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Original Research Article

Correlation of proliferative marker (Ki67), p53 expression and histomorphology in colorectal carcinoma

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Abstract

Aim: To investigate combined immune-expression of Ki67 and p53 proteins in colorectal carcinoma and to correlate expression patterns of these markers with histopathological variables of colorectal carcinoma.
Material and Methods: We determined immunohistochemical expression of proliferative expression of Ki67 and p53 in 47 cases of colorectal carcinoma in our set up.
Results: Mean Ki67 index in our study was 34.7%. Mean Ki67 index of Adenocarcinoma NOS was observed to be 38.2%, which was higher than that of mucinous adenocarcinoma (28.3%) and signet ring cell carcinoma (11%). Mean Ki67 increased with dedifferentiation of tumor (Grade I-22.5%, Grade II-34.3%, Grade III-74.6%). p53 positivity rate was 72.3%. p53 positivity rate was higher in Adenocarcinoma NOS (72.9%) as compared to mucinous adenocarcinoma (57%). Percentage of diffuse p53 positivity increased with dedifferentiation of tumor. All cases of signet ring cell carcinoma showed diffuse p53 positivity. Correlation between Ki67 and p53 expression was not significant statistically.
Conclusion: Mean Ki67 labelling index correlated with histological grade of colorectal carcinoma but not the histological type of tumor. p53 positivity rate did not correlate with histological grade nor the histological type. The combined expression of Ki67 and p53 in colorectal carcinomas did not correlate in the present study.
Keywords: Colorectal carcinoma, Ki67, p53.

1. Introduction

Cellular proliferation is fundamental to maintain tissue homeostasis and is important in oncogenesis. Quantification of cell proliferation activity in neoplasms has been targeted with the help of Ki67 antibody. Loss of p53 gene is crucial for transformation of colorectal adenoma in to colorectal carcinoma. The immunohistochemical expression of p53 may have prognostic value among the patients with colorectal carcinoma. This study was conducted to investigate combined immune-expression of Ki67 and p53 proteins in colorectal carcinoma 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.

2. Material and Methods

This cross-sectional study was conducted at the Department of Pathology, Medical College Baroda, Vadodara, from the year September 2013 to October 2015. The study consisted of 47 specimens of colorectal carcinomas of which 20 were biopsy specimens and 27 were resected colectomy specimens. The tumor was then graded as well, moderately and poorly differentiated according to WHO grading criteria. Immunohistochemical staining was performed using Peroxidase anti peroxidase method (PAP) using paraffin embedded blocks cut into 3–4 µm thick sections. Biogenex reagents were used for the antigen retrieval and IHC staining process. Tris-EDTA based antigen retrieval solution with a pH 6 was used for Ki67 and p53 staining. The heating cycles followed in the AG unit (Biogenex EZ-Retriever System v.2.1) were two cycles of 10 minutes and 5 minutes each at 95°C, with intermittent refilling of the antigen retrieval solution. AG unit works on the principle of application of heat energy for varying length of time on formalin-fixed, paraffin-embedded tissue sections immersed in 'Antigen retrieval solution' with a unique feature of temperature monitoring system.

Thereafter the slides were brought down to room temperature and taken through the steps of wash with PBS, peroxide block, power block and incubated with only one drop of primary antibodies on the tissue section: Ki-67 (clone BGX-Ki67, IgG1, Kappa class immunoglobin with protein concentration of 10-15mg/ml) and p53 (clone BP53-12,

IgG2a class immunoglobin with protein concentration of 10-15mg/ml) for 1 hour in humidity chamber at room temperature. After this, slides were again washed with PBS, and treated with super enhancer and polymer HRP and then again washed with PBS and then the secondary antibody to be exhibited, thereafter DAB chromogen was added. The slides were then washed with water, counterstained with Hematoxylin and blued. Then slides were serially dehydrated in alcohol, cleared in xylene and thereafter mounted using DPX. After drying, the test slides were examined along with the control sections stained simultaneously.

Negative external control sections for each case were treated identically except that the primary antibody was replaced with phosphate buffer saline (PBS). Positive external control sections containing tissue from a tonsil (for Ki-67), and from an invasive adenocarcinoma sample known to be positive for p53, were included in each staining run.

2.1 Assessment of Ki-67

The MIB-1 immunostained sections were light-microscopically evaluated using a total magnification of 400x and 6-9 sites within the tumour were examined, excepting the areas with tissue enfolding, necrosis and haemorrhagic infiltrate. Nuclear staining was regarded as positive whereas cytoplasmic staining was considered as artefact. MIB-1 labelling index (LI) was determined by counting about 500 tumor cells, and was calculated as the percentage of positive labelled nuclei. 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 [1-3].

