Original Research Article

An expression of p53 marker in colorectal cancer with histopathological correlation

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Abstract

Introduction: Colorectal cancer is the major cause of mortality and morbidity worldwide. Colorectal cancer is the malignant epithelial tumor of the colon and rectum. Incidence in India are quite low about 2 to 8 per 1,00,000. Colorectal cancer develop through a multistep process as characterized by histopathological precursors lesion and molecular genetic alterations including adenomatous polyposis coli 9APC), K-RAS, and p53. The p53 mutations seem to be associated with poor prognosis in colorectal cancer, p53 protein overexpression has been shown to correlate with patient survival.

Aim and objectives: To diagnose colorectal carcinomas on H&E sections, to perform and interpret p53 immunostaining on all diagnosed colorectal carcinomas, to infer the rate of positivity and negativity of the P53 mutations in the colorectal lesions, to correlate the p53 mutations with the grade and stage of the colorectal carcinomas, to indicate the prognostic value of p53 mutations in colorectal carcinomas

Materials and methods: A prospective study was done for duration of 3 years i.e., 2014- 2016 on in MGM Hospital Warangal. All the colorectal biopsies and resection specimens in all age groups, received in the pathology department during this period were considered after a histopathological diagnosis of the lesion was made, the paraffin blocks of the samples which had met the criteria of inclusion are collected and proceed for immunohistochemical marker.

Results: In our study the commonest age group were from 60-69 years with majority of the lesions diagnosed as adenocarcinomas. Males contributed to large number of cases (36) and rectum is the most common site in the present study. Most of cases in this study are of stage IV (AJCC) (30%) and are well differentiated carcinomas (43.3%). p53 overexpression was seen in 40/60 cases. In present study there is statistically significant correlation between p53 overexpression and stage of tumor p value 0.04. In rectal cancers p53 overexpression was more common that colon cancers. Rectum showed 47.5% positivity and left colon 35%. Thus in present study rectal cancers showed high p53

expression. There was no significant correlation of p53 over expression and grade of colorectal Adenocarcinoma but statistically significant correlation was observed with advance stage and p53 overexpression in conventional adenocarcinomas, thus p53 overexpression serves as poor prognostic marker in colorectal adenocarcinomas and it may help to assess the responsiveness of patients to standard chemotherapy.

Conclusion: In our study, we noted p53 overexpression in 66.6 % of colorectal cancer. There is an increase in p53 expression with increasing stage of tumor. There is statistically significant correlation between stage and p53 expressions. In conclusion p53 seem to have an important role in the carcinogenesis of colorectal cancers. The evaluation of p53 over expression using a standardized IHC procedure could be clinically useful marker for identification of colorectal cancer patients likely to benefit from Standard chemotherapy regime currently used for this disease.

Key words

Histopathology, p53 marker, Colorectal cancer.

Introduction

Colorectal cancer is the major cause of mortality and morbidity worldwide. Colorectal cancer is the malignant epithelial tumor of the colon and rectum. Only tumor that had penetrated through muscularis mucosae into submucosa is considered malignant at this site [1]. Globally cancer of the colon and rectum is the fourth most common cancer in males and third leading cause of cancer in females with mortality paralleling incidence [2]. Incidence in India are quite low about 2 to 8/1,00,000 [3]. Incidence in male is 4.3/1,00,000, incidence in females- 3.4/1,00,000 [3]. Male sex, increasing age, presence of long standing IBD, and familial predisposition beside are strong risk factors

The etiology of colorectal cancer is complex, involving interplay of environmental and genetic factors. Colorectal cancer develops through a multistep process as characterized by histopathological precursors lesion and molecular genetic alterations including adenomatous polyposis coli (APC), K-RAS, and p53 [4]. The mutations of APC and K-RAS genes occur early in the carcinogenesis, whereas p53 mutations are late events. Mutations in the tumor suppressor gene TP53 are found in almost half of CRC [5]. Therefore, considerable interest has focused on the identification of novel tumor-based markers that can more accurately predict the course of this malignancy, as well as determination of optimal adjuvant therapy approaches.

Role of p53 mutations in colorectal cancers

The p53 suppressor gene, located on the short arm of chromosome 17, encodes a 53-kd nuclear phosphoprotein that regulates the cell cycle. P53 is a transcription factor that is at the center of a large network of signals that sense cellular stress, such as DNA damage, shortened telomeres, and hypoxia. Mutations in this gene constitute some of the most frequently occurring genetic changes found in human malignancies [6].

- They are thought to be a late development in the adenoma-carcinoma sequence in colorectal cancer [7].
- It has been reported that p53 mutations seem to be associated with poor prognosis in colorectal cancer [8-10]. However, conflicting results have also been claimed [11].
- Over expression of the p53 protein is detectable in 30% to 70% of the tumors, using immune-histochemical (IHC) methods. In a great majority of studies, p53 protein over expression has been used as a surrogate marker for p53 mutations, an assumption that is not entirely correct, although it may sometimes be justified for practical and economic reasons

- In some studies, p53 protein over expression has been shown to correlate with patient survival, a finding that has not been observed in other 39 studies [12]. Mutations in the tumor suppressor gene TP53 are found in almost half of CRC.
- Mutations in different domains of the gene lead to a variable prognosis [13, 14].
- TP53 mutations are found more commonly in distal CRC [8, 9]. Proximal tumors found to have mutations in TP53 were more likely to exhibit lymphatic invasion and be more responsive to 5-FU therapy.
- Mutation in exon 5 of the TP53 gene is associated with a poorer outcome [15].
- Individuals with wild type TP53 have a superior survival rate with 5-FU therapy in rectal cancer [16].

