

P53 Expression in Sporadic Colorectal Carcinoma

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Author's Contribution

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ABSTRACT

Objective: To determine expression of p53 in colorectal carcinoma (CRC) tissues and to find the frequency of p53 over-expression in various stages and grades of tumor.

Methodology: A prospective observational design study was conducted at BMSI JPMC between January 2023 till October 2023, after obtaining approval from ethics review committee. Fifty-six patients of CRC were recruited and the tumor tissue was subjected to H&E staining. The extent of nuclear immunoreactivity of p53 was scored from 0-3. The intensity was score from mild to strong, with score zero for no intensity. The results of immunohistochemistry were used to determine frequency of p53 over-expression with Astler and Coller grading and staging system and TNM staging.

Results: There were 30 cases (53.5 %) cases which showed p53 over-expression. The Astler and Coller grading and staging system showed that the majority of cases were in stage B2 (44.6%) followed by C2. As compared to other stages, TNM stage 3 showed higher frequency of p53 overexpression.

Conclusion: there is high frequency of p53 over-expression which highlights its significance. The over-expression has been found in higher stage and grade which indicates that it may form a key molecular event that may act as a prognostic as well as possible therapeutic marker.

Keywords: Tp53, colon cancer, mutation.

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Introduction

Colon carcinoma also called colorectal cancer or colorectal carcinoma (CRC) is the commonest malignant tumor of gastrointestinal tract.¹ Colon cancer and rectal cancer are often grouped together because of their common features. Globally, CRC is the third most common cancer and the fourth leading cause of cancer related deaths.² The incidence of CRC and its associated mortality is the highest in Asia.³ Pakistan has a growing burden of CRC however there is dearth of comprehensive national cancer registry to document on actual magnitude of burden or control programs to curb development of CRC.⁴ The mainstay of treatment of CRC remains surgery, where drug treatment is required 5- Fluorouracil(5-FU)-based adjuvant chemotherapy is considered to be the standard of care.⁵ However, the response to adjuvant

chemotherapy is seen in only 10–15% of individuals and the five year relapse rates is about 60% .⁶ In addition, there are various toxic effects associated with over-treatment with adjuvant chemotherapy (85–90% of patients). As a result, substantial work have been directed towards identification of tumor-based molecular markers that can complement standard clinical and pathological staging systems to more accurately predict disease outcome and determine optimal adjuvant treatment approaches.⁵

CRC cell lines-based studies suggest that the response to chemotherapy depends on whether the *TP53* gene is wild type or mutated. The Tp53 gene, a tumour suppressor gene, is located on the short arm of chromosomes 17 and encodes for p53. When cells are exposed to stress like damaged DNA, shortening of telomeres, hypoxia,

abnormal growth signals and chemotherapy, there is induction of p53. Activated p53 halts the cell cycle, allowing time for DNA repair and impedes proliferation of compromised cells.⁷

In normal cells, p53 is present in low concentration, has a short half-life and plays a major role in the cellular response to DNA damage.^{8, 9} Mutant p53 protein has an increased half-life and accumulates, primarily within the nucleus of active proliferating cells, where it can then be detected by immunohistochemistry (IHC).⁸ Loss of function of p53 is a late event in adenoma-carcinoma progression and has been identified in up to 60% of colon cancers.¹⁰ The IHC expression of p53 has been associated with overall survival (OS) and is reported to be an independent prognostic marker of CRC.¹¹ There is dearth of data on p53 immunohistochemistry of colon carcinoma in our part of world. Objectives of the current study were to assess the expression of p53 on immunohistochemistry in primary colon adenocarcinoma cases received in Pathology Department BMSI-JMPC, Karachi and to find the relationship of p53 expression with Astler and Coller grading and staging system and TNM staging.