The positive nuclear staining was observed in the epithelial cells of normal colonic mucosa and in the lymphoid cells and they also served as internal positive control.

2.2 Assessment of p53

p53 immunostaining was assessed using a light microscope and a visual grading system based on the number of positively stained nuclei of the malignant cells in each section. If 5% or more of the malignant nuclei were stained, the slide was scored as positive. If fewer than 5% of the nuclei were stained, the slide was scored as negative. The staining distribution for p53 was determined for each slide. The staining distribution was either focal or diffuse. The samples with 5-50% stained malignant cells were considered focal, and those with more than 50% stained malignant cells were scored as diffuse.

2.3 Statistical method

The data were tabulated and frequencies and percentages were calculated for qualitative variables. Quantitative variables were reported as mean and standard deviation. Categorical variables were compared using Chi-square test and p value was calculated. McNemar test was applied for agreement between p53 and Ki67. p-value less than 0.05 was considered statistically significant. The statistical analysis was done by using MED Calc v.12.5.0 software.

3. Results and Discussions

In the present study, mean age was observed to be 51.7 years and males were more commonly affected than females. The most common site of involvement was observed to be Rectum.

Mean Ki67 index was observed to be 34.7%. The percentage of cases positive for Ki67 increased from moderate (42.8%) to poor (60%). The mean Ki67 index was higher in Adenocarcinoma NOS 38.2%, as compared to mucinous adenocarcinoma 28.3%. This difference was statistically not significant. Mean Ki67 index was 22.5% in grade I, 34.3% in grade II, 74.6% in grade III. The difference in mean Ki67 index is statistically significant between moderate and poor differentiation.

p53 positivity rate was 72.3%. p53 was more frequently seen in Adenocarcinomas NOS (72.9% positive rate), than in mucinous carcinomas (57% positive rate).The difference in number of p53 positive cases amongst the histological type of tumor is not statistically significant. p53 positivity rate was observed in 75% cases of well differentiated carcinoma and increased from 67.8% to 100% in moderately to poorly differentiated carcinoma. The difference was statistically insignificant. The p53 staining distribution was diffuse (i.e. more than 50% of the cells were stained) in 82.35%cases and focal (i.e. 5-50% of the cells were stained) in 17.65% cases. 81.5% Adenocarcinoma NOS cases showed diffuse p53 positivity. All the cases of signet ring cell carcinoma showed diffuse positivity.

McNemar test was applied for agreement between p53 expression and Ki67. p-value was 0.05. But p value less 0.05 is considered statistically significant. Hence, the comparison between p53 expression and Ki67 was statistically insignificant

Table 1: Comparison of Mean Ki67 index in different studies

	Total no of cases	**Mean Ki67 index**
Saleh *et al*[4]	52	38.12%
Ihmann *et al*[5]	43	32.8%
Claudia *et al*[6]	41	48%
O Petrisor *et al*[7]	30	55.8%(colonic) 59.6%(rectal)
Present study	47	34.7%

CRCs showed a wide range of Ki-67 LI, ranging from 32-60%[4,7] indicating a variation in proliferative activity. The mean Ki67 index in our study was 34.7%. Explanation for this wide-ranged variation in the proliferative activities of CRC, as measured by MIB-1 antibody, among studies could be due to a difference in epitope preservation, in methods of evaluation or quantification of Ki-67 immunostaining and in study population.

Table 2: Comparison of Mean Ki67 index with histological grade of tumor

Mean Ki67 index/ Grade of tumor	Saleh *et al*[4]	Ishida *et al* [8]	Claudia *et al* [6]	Petrisor *et al*[7]		Present study
				Colonic	Rectal	
Well	35.7%	57.7%	20%	48%	35.2	22.5%
Moderate		60.9%	34%	58.6%	60.2	34.4%
Poor	48.3%	46.6%	57%	60.8%	84.4	74.6%

In the present study, mean Ki67 index increased with tumor grade. The difference in values of mean Ki67 index amongst moderate and poorly differentiated tumors was statistically significant. The stepwise increase of mean Ki-67 LI with the dedifferentiation of the colorectal adenocarcinomas was similar to that studied by Claudia *et al*[6]. This is also in accordance with studies by Saleh *et al*[4], Simona *et al*[1], Azza *et al*[9] where the mean Ki67 index increased with grade of tumor. However, other studies by Uzma *et al*[10], Ishida *et al*[8] found that Ki67 index was lower in cancers with poor differentiation. Recent studies [9], established the fact that an increased expression of Ki67 indicates a better survival in rectal and recto sigmoid cancer as these tumors have better response to radiotherapy, as irradiation destroys preferentially the quick dividing cells.