Aim and objectives

- To diagnose colorectal carcinomas on H & E sections.
- To perform and interpret p53 immunostaining on all diagnosed colorectal carcinomas.
- To infer the rate of positivity and negativity of the P53 mutations in the colorectal lesions.
- To correlate the p53 mutations with the grade and stage of the colorectal carcinomas.
- To indicate the prognostic value of p53 mutations in colorectal carcinomas

Materials and methods

A prospective study was done for duration of 3 years i.e., 2014-2016 in MGM Hospital Warangal. All the colorectal biopsies and resection specimens in all age groups, received in the Pathology Department during this period were considered.

Inclusion criteria

- Only samples with definite histopathological diagnosis of carcinoma were considered.
- Representative areas in the biopsies were only included.

Exclusion criteria

- Non neoplastic lesions
- Congenital lesions like Hirschsprungs disease were excluded.
- Inadequate samples were excluded.

Specimen handling

Colorectal biopsies and the resection specimens were fixed in 10%formalin and then sent for routine histopathological processing. After a histopathological diagnosis of the lesion was made, the paraffin blocks of the samples which had met the criteria of inclusion are collected. Basic details of the each case like the biopsy no, age and sex, clinical details, histopathological diagnosis were recorded. Sections were made from the paraffin embedded tissue block as follows: One 5-micron section taken for staining with H&E, Two 5-micron sections taken on the polylysine coated slides stained for p53 IHC. The results were recorded for individual case.

p53 immunostaining using p53 antibody (DAKO)

- Sections underwent histologic evaluation to select blocks without necrotic and hemorrhagic areas.
- Consecutive 3-4 μm sections were taken on polylysine coated slides at the next stage, sections were deparaffinized and Antigen-retrieval procedure was performed by trilogy solution using microwave method. Sections were thoroughly washed with wash buffer in between every step.
- Endogenous peroxidase blocking was done by horse raddish peroxidase. Then, monoclonal antibody against p53 protein (clone DO-7; Dako), was applied to the sections and incubated for 30 minutes at room temperature.

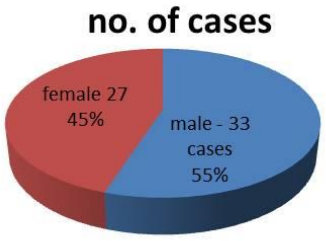
- Then, secondary antibody was added and incubated for 20 minutes.
- Then freshly prepared diaminobenzidine (DAB) was added to the sections for 10 minutes and the sections were lightly counterstained with hematoxylin.
- Slides were then dehydrated, cleared and mounted.

Results

In our study we evaluated colorectal resections in 60 patients between age groups 20 years to 90 years from July 2014 to November 2016 in MGM Hospital, Warangal.

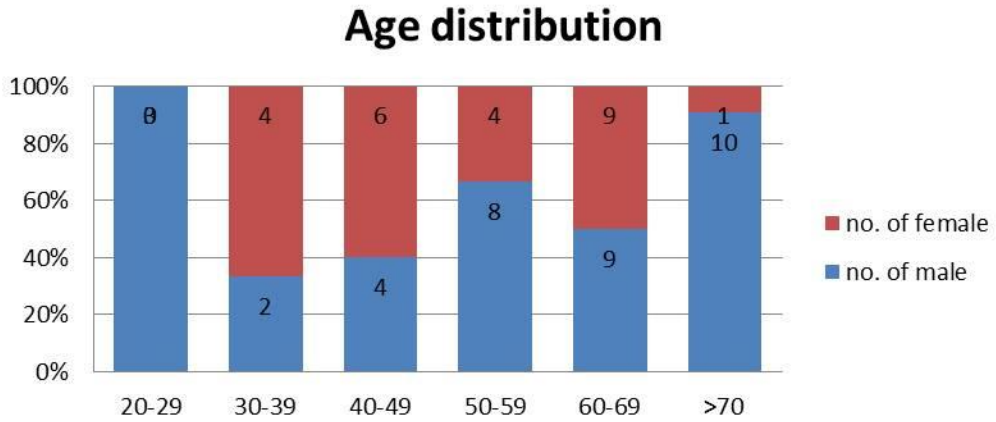
Out of 60 patients of colorectal carcinomas males were 33 which accounts for 55% cases and females were 27 which accounts for 45% cases. Males accounted for larger number of cases compared to females. Male to Female ratio was 1.2:1. There was slightly male preponderance (**Graph – 1**).

Graph - 1: Sex distribution of colorectal carcinoma.



Mojority of colorectal carcinoma in this study were in the age group 60-69 years which accounts for 30% of all ceases. Males and females accounted for equal number of cases in this group. In age group of 20-29, only 3 cases were seen and all were males. In age group of 30-39, females accounted for 4 cases and males accounted for 2 cases. In age group 40-49 years, females accounted for 6 cases and males accounted for 4 cases. In this study in age group 30-49, females accounted for more number of cases than males. After 70 years there were 10 males and 1 female. Thus in this study <30 and >70 years males accounted for more number of cases (**Graph – 2**).

Graph - 2: Age distribution of colorectal carcinoma.