Methodology

A prospective observational study was conducted at the BMSI - Jinnah Postgraduate Medical Center (JPMC) between January 2023 and October 2023, following approval from the ethics review committee. A total of 56 colectomy cases were included using consecutive sampling technique during the aforementioned period. Comprehensive demographic and clinical data were meticulously recorded on a designed proforma. Tissue specimens obtained from excised adenocarcinoma were meticulously examined using Hematoxylin & Eosin staining to evaluate morphologic features. Tissues exhibiting poor fixation or necrosis were excluded from the analysis. Immunohistochemical staining was subsequently performed on all selected cases using monoclonal antibodies targeting p53 (Cell Marque Cat#04-035). Microscopic evaluation was conducted using a scanner (4x), low-power (10x), and high-power (40x) lenses. The extent of reactivity, expressed as the percentage of immunoreactive nuclei, was categorized as

follows: 0 (<5%), 1+ (5-25%), 2+ (25-75%), and 3+ (>75%). Additionally, the intensity of reactivity was graded as: 0 (no staining), 1+ (weak nuclear staining), 2+ (moderate nuclear staining), and 3+ (strong nuclear staining).

The scoring process was independently performed by two histopathologists, and any discrepancies in reporting were resolved through discussion with all authors prior to assigning a final score to each case. Data were meticulously entered into MS Excel, and statistical analysis was performed using SPSS version 21 software.

Results

The sample comprised of 56 participants ranging from 20 to 75 years of age with mean age of 43.80+ 14.87. A total of 34 (60.7%) participants were male while 22 were females (39.3%). most of the cases belonged to descending colon (n=45; 80.4%) as compared to ascending colon (n=10; 17.9%) and transverse colon (n=1; 1.8%). On histopathology the most commonly encountered lesion was non-mucinous adenocarcinoma with 42 (75%) cases, while 12 cases (21.4%) of mucinous adenocarcinoma and two cases (3.6%) of signet ring cell carcinoma were seen. Grading was done on H&E which revealed 8 cases (14.3%) of well differentiated, 34 cases (55.35%) of moderately differentiated and 14 cases (25%) of poorly differentiated adenocarcinoma. There were 30 cases (53.5%) of p53 over-expression out of which majority of the patients (66.67%) were above 40 years of age.

The Astler and Coller grading and staging system showed that majority of cases, (n=25; 44.6%) were stage B2 followed by C2 stage (n=19; 33.9%). There were 11 Stage C1 (19.6%) and one case of stage B1 (1.8%). The TNM staging revealed one case of stage I (1.8%), 25 cases of stage II (44.6%) and 30 stage III cases (53.6%).

Table I shows that 30 cases had varying degree of intensity for p53 expression. 16 cases of colorectal carcinoma showed strong (+3) nuclear staining for p53. All these 16 cases also show strong reactivity (Extent) in more than 75% of neoplastic cells. Ten out of 30 cases showed moderate nuclear staining with 09 cases showing strong reactivity in between 25-75% of neoplastic cells. In one of

Table I: Nuclear Intensity and Extension of p53 Immunoreactivity in Colorectal Adenocarcinoma. (n=56)

| Lesion | Extent* | | | | Intensity** | | | |
|------------|----------|---------|----------|----------|-------------|---------|----------|----------|
| | 0 | 1+ | 2+ | 3+ | 0 | 1+ | 2+ | 3+ |
| Colorectal | 26 | 05 | 09 | 16 | 26 | 04 | 10 | 16 |
| Carcinoma | (46.43%) | (8.93%) | (16.07%) | (28.57%) | (46.43%) | (7.15%) | (17.85%) | (28.57%) |

* Extent of reactivity (% of immunoreactive nuclei) was as follows: 0, <5%; 1+, 5-25%; 2+, 25-75%; 3+, >75%. ** Intensity of reactivity was as follow: 0, no staining; 1+, weak nuclear staining; 2+ moderate nuclear staining; 3+ strong nuclear staining.

