (C)	17	37.53±25.57	0-90	

s =significant
ANOVA followed by Bonferroni test was performed to compare between groups.

Table IV: Association between staging pN with P53 expression (n=50)

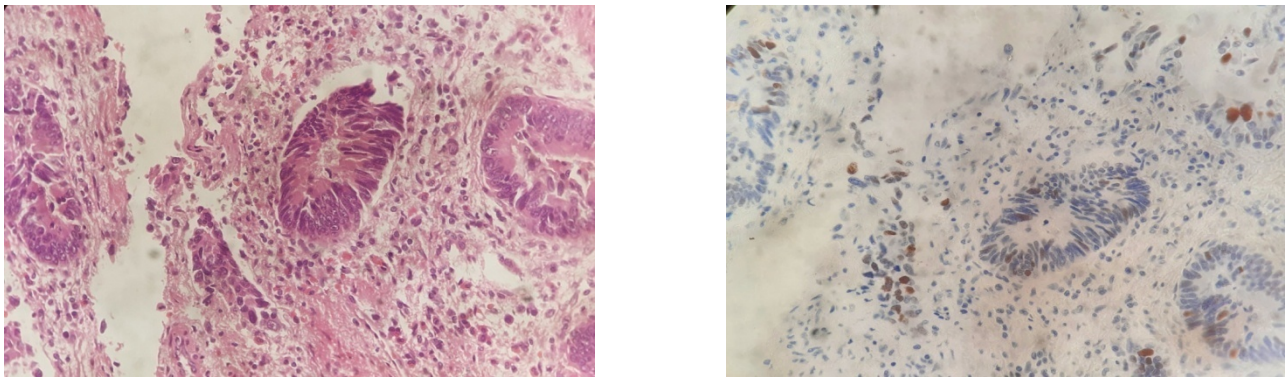
Staging N	P53 Expression N	Mean±SD	Min-max	P value
N0 (A)	24	18.29±12.23	0-80	0.019[s]
N1 (B)	13	22.54±14.98	0-80	
N2 (C)	13	32.69±16.9	0-80	

s =significant
ANOVA followed by Bonferroni test was performed to compare between groups.

Table V: Association between Dukes' staging with P53 expression (n=50)

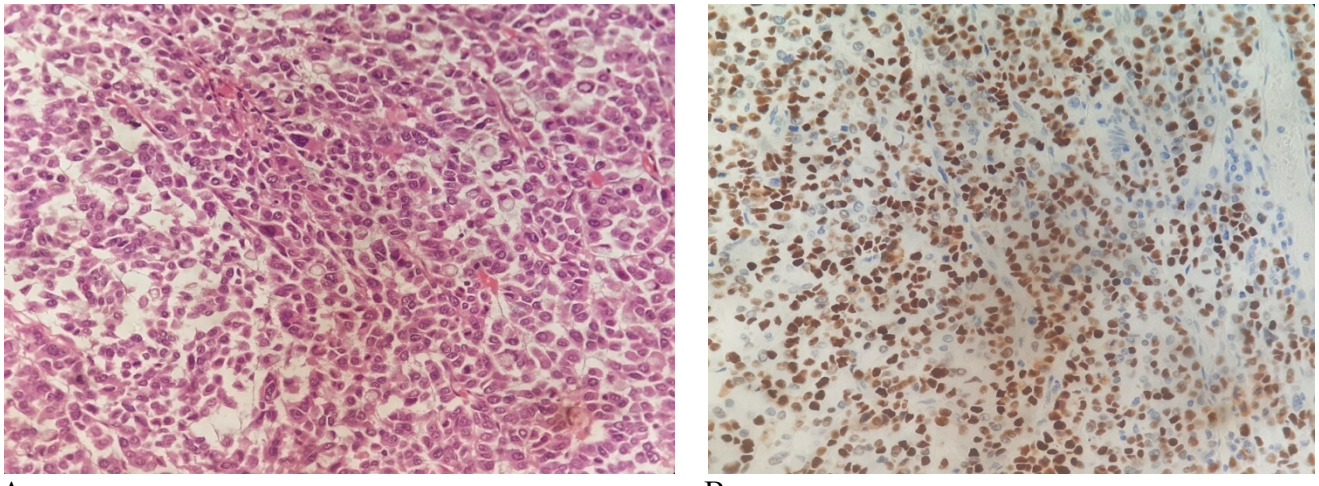
Duke's Staging	P53 Expression			P value
	n	Mean±SD	Min-max	
A	7	12.31±13.15	0-80	0.001[s]
B1	13	13.71±13.71	0-90	
B2	4	22.54±14.98	0-90	
C1	13	32.69±16.9	0-90	
C2	13	45.75±10.5	0-80	

s =significant
ANOVA followed by Bonferroni test was performed to compare between groups.