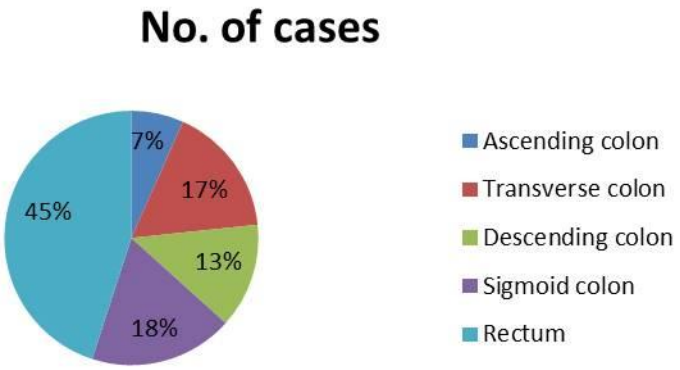


In this study more number of cases were located in rectum 27 cases (45%), followed by sigmoid colon 11 cases (18.3%), transverse colon 10 cases (16.6%), descending colon 8 cases (13.3%) and ascending colon 4 cases (6.6%). Thus in this study rectum and left colon accounted for large number of cases (**Graph – 3**).

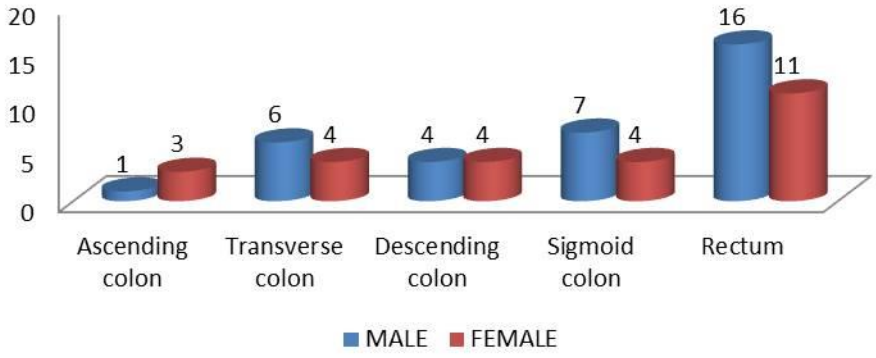
Majority of colorectal cancers in this study were located in rectum 27cases (5%). In both sexes rectum was the most common site followed by sigmoid colon. In rectum males accounted for more number of cases (16) than female (11). Sigmoid colon accounted for more number of cases next to rectum, males accounted for 7 cases and females accounted for 4 cases. In descending

colon males and females accounted for same number of cases. In transverse colon males accounted for 6 and females accounted for 4 cases. In ascending colon males accounted for 1 case and females accounted for 3 cases. Males accounted for large number of cases in rectum, sigmoid colon and transverse colon. Only in ascending colon females accounted for more number of cases than males (**Graph – 4**).

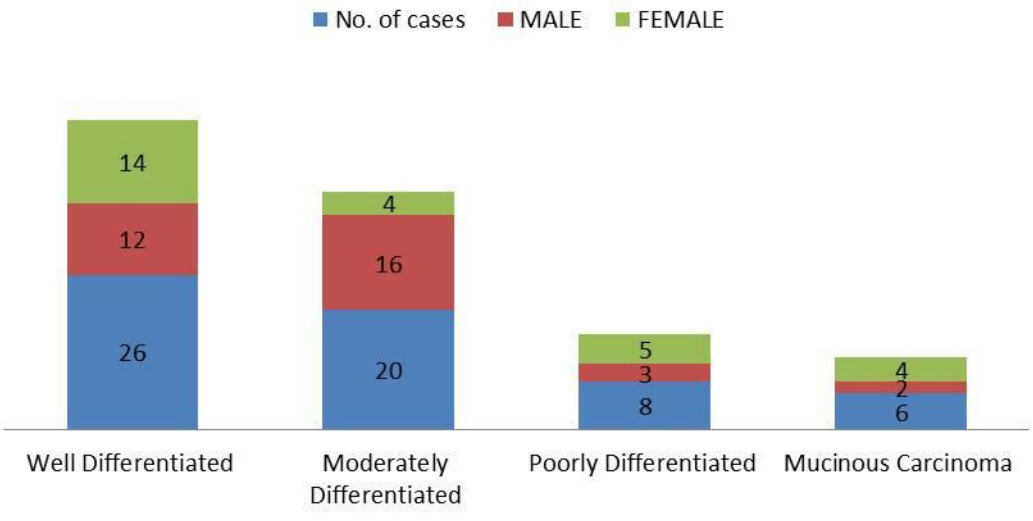
Graph - 3: Distribution of colorectal carcinoma based on their location.



Graph - 4: Number of colorectal cases in males and female.



Graph - 5: Colorectal carcinoma histological grading and number of males and females in each grade.



Colorectal carcinoma - Microscopic findings

Among 60 cases studied 26 cases (43.3%) were well differentiated adenocarcinomas. 20 cases (33.3%) were moderately differentiated adenocarcinomas, 8 cases (13.3%) were poorly differentiated adenocarcinomas and 6 cases (10%) were mucinous carcinomas (**Table – 1**).

Table – 1: Grading of colorectal carcinoma.

Grade	No. of cases	Male	Female	Total %
Well Differentiated	26	12	14	43.3%
Moderately Differentiated	20	16	4	33.3%
Poorly Differentiated	8	3	5	13.3%
Mucinous Carcinoma	6	2	4	10%

Table – 2: Number of cases based on staging and sex distribution in each stage.

Stage (AJCC)	No. of cases	Male	Female	Percentage
I	15	8	7	25%
II	15	7	8	25%
III	12	9	3	20%
IV	18	9	9	30%

Table - 3: p53 expression in colorectal adenocarcinomas.

P53 expression	Positive	Negative	Total
Conventional	38(95%)	16	54
Mucinous	2(5%)	4	6
Total	40	20	60

Table - 4: p53 score in relation to subtypes.