Table II: Nuclear Intensity and Extent of p53 Immunoreactivity in Different Histopathological Grades of Colorectal Adenocarcinoma (n=56)

| Grades of Colorectal Adenocarcinoma | N | Extent | | | | Intensity | | | |
|--|-----------|----------------|---------------|----------------|----------------|----------------|---------------|----------------|----------------|
| | | 0 | 1+ | 2+ | 3+ | 0 | 1+ | 2+ | 3+ |
| Grade 1 (well differentiated) | 11 | 07 (64%) | 01 (09%) | 01 (09%) | 02 (18%) | 07 (64%) | 00 (00%) | 02 (18%) | 02 (18%) |
| Grade 2 (Moderately differentiated) | 31 | 12 (38.70%) | 03 (9.68%) | 05 (16.13%) | 11 (35.49%) | 12 (38.70%) | 03 (9.68%) | 05 (16.13%) | 11 (35.49%) |
| Grade 3 (Poorly differentiated) | 14 | 07 (50%) | 01 (7.16%) | 03 (21.42%) | 03 (21.42%) | 07 (50%) | 01 (7.16%) | 03 (21.42%) | 03 (21.42%) |

Table III: Nuclear Intensity and Extent of p53 Immunoreactivity in Different Astler & Coller Grade & Stage of Colorectal Adenocarcinoma. (n=56)

| Astler & Coller Grade & stage System | N | Extent | | | | Intensity | | | |
|--------------------------------------|----|----------------|----------------|---------------|---------------|----------------|---------------|---------------|---------------|
| | | 0 | 1+ | 2+ | 3+ | 0 | 1+ | 2+ | 3+ |
| B1 | 01 | 01 | 00 | 00 | 00 | 01 | 00 | 00 | 00 |
| B2 | 25 | 11 (42%) | 2 (8%) | 5 (21%) | 7 (29%) | 11 (42%) | 2 (8%) | 5 (21%) | 7 (29%) |
| C1 | 11 | 5 (45.45%) | 00 | 4 (36.36%) | 2 (18.19%) | 05 (45.45%) | 00 | 4 (36.36%) | 2 (18.19%) |
| C2 | 19 | 09 (47.47%) | 03 (15.78%) | 00 | 7 (36.85%) | 09 (47.47%) | 2 (16.52%) | 1 (5.26%) | 7 (36.85%) |

Table IV: Nuclear Intensity and Extent of p53 Immunoreactivity in Different TNM Stage of Colorectal Adenocarcinoma (n=56)

| TNM Stage | N | Extent | | | | Intensity | | | |
|------------------|----|-------------|----------|-------------|----------|-------------|------------|-------------|----------|
| | | 0 | 1+ | 2+ | 3+ | 0 | 1+ | 2+ | 3+ |
| Stage I | 01 | 01 | | | | 01 | | | |
| Stage II | 25 | 11 (44%) | 02 (08%) | 05 (20%) | 07 (28%) | 11 (44%) | 02 (08%) | 05 (20%) | 07 (28%) |
| Stage III | 30 | 14 (46.66%) | 03 (10%) | 04 (13.73%) | 09 (30%) | 14 (46.66%) | 02 (6.76%) | 05 (16.76%) | 09 (30%) |
| Stage IV | 00 | | | | | | | | |

case extent was <5% of neoplastic cells. 04 cases showed weak nuclear staining with 05 cases showing weak reactivity in between 5%-25% of neoplastic cells.

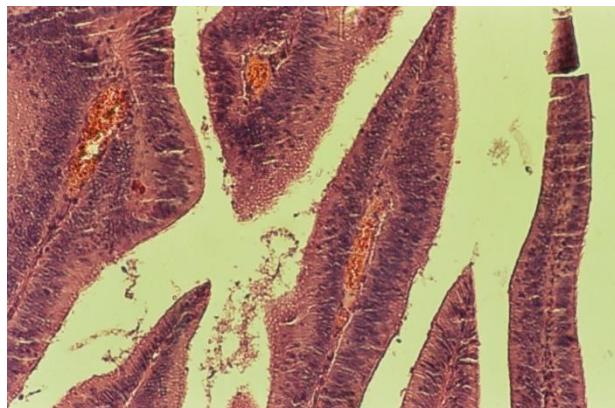
Comparing the extent and intensity of p53 immunoreactivity according to the degrees of differentiation; Table II shows that 11 colorectal carcinoma cases were of Grade 1 (well differentiated) adenocarcinoma. Among these, 02 cases showed strong intensity of staining for p53 with 02 cases showing strong reactivity (Extent) in more than 75% of cells while 02 out of 11 cases showed moderately intensity of staining and 01 case showing moderately reactivity while 01 case showed weak reactivity in neoplastic cells. Among 31 cases of Grade 2 (Moderately differentiated), 11 showed strong intensity of staining for p53 with 11 cases with strong reactivity (Extent) in more than 75% of cells. 08 out of 31 cases showed weak to moderately intensity of staining with 03 out of 14 cases of Grade 3 (Poorly differentiated) showed strong intensity of staining for p53 with 03 cases

showing strong reactivity while 04 cases show weak to moderately intensity of staining.