Table 3: Comparison of Mean Ki67 index with histological type of tumor

Mean Ki67 index/ Types of tumor	Claudia *et al* 2007[6]	Present study
Adenocarcinoma NOS	37%	38.2%
Mucinous adenoCa	32%	28.3%
Signet ring cell Ca	45%	11%

Mean Ki67 index was higher in non-mucinous tumors rather than mucinous tumors. This was in accordance with study by Uzma *et al*[10] and Nadya *et al*[3], but disagreed with the study carried out by Lanza *et al*[11] where mucinous carcinoma showed higher levels of Ki-67 reactivity than non-mucinous adenocarcinomas.

Mean Ki67 index was low (11%) in signet ring cell carcinomas in the present study. This was similar to that studied by Cabibi *et al*[12] that states that signet ring cell are in post-mitotic phase of cell cycle and show low proliferation index.

Table 4: Comparison of p53 positivity rate in different studies

	Total no. of cases	No of positive cases	p53 positivity rate
Claudia *et al*[6]	41	24	58.5%
Gurzu *et al*[1]	38	35	92%
Hunaldo *et al*[2]	82	70	85.4%
Zhenyuhu *et al*[13]	126	70	55.5%
Yong Shin *et al*[14]	266	162	60.9%
Present study	47	34	72.3%

The p53 over-expression was detected in our study in 34 cases (72.3%). The frequency of p53 over expression varied from 55% to 92% in different studies as mentioned in the table. This wide range is due to inter-study variations, including different antibodies, scoring systems, cut-off values, and study populations.

The p53 over-expression detected by immunohistochemistry is based on the accumulation of p53 protein in cells. But the wild p53 type can also accumulate in the nucleus in the case of cellular hypoxia or DNA alterations. Moreover, not all aberrant mutations of p53 cause p53 accumulation and this can cause false negative results [6]. A correlation of 70% is found between p53 immunohistochemical over expression and p53 mutation [15].

Table 5: Comparison of p53 positivity rate with histological grade

Histological grade	Claudia *et al*[6]	Hany E Saleh *et al*[15]	Yong Shin *et al*[14]	Present study
p53 nuclear positivity cut off	5%	5%	10%	5%
Well	60%	52%	62%	75%
Moderate	61.9%	39%	59.3%	67.8%
Poor	77.77%	38%	66.66%	100%

Regarding the cell differentiation in adenocarcinomas, we observed that the p53 positive rate did not correlate with grade of tumor. This was in accordance to study by Yong Shin *et al* [14]. However, Claudia *et al*[6] studied that p53 positivity rate increased with dedifferentiation of tumor and Hany E Saleh *et al*[15] studied that p53 positivity rate decreased with dedifferentiation though it was not statistically significant in either of the studies.

Table 6: Comparison of p53 positivity rate with histological type of tumor

p53 positivity rate/ Histological type of tumor	Claudia *et al* [6]	Azza*et al*[9]	Yong Shin *et al*[14]	Present study
AdenoCa NOS	65.7%	27.7%	60%	72.9%
Mucinous adenoCa	0	41.6%	77%	57%
Signet ring cell Ca	50%			100%

57% of the cases of carcinomas with mucus secreting cells over-expressed p53, while the Adenocarcinomas NOS over expressed this protein in many more cases (72.9%). This finding is consistent with previously reported results by Claudia *et al* [6]. The lower frequency of p53 alteration in carcinomas with mucus secreting cells suggests that these carcinomas occur in a different way as compared to Adenocarcinoma NOS. This was in contrast to that studied by Azza *et al*[9], Yong Shin *et al*[14] where mucinous tumors showed higher p53 positivity than adenocarcinoma NOS.

Table 7: Comparison of cases with focal and diffuse p53 positivity

	Percentage of cases with focal p53 positivity	Percentage of cases with diffuse p53 positivity
Claudia *et al*[6]	58.33%	41.67%
Present study	17.65%	82.35%

82.35% cases in the present study showed diffuse p53 positivity. This was in contrast to that studied by Claudia *et al* where only 41.67 % cases showed diffuse p53 positivity.

Table 8: Comparison of Percentage of cases with diffuse p53 positivity with histological grade of tumor

Percentage of cases with diffuse p53 positivity/ Grade of tumor	Claudia *et al*[6]	Present study
Well	37.5%	66.6%
Moderate		78.94%
Poor	57.14%	100%

The p53 staining distribution was diffuse in 66.6% of the cases of grade I, 78.94% cases of grade II, and in 100% of the cases of grade III adenocarcinomas. The percentage of diffuse