Adenocarcinoma	P53 score				Total positive
	0	1	2	3	
Conventional	16	8	13	17	38
Mucinous	4	2	0	0	2
Total	20	10	13	17	40

Table - 5: p53 score in relation to histological grade.

Histological grade	P53 score				Total	Total positive
	0	1+	2+	3+		
Well differentiated	7	4	6	9	26	19(73%)
Moderately differentiated	6	3	5	6	20	14(70%)
Poorly differentiated	3	2	2	1	8	5(62%)
Mucinous carcinoma	4	1	1	0	6	2(33.3%)
Total	20	10	14	16	60	40(66.6%)

Well differentiated adenocarcinomas account for 26 cases (43.3%), followed by moderately differentiated adenocarcinoma 20 cases (33.3%), poorly differentiated adenocarcinoma 8 cases (13.3%) and mucinous carcinomas 6 cases (10%) as per **Table – 1**.

In well differentiated adenocarcinoma males accounted for 12 cases and females 14 cases. In

moderately differentiated adenocarcinoma males accounted for 16 cases and females 4 cases. In poorly differentiated adenocarcinoma males accounted for 3 cases and females 5 cases. In mucinous adenocarcinoma males accounted for 2 cases and females 4 cases. Males accounted for larger number of cases in moderately differentiated carcinoma cases and in remaining females accounted for more number of cases than males (**Graph – 5**).

Colorectal carcinoma AJCC staging

Majority of cases in this study were in stage IV 18 cases which accounted for 30%. Stage I and II accounted for equal number of cases 25% each. Stage III accounted for only 12 cases (20%). In stage I there were 8 males and 7 females. In stage II there were 7 males and 8 females. In stage III there were 9 males and 3 females. In stage IV there were 9 males and 9 females. In stage IV males and females accounted for equal number of cases. Only in stage II females accounted for slightly more number of cases than males. In satge I and III males were more than females (**Table – 2**).

p53 immunostaining results on colorectal carcinomas

p53 IHC was done on all the cases. Out of 60 cases, 40 were positive in which 38 were colorectal adenocarcinomas and 2 were mucinous carcinomas (**Table – 3**). In this study out of 60 cases 40 cases showed p53 nuclear positivity accounts for 66.6% cases. Out of this conventional adenocarcinoma accounts for 95% cases and mucinous adenocarcinoma accounted for 5%. Sample size of mucinous carcinoma was also low (6 cases) in this study.

Mucinous carcinoma showed lower level of p53 expression than conventional adenocarcinoma. In caonventional adenocarcinoma 38 cases were positive (70.4%) and 16 (29.6%) were negative. In mucinous carcinoma only 2 cases were positive (33.3%) and 4 cases were negative (66.6%) as per **Table – 3**.

In conventional adenocarcinoma 16 cases showed score 0 (negative), 8 cases (21%) showed score 1, 13 cases (34%) showed score 2 and 17 cases showed score 3 (44%). More cases showed score 3 in conventional adenocarcinoma. In mucinous carcinoma, 4 cases showed score 0 (negative) and 2 cases showed score 1(33.3%) as per **Table - 4**.

p53 score of at least 1+ was considered positive. 73% of well 70% of moderately and 62% of poorly differentiated carcinomas showed p53 positivity. Only 33.3% of mucinous carcinomas showed p53 positivity. p53 score decreases from well to poorly differentiated adenocarcinoma. Higher scores were seen in well to moderately differentiated carcinoma than poorly differentiated carcinoma. In well differentiated carcinoma 9 cases showed score 3, 6 cases showed score 2 and 4 cases showed score 1. In moderately differentiated carcinoma 6 cases showed score 3, 5 cases showed score 2 and 3 cases showed score 1. In poorly differentiated carcinoma, 1 case showed score 3, 2 cases showed score 2, 2 cases showed score 1. In mucinous carcinoma, 2 cases showed score of only 1 (**Table – 5**).

Majority of the cases in stage IV showed p53 positivity followed by stage III. There was progressive increase in p53 scores as stage increases.

P53 expression in relation to stage in conventional adenocarcinoma

Stage I showed only 50% p53 positivity (7 cases)
Stage II showed 66.6% p53 positivity (12 cases)
Stage III showed 80% p53 positivity (10 cases)
Stage IV showed 83% p53 positivity (18 cases)
From stage I to IV there was progressive increase in p53 positivity cases (**Table – 6**).

Only 2 mucinous CRC showed P53 positivity. They showed score of 1.4 mucinous CRC were P53 negative. Mucinous CRC showed lower level of P53 expression (**Table – 7**).

Table - 6: p53 score in conventional adenocarcinoma in relation to stage.

Stage (AJCC)	Score				Total no. of cases	Total no. of positive cases
	0	1	2	3		
I	7	3	2	2	14	7(50%)
II	4	4	2	2	12	8(66.6%)
III	2	1	2	5	10	8(80%)
IV	3	0	7	8	18	15(83%)

Table - 7: p53 score in mucinous carcinomas in relation to stage.

Stage (AJCC)	Score				Total
	0	1	2	3	
I	1	-	-	-	1
II	2	1	-	-	3
III	1	1	-	-	2
IV	-	-	-	-	-
Total	4	2	-	-	6

Table - 8: p53 score in male and female based on stage (AJCC).

Stage	Male		Female	
	Positive	Negative	Positive	Negative
I	5	3	2	5
II	4	3	4	4
III	8	1	2	1
IV	8	1	7	2
Total	25	8	15	12

Out of 6 mucinous carcinoma, only 2 showed p53 nuclear positivity which accounted for 33.3% cases and 66.6% showed nuclear negativity. Out of 2 positive cases they showed p53 score of only 1.