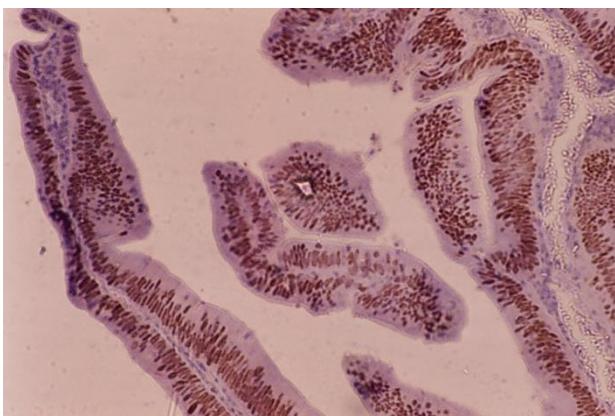
Table III shows the comparison of extent and intensity of p53 immunoreactivity according to the Astler and Coller grading and staging system in colorectal adenocarcinoma. Among 56 cases of colorectal adenocarcinoma, 01 case was stage B1, which showed no staining and no reactivity in neoplastic cells. Of 25 stage B2 cases, 14 showed weak to strong intensity of staining for p53 with 07 cases showed strong reactivity in more than 75% of cells while 07 cases showed weak to moderate reactivity. Cases of stage C1 were 11 and among these, 06 showed moderate to strong intensity of staining with moderate to strong reactivity in neoplastic cells. 19 cases were stage C2, 08 out of 19 cases showed moderate to strong intensity while 02 cases showed weak staining. 03 cases showed weak reactivity while 07 cases showed strong reactivity in more than 75% neoplastic cells.

Table IV shows comparison of the extent and intensity of p53 immunoreactivity according to the TNM staging system in colorectal adenocarcinoma. Among 56 cases of

colorectal adenocarcinoma, 01 case was stage-I, which showed no staining and no reactivity in neoplastic cells. 25 cases were stage-II, 07 out of 25 cases showed strong intensity while 07 case showed moderate to weak intensity of staining for p53. Seven cases showed strong reactivity in more than 75% of cells while 07 cases showed weak to moderate reactivity. 30 cases were stage-III, 09 out of 30 showed strong intensity of staining with 09 cases showing strong reactivity. Seven cases showed moderate to weak intensity and showed weak to moderate reactivity in more than 75% neoplastic cells.



Photomicrograph 1 (S.P. No. 08-2642): Well differentiated colorectal adenocarcinoma. H&E X 10

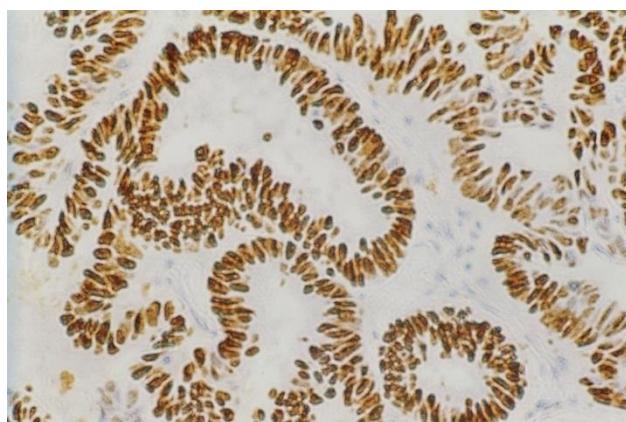


Photomicrograph 2 (S.P. No. 08-2642): Well differentiated colorectal adenocarcinoma showing strong p53 staining in more than 75% of nuclei. IHC X 10