A B

Figure 1. Photomicrograph showing (A) H & E stained section (400x) and (B) p53 immunostain in well differentiated adenocarcinoma of colon, (400x)



A B

Figure 2. Photomicrograph showing (A) H & E stained section (400x) (B) p53 immunostain in poorly differentiated adenocarcinoma of colon, (400x)

Discussion

Many studies reveal that the mutant p53 immunostain is very high in CRC. The positivity rate is reported being between 43% and 86.36%.[14,15,16] In the present study, out of 50 cases of colorectal carcinoma samples, 29 cases (58%) displayed mutant p53 protein overexpression. The over expression of mutant p53 seen in this study and other previous studies, support the hypothesis that *p53* gene mutations are important in colorectal carcinogenesis. The results from a number of studies showing that *p53 protien immuno*expression is positively correlated with *p53* gene mutation.[17,18]

In the present study, correlation between p53 staining and age (<40 years vs. ≥40 years; p=0.032) was statistically significant. The study done by Kavita et al. (2017) detected significant relationship between age and p53 expression.[19] Although Prasad and Banda (2017) detected the age factor was not significant.[20]

The present study revealed p53 positivity in 65.5 % of all male patients and 34.5 % of all female patients. There was no significant correlations between p53 staining and gender (P =0.108). Present result is consistent with other studies [21,19] and is different from a study that showed a significant correlation with male gender.[10]

Present findings of a higher proportion of *p53* positive tumors in the left colon compared to the right colon is statistically significant (p=0.028) which is consistent with the study of Rambau et al.(2008).[8] This implies that the pathogenesis of left and right sided colonic tumors are different, and possibly the reason for differences in their prognosis.

It was observed that p53 expression in relation to morphological type is not significant (p= 0.108). Present result is consistent with studies of Prasad and Banda (2017) and Rambau et al.(2008)[20,8] but differs from the study by Shin et al. (2014) which had reported significant correlation.[22]

Compared with the p53 negative group, patients in the p53 positive group showed a greater tendency toward poor differentiation (p=0.039). Present result is consistent with Wang et al. (2017) study.[5] With regard to grade Kapiteijn et al. (2001), found that in poorly differentiated colorectal carcinomas, over expression of *mutant p53* occurred in more than 70% of tumors compared to less than 30% in those with well or moderately differentiated tumors.[24] In contrast to present findings Carneiro et al. (2006) found no relationship between *p53* expression and histological differentiation.[25]

In present study, patients in the p53 positive group showed a greater tendency toward advanced pT stage (p=0.003) and pN stage (p=0.019) that is consistent with Wang et al. study(2017).[5] No correlation was found between p53 expression and pT or pN factors in the study of Prasad and Banda (2017).[20] Studies done by Ghavam et al.(2007) and Theodoropoulos et al.(2009), in which p53 positivity increased with the Dukes stages of tumor.[21,26] Present study had higher percentage of p53 positive cells in higher Dukes stages, which is statistically significant(p=0.001). Meanwhile, multivariable analysis showed that p53 positive together with higher malignant pathological pattern, poor differentiation degree, advanced pT stage and pN stage, were all unfavorable prognostic predictors of CRC patients.[5]

In our study, the p53 positive group, the mucinous adenocarcinoma type and which meant a higher malignant degree compared with non mucinous type, took up a larger proportion than in the p53 negative group (p

=0.040). Our result is consistent with Peng et al. (2017) study.[5] However, Kavita et al. (2017) and Ghavam et al. (2007) found non mucinous adenocarcinomas had expressed more p53 than mucinous adenocarcinomas.[19,21]

It is now generally accepted that p53 signaling is frequently dysregulated in CRC.[19] 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.[27] 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.[28] Several studies have shown that tumor cells with impaired p53 function have poor response to adjuvant or neo-adjuvant therapy.[9,10] To assess the utility of mutant p53 expression in prognosis of colorectal carcinoma this study was conducted to correlate the expression of mutant p53 with various clinicopathological parameters.