In stage I out of 7 positive cases 5 were males and 2 were females. Out of 8 negative cases 5 were females and 3 were males.

In stage II out of 8 positive cases 4 were males and 4 were females. Out of 7 negative cases 3 were males and 4 were females

In stage III out of 10 positive cases 8 were males and 2 were females. Out of 2 negative cases 1 was male and 1 was female. In stage IV out of 15 positive cases 8 were males and 7 were females. Out of 3 negative cases 1 was male 2 were females (**Table – 8**).

Males accounted for larger number of p53 positive cases in all stages. In this study p53 expression was higher in males compared to females. Out of 33 cases males showed 25 positive cases and 8 negative cases. Out of 27 cases females showed 15 positive and 12 negative cases (**Figure – 6 to 19**).

Figure – 6: Ascending colon growth.

Figure – 7: Rectosigmoid stricture.

Figure – 8: Concentric growth.

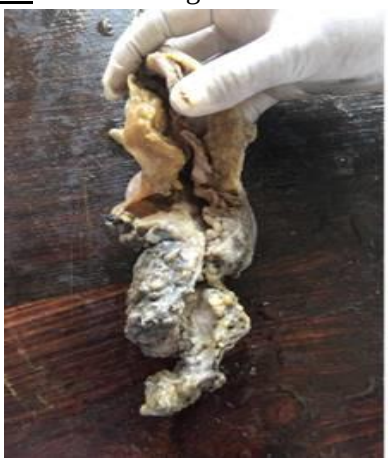


Figure – 9: Well differentiated.

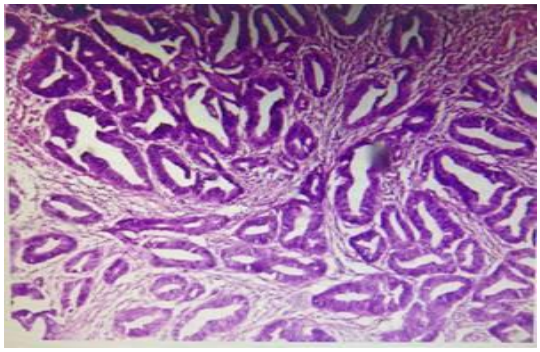


Figure – 10: Moderately differentiated.

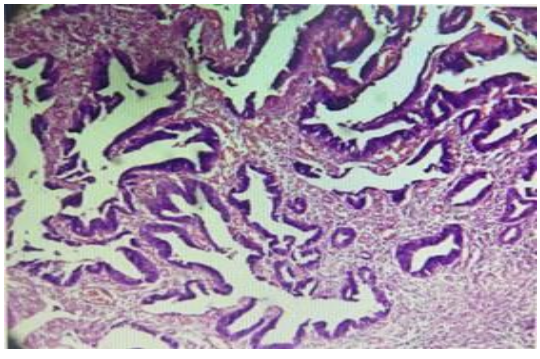


Figure – 11: Poorly differentiated.

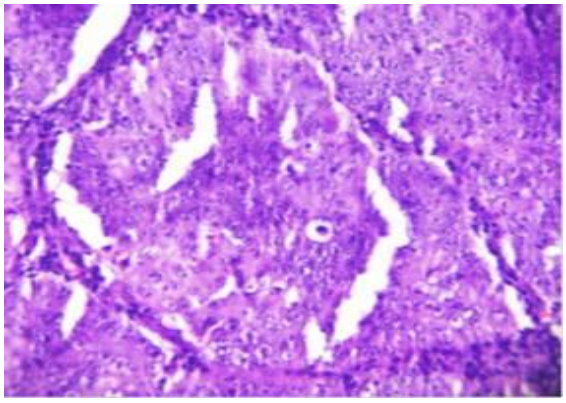


Figure – 12: Mucinous adenocarcinoma.

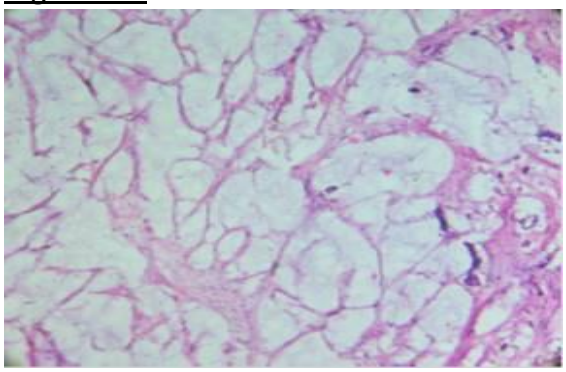


Figure – 13: Papillary adenocarcinoma.

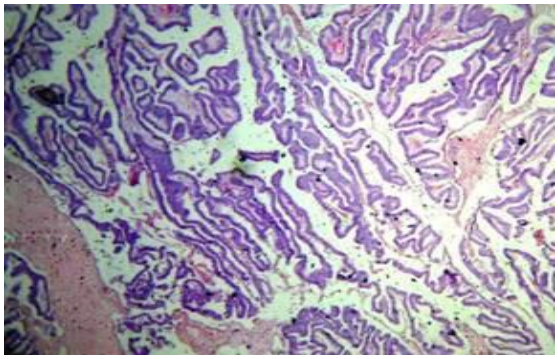


Figure – 14: Grade 3 adenocarcinoma, p53- , Score 0 IHC: 100X.