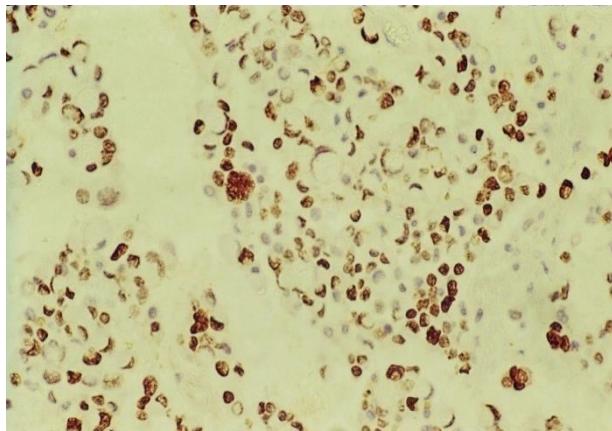
Discussion

This study focusses on in-depth analysis of p53 expression in CRC in the local population. In this study the expression of p53 and its relationship with staging and grading was studied. In the present study, 30 (53.5%) cases of colorectal carcinoma showed p53 over expression. This concurs with literature which documents p53 expression to be found in about 50-60% of CRC cases.¹² The CRC cases showing positive p53 expression comprised of 76.66% non-mucinous, 16.67% mucinous adenocarcinomas and

6.67% signet ring cell carcinoma. This finding concurs with the documented literature which reports that p53 expression is significantly associated with less mucin production of tumor.¹³ Most of cases in this study showed p53 over expression involving left side of colon i.e. 86.6% and right side of colon were 13.4%. The left-sidedness of p53 positive cases is in contrast to the reported literature.^{13, 14} This may be due to the fact that the current study sample contained more left sided colon cancers cases.



Photomicrograph 3 (S.P. No. 10-1568 B): Well differentiated colorectal adenocarcinoma showing strong p53 staining in more than 75% of nuclei. IHC X 10



Photomicrograph 4 (S.P. No. 08-5504): Signet ring cell carcinoma showing strong p53 expression. IHC X 20

In our study, p53 over expression was correlated with degree of differentiation of the tumor showing 13.33% of well differentiated, 63.34% in moderately differentiated and 23.33% in poorly differentiated adenocarcinoma. Our findings agreement with study by Georgescu et al showed majority of cases were moderately differentiated as 54.1% followed by poorly differentiated and well differentiated as 29.16% and 12.5 % respectively.¹⁶

In our study, most of cases showing p53 over expression were found in Astler and Coller stage B2 i.e. (46.67%) followed by C1 and C2 stage that in aggregate showed 53.33%. A study conducted by Nasiri-Ghavan et al using

Dukes method for staging and stage B showed 52.8% cases with p53 over expression in stage B and 47.2% in stage C which almost corresponds to the present study.¹⁸ Over expressed p53 colorectal carcinoma with Dukes stage and stage C patients have bad prognosis than patient with p53 normal tumor in same stages¹⁹ (Allegra et al 2003).

The p53 expression has been associated with poor patient outcome.¹⁵ We studied the p53 overexpression in different TNM stages and found that out of total cases (n=30) of p53 overexpression, majority were found in TNM stage-II as (44.65%) and the rest in stage- III (53.57%). Moreover, the majority of p53 positive cases (66.67%) were found in patients above 40 years (10 patients) while 5 cases (33.33%) were in younger age group (<40 years). In older age group majority (8/10) cases were in Astler and Coller C1/C2 stage and TNM stage-III suggesting poor prognosis. Similarly, in younger age group 02/5 cases show stage C2 Astler & Coller and TNM stage-III. In both age groups a high Astler and Coller/ TNM is seen which is in conformity with documented literature regarding p53 expression showing poor prognosis.¹⁵

In this new era of cancer management, several studies are being carried out on role of p53 as possible therapeutic target.^{19, 20} Our study opens avenues towards advanced studies on genetically distinct population by building basic data regarding p53 expression in the local population.

Conclusion

High p53 overexpression was found in this study which reflects significance of p53 testing in routine immunohistochemistry. The p53 over-expression maybe an indicator of need for aggressive therapy as these mutations are associated with higher stages. The patients with p53 over-expression may respond well to the recent target therapies that are in trial for better management of CRC patients.

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