Conclusion

The present study was intended to find out the association of p53 protein in colorectal carcinoma. CRC patients with mutant p53 positive tended to be related to a higher degree of grade, advanced TNM and Dukes' stage and mucinous morphology. The p53 positivity was an unfavorable prognostic marker for CRC patients. The IHC method is acceptable for detection of p53 mutation due to its reliability and feasibility. This technique may be expected to serve as a useful genetic marker for predicting the recurrence of CRC. It can also predict the response to chemotherapy in patients with CRC and identify patients who might be benefited from adjuvant therapy. So, p53 can be considered as an important prognostic marker in colorectal carcinoma.

References

1. Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, Vignat J, Ferlay J, Murphy N, Bray F. Global burden of colorectal cancer in 2020 and 2040: Incidence and mortality estimates from GLOBOCAN. Gut. 2023 Feb 1; 72(2):338-44.
2. Thrumurthy SG, Thrumurthy SS, Gilbert CE, Ross P, Haji A. Colorectal adenocarcinoma: risks, prevention and diagnosis. Bmj. 2016 Jul 14; 354.
3. Compton CC. Colorectal carcinoma: diagnostic, prognostic, and molecular features. Modern Pathology. 2003 Apr 1; 16(4):376-88.
4. Leslie A, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma–carcinoma sequence. British Journal of Surgery. 2002 Jul; 89(7):845-60.
5. Wang P, Liang J, Wang Z, Hou H, Shi L, Zhou Z. The prognostic value of p53 positive in colorectal cancer: A retrospective cohort study. Tumor Biology. 2017 Apr; 39(5):1010428317703651.
6. Kumar V, Abbas AK, Astor JC. Neoplasia. In: Robbins and Cotran Pathologic Basis of Disease. South Asian Ed., Vol. I. Elsevier; 2015. p. 293-294.
7. Li T, Kon N, Jiang L, et al. Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and Senescence. Cell 2012; 149(6): 1269–1283.
8. Rambau PF, Odida M, Wabinga H. p53 expression in colorectal carcinoma in relation to histopathological features in Ugandan patients. African health sciences. 2008;8(4):234-8.
9. Elsaleh, H., Powell, B., McCaul, K., Grieu, F., Grant, R., Joseph, D. and Iacopetta, B., 2001. P53 alteration and microsatellite instability have predictive value for survival benefit from