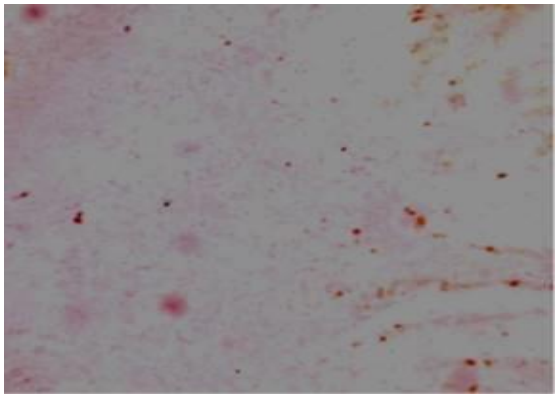


Figure – 15: Grade 3 adenocarcinoma, p53+, Score 1 IHC: 100X.

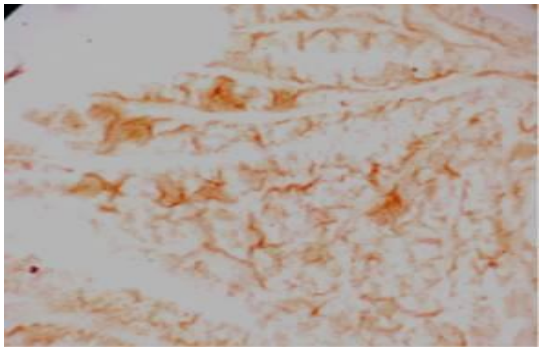


Figure – 16: Grade 2 adenocarcinoma, p53+, Score 2 IHC: 100X.

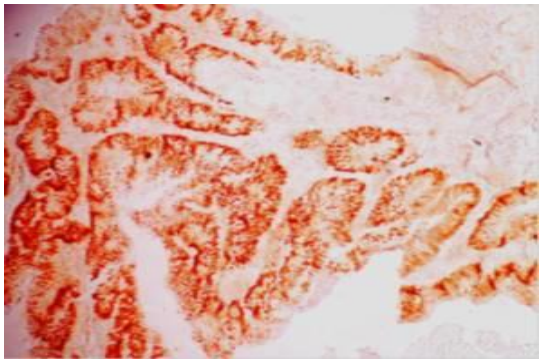


Figure – 17: Grade 3 adenocarcinoma, p53+, Score 3 IHC: 100X.

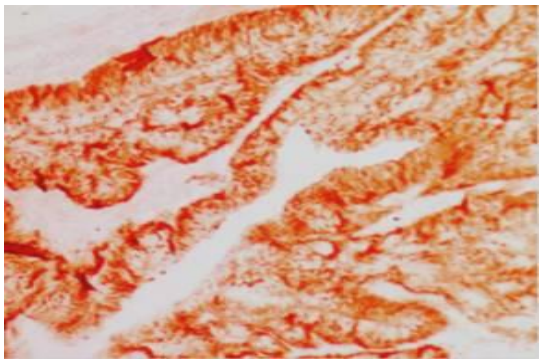


Figure – 18: Grade 1 adenocarcinoma, p53+, Score 3 IHC: 100X.

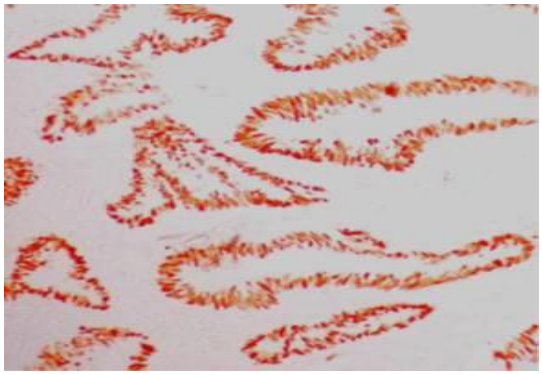
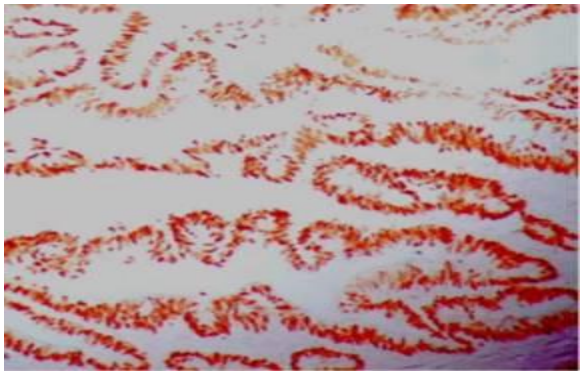


Figure – 19: Grade 2 adenocarcinoma, p53+, Score 3 IHC: 100X.



Discussion

Colorectal carcinoma is by far most common and most curable cancer of GIT. More than 90 % of cancers in this region are adenocarcinomas. In this study age group ranges from 24-90 years and most common age group is 60-69 years. This is consistent with studies done by Ahsan, et al. [17] and Neagoes, et al. [18] who reported that most of CRC cases are in 6^{th} and 7^{th} decades of life (**Table – 8**).

Table - 8: CRC most common age group comparison.

Study	Most common age group
Ahsan, et al. [17]	6^{th} and 7^{th} decade
Neagoes, et al. [18]	6^{th} and 7^{th} decade
Present study	6^{th} decade (60 – 69)

Most of colorectal carcinomas exhibited ulceroproliferative growth grossly. In this study there is male preponderance36 (60%) and females24 (40%). M:F ratio is 1.2:1. In the present study no significant relationship between p53 and age or gender, and is consistent with Mohammad-Reza Ghavam-Nasiri, et al. [19] who found no relationship between these variables. Slattery, et al. [20] detected significant relationship between p53 expression and age or gender, which is inconsistent with our results.

In colorectal carcinogenesis two distinct pathways are involved. APC (adenomatous polyposis coli)/ beta catenin pathway is associated with classic adenoma–carcinoma sequence and p53 mutation occurs in late stages

of tumor progression. Second pathway is microsatellite instability pathway in which tumors often have mucinous differentiation and are frequently located in right colon Hence mucinous carcinomas tend to have lower frequency of p53 mutation.

In this study adenocarcinoma accounts for 90% of cases and mucinous carcinoma accounts for 10% of cases. This is consistent with study done by Georgescu, et al. [21], they reported that in their study 85% are adenocarcinomas, 10% mucinous and 5% signet ring carcinomas. Rectum was most common site in both sexes and in all age groups. p53 overexpression and mutation in colorectal cancers in various studies were as per **Table – 9**.

Table - 9: p53 overexpression and mutation in colorectal cancers in various studies.

Study	**No. of patients**	**P53 positivity %**	**Method employed**
M Morrin, et al. [22]	52	62%	IHC With p53 antibody
Zhi – Zhong Pan, et al. [23]	97 (rectal cancer)	62.9%(61) 52.6% (51)	IHC with p53 antibody PCR-SSCP
Silvia Tortola, et al. [24]	140	50%(66)	PCR-SSCP
Must afa Akkiprik, et al. [25]	43	32.5%(14)	PCR-SSCP followed by sequencing
Mohammad- Reza Ghavam-Nasiri, et al. [19]	100	59%	IHC with p53 antibody
Present study	60	40(66.6%)	IHC with p53 antibody

Table - 10: Comparison of p53 overexpression in CRC in present study with other study.

		M Morrin, et al. [22] (62%)		Present study (57%)	
Variables		Cases	No. of p53 positivity	Cases	No. of p53 positivity
location	Right colon	11	6(55%)	12	7(58%)
	Left colon	25	13(52%)	21	14(66%)
	Rectum	15	11(73%)	27	19(70%)
Differentiation	Well	27	17(63%)	26	19(73%)
	Moderate	21	12(57%)	20	14(70%)
	Poor	4	2(50%)	8	5(62%)

Extent of p53 expression varies during different phases of tumor oncogenesis. In colorectal carcinoma the occurance of p 53 mutation is variable among different series and has been found in 50-70% cases [26, 27].

Our results are comparable with the above studies using IHC for overexpression of p53. But the incidence of p53 mutations as detected by molecular methods are little lower then that detected by IHC. This discrepancy may be due to two reasons:

- Those wild type p53 proteins could combine with viral oncoproteins or cellular oncoproteins to enhance their stability and prolong their half-life, leading to p53 protein accumulation in cells. In such cells, IHC staining was still positive even without p53 gene mutations
- Furthermore, about 10% p53 gene mutations could take place outside of exons 5-8. Therefore the positive rate of PCR-SSCP targeting only exons 5-8 was usually lower than that of IHC [28]. The variance in p53 gene mutations and p53 protein accumulation indicated that dysfunction of p53 gene might be caused by mechanisms other than mutations.

Comparison of p53 overexpression in CRC in present study with other study was as per **Table – 10**.

P53 expression in relation to histological grade

Morrrin, et al. [22] studied p53 expression by immunostaining in 52 cases of colorectal cancers and correlated thep53 overexpression with the survival rates. 62% cases were positive. Most of the rectal carcinomas showed p53 overexpression. 63% per cent of the well differentiated tumor were positive for p53 overexpression, while 57% of the moderately and 50% of the poorly differentiated tumor showed overexpression. They found no statistical significant correlation to p53 status and survival rates.

In our study, 19(73%) of well differentiated, 14(70%) of moderately differentiated and 5(62%) poorly differentiated adenocarcinomas were positive for p53.

The present study showed no significant association for increasing grade of tumor with p53 expression which correlates well with results of studies by Yamaguchi A, et al. [29], Soong R, et al. [30], Yuan-tzu Lan [31] and C. Hanski, et al. [32], Georgescu, et al. [21] and Asaad, et al. [33].

But higher scores were found commonly in well to moderately differentiated tumors than poorly differentiated tumors. This is in concordance with study by Yuan-Tzu-Lan [31] who found p53 over expression in 60% of well to moderate versus 40% of poorly differentiated tumors. This may be due to fact that p53 expression may be reduced as cells become less differentiated. So p53 can serve as differentiation marker in colorectal carcinoma. Comparison of p53 overexpression association with grade of CRC in various studies was as per **Table – 11**.

Present study is comparable with the above study results. There is no significant relation between p53 expression and histological grade of tumor. P53 expression decreases from well differentiated adenocarcinomas (73%) to moderately differentiated adenocarcinomas (70%) to poorly differentiated Adenocarcinoma (62%) but not statistically significant p value > 0.05. p53 positivity in colorectal carcinoma in various studies was as per **Table – 12**.

Table - 11: Comparison of p53 overexpression association with grade of CRC in various study.

Study	**P53 association with grade**
Yamaguchi A, et al. [29]	No significant association
Soong R, et al. [30]	No significant association
Yuan-tzu Lan [31]	No significant association
C Hanski, et al. [32]	No significant association
Georgescu, et al. [21]	No significant association
Asaad, et al. [33]	No significant association
Present study	No significant association

George E. Theodoropoulos, et al. [34] reported nuclear positivity for p53 in 63.4% of colorectal adeno- carcinomas. Yamaguchi, et al. [29] found p53 immunoreactivity in 61% of colorectal carcinoma. This study shows p53 positivity in 66.6% cases which is slightly higher than above studies.