chemotherapy in stage III colorectal carcinoma. Clinical cancer research, 7(5), pp.1343-1349.
10. Liang, J.T., Huang, K.C., Cheng, Y.M., Hsu, H.C., Cheng, A.L., Hsu, C.H., Yeh, K.H., Wang, S.M. and Chang, K.J., 2002. P53 overexpression predicts poor chemosensitivity to high-dose 5-fluorouracil plus leucovorin chemotherapy for stage IV colorectal cancers after palliative bowel resection. International journal of cancer, 97(4), pp.451-457.
11. Adell, G., Sun, X.F., Stål, O., Klintenberg, C., Sjödahl, R. and Nordenskjöld, B., 1999. p53 status: an indicator for the effect of preoperative radiotherapy of rectal cancer. Radiotherapy and oncology, 51(2), pp.169-174.
12. Dignam JJ, Ye Y, Colangelo L, Smith R, Mamouncs EP, Wie HS, Wolmark N. Prognosis after rectal cancer in blacks and whites participating in adjuvant therapy. Clin Oncol. 2003;21:413–420.
13. Levine AJ, Perry ME, Chang A, Silver A, Dittmer D, Wu M, Welsh D. The role of p53 tumor suppressor genes in tumorigenesis. Br J Cancer. 1994;57:1–9.
14. Akshatha C, Mysorekar V, Arundhathi S, Arul P, Raj A, Shetty S. Correlation of p53 overexpression with the clinicopathological prognostic factors in colorectal adenocarcinoma. Journal of clinical and diagnostic research: JCDR. 2016 Dec;10(12):EC05.
15. BAN, Q., HUSAM, A. and ALAA, H., 2012. Immunohistochemical expression of p53 and Bcl-2 in colorectal adenomas and carcinomas using automated cellular imaging system.
16. Ghiţă C, Vîlcea ID, Dumitrescu M, Vîlcea AM, Mirea CS, Aşchie M, Vasilescu F. The prognostic value of the immunohistochemical aspects of tumor suppressor genes p53, bcl-2, PTEN and nuclear proliferative antigen Ki-67 in resected colorectal carcinoma. Rom J Morphol Embryol. 2012 Jan 1;53(3):549-56.
17. Colomer, A., Erill, N., Verdú, M., Roman, R., Vidal, A., Cordon-Cardo, C. and Puig, X., 2003. Lack of p53 nuclear immunostaining is not indicative of absence of TP53 gene mutations in colorectal adenocarcinomas. Applied Immunohistochemistry & Molecular Morphology, 11(2), pp.130-137.
18. Huang, C.C., Cheng, Y.W., Chen, M.C., Lin, Y.S., Chou, M.C. and Lee, H., 2006. Different p53 mutation patterns in colorectal tumors from smokers and nonsmokers. Environmental and Molecular mutagenesis, *47*(7), pp.527-532.
19. Kavita M., Sharma, M., Bhardwaj, M. and Rao, M., 2017. p53 expression in colorectal carcinomas and its correlation with clinicopathological parameters. Clinical Cancer Investigation Journal, *6*(1), p.26.
20. Raja Prasad, G. and Banda, S., Evaluation of Immunoreactivity of p53 in Colorectal Adenocarcinomas.
21. GHAVAM NM, SEYLANIAN TM, Rezaei E, GHAFARZADEGAN K, Malekifard H. Expression of p53 in colorectal carcinoma: correlation with clinicopathologic features.
22. Shin IY, Sung NY, Lee YS, Kwon TS, Si Y, Lee YS, Oh ST, Lee IK. The expression of multiple proteins as prognostic factors in colorectal cancer: cathepsin D, p53, COX-2, epidermal growth factor receptor, C-erbB-2, and Ki-67. Gut and liver. 2014 Jan;8(1):13.
23. Zhao DP, Ding XW, Peng JP, Zheng YX, Zhang SZ. Prognostic significance of bcl-2 and p53 expression in colorectal carcinoma. Journal of Zhejiang University Science B. 2005 Dec; 6:1163-9.
24. Kapiteijn E, Liefers GJ, Los LC, Klein Kranenbarg E, Hermans J, Tollenaar RA,

Moriya Y, Van De Velde CJ, Van Krieken JH. Mechanisms of oncogenesis in colon versus rectal cancer. The Journal of pathology. 2001 Sep; 195(2):171-8.
25. Carneiro FP, Ramalho LN, Britto-Garcia S, Ribeiro-Silva A, Zucoloto S. Immunohistochemical expression of p16, p53, and p63 in colorectal adenomas and adenocarcinomas. Diseases of the colon & rectum. 2006 May; 49:588-94.
26. Theodoropoulos, G.E., Karafoka, E., Papailiou, J.G., Stamopoulos, P., Zambirinis, C.P., Bramis, K., Panoussopoulos, S.G., Leandros, E. and Bramis, J., 2009. P53 and EGFR expression in colorectal cancer: a reappraisal of 'old'tissue markers in patients with long follow-up. *Anticancer research*, *29*(2), pp.785-791.
27. Zopf, S., Neureiter, D., Bouralexis, S., Abt, T., Glaser, K.B., Okamoto, K., Ganslmayer, M., Hahn, E.G., Herold, C. and Ocker, M., 2007. Differential response of p53 and p21 on HDAC inhibitor-mediated apoptosis in HCT116 colon cancer cells in vitro and in vivo. International journal of oncology, 31(6), pp.1391-1402.
28. Jerjees DA, Bedoor A, Irhayim-AL. P53 expression in colonic carcinoma - immunohistochemical study. Ann Coll Med Mosul.2009;35:111-16