In the present study mucinous tumors showed lower rate of p53 positivity (33.3%). Among 2 positive cases they had score of 1but significant correlation could not be obtained due to smaller sample size. In this study 33.3% of mucinous carcinomas and 70% of non-mucinous carcinomas showed p53 positivity by IHC. This is in concordance with study done by C. Hanski, et al. [32] who found that only 36% of mucinous carcinoma shows p53 positivity by IHC but 76% of non-mucinous adenocarcinoma showed p53 positivity suggesting that mucinous carcinoma develop by different pathway. Satoshi Ikeda et al also has observed similar findings in their study [35].

Table - 12: p53 positivity in colorectal carcinoma in various studies.

Study	No. of cases	P53 – Positivity
Silivia Tortala, et al. [24]	132	66(50%)
M Morrin, et al. [22]	52	32(62%)
Mustafa Akkiprik, et al. [25]	43	14(32.5%)
Zhi-Zhong Pan, et al. [23]	97 (rectal cancers)	51(52.6%)
Present study	60	40(66.6%)

Table - 13: Comparison of p53 overexpression association with stage of CRC in various studies.

Study	Significant association with stage
Flamini, et al. [26]	Present
George E. Theodoropoulos, et al. [34]	Present
Jackson, et al. [36]	Present
Starzynska, et al. (1992) [37]	Present
Present study	Present
By Demirbas, et al. [38]	Present

P53 with stage of tumor

In this study stage I shows 50% positivity. Stage II shows 66.6% positivity. Stage III shows 80% positivity. Stage IV shows 83 % positivity. In this study there was a progressive increase in p 53 score as the stage of colorectal adenocarcinoma increases. Statistical analysis using chi-square test was done which revealed a p value of 0.04 which is statistically significant. In our study statistically significant correlation was obtained for p53 over expression and advanced stage (p value 0.04) but not for grade of tumor This is in accordance with studies by Flamini, et al. [26], and George E. Heodoropoulos [34], Jackson, et al. [36]; Starzynska, et al. (1992) [37], but inconsistent with studies done by Demirbas, et al. [38] who found no significant relationship between stage and p53 expression.

In our study most of mucinous carcinomas were in low stage and sample size was too low to get statistically significant results. J Walker, et al. [39] in his study concluded that stage is the most accurate prognostic marker for survival and recurrence. Comparison of p53 overexpression association with stage of CRC in various studies was as per **Table – 13**.

Since p 53 expression has shown significant association with stage of disease and since stage is a proven prognostic factor in colorectal adenocarcinoma, P53 overexpression can be used as a poor prognostic marker.

P 53 expression in relation to sex

In this study p53 expression is higher in males compared to females. Out of 33 cases males show 25 positive cases and 8 negative cases. Out of 27 cases females show 15 positive and 12 negative cases. In stage I out of 7 positive cases 5 were males and 2 were females. In stage II out of 8 positive cases 4 were males and 4 were females. In stage III out of 10 positive cases 8 were males and 2 were females. In stage IV out of 15 positive cases 8 were males and 7 were females. Males account for larger number of p53 positive cases in all stages.

p53 expression in relation to tumor location

P53 nuclear positivity in this study was commonly seen in left side tumors with rectum predominating (70%) and left colon (66%).Similar results were found in study by Antonio Russo [40] and Yuan –Tzu Lan [31]. M Morrin, et al. [22] found 73% p53 nuclear positivity in rectum, present study result is comparable with their results in rectum. But in present study left colon accounts for more no of

p53 positive cases next to rectum. While study done by M Morrin, et al. [22] shows that right colon accounts for more no of p53 positive cases next to rectum. Rectum shows higher p53 expression in present study and is comparable with above studies. Bell, et al. (1993) [41], Borresen –Dale, et al. (1998) [42], Manne, et al. (1998) [43], Kressner, et al. (1999) [44], Soong, et al. (2000) [30], Diez, et al. (2000) [45] shows higher expression of p53 with distal tumors.

Investigations have demonstrated that mutant p53 renders malignant cells less sensitive to most chemotherapeutic agents. Patients with p53 mutations are relatively resistant to chemotherapy and radiotherapy. Irradiation and chemotherapy, the two common modalities of cancer treatment, mediate their effects by inducing DNA damage and subsequent apoptosis. Tumors that retain normal p53 are more likely to respond to such therapy than tumors that carry mutated alleles of the gene.

Conclusion

In our study we noted p53 overexpression in 66.6 % of colorectal cancers. Majority of p53 positive cases are conventional adnocarcinomas (38/40) 95% and only 2 are mucinous carcinomas (2/40) 5%. There is an increase in p53 expression with increasing stage of tumor. There is statistically significant correlation between stage and p53 expression. Since stage is proven prognostic marker in colorectal cancers p53 overexpression can be used as poor prognostic marker. Alteration in this gene appear to have little or no prognostic value in patients treated with surgery alone, but are associated with worse survival for patient treated with chemotherapy. Several studies have shown that normal p53 is required for response of colorectal cancers to 5-Fluorouracil based chemotherapy. (Normal p53 function is required to trigger apoptosis in cells damaged by chemo-radiotherapy). In conclusion p53 seem to in have an important role in the carcinogenesis of colorectal cancers. The evaluation of p53 over expression using a standardized IHC procedure could be clinically useful marker for identification of colorectal cancer patients likely to benefit from Standard chemotherapy regime currently used for this disease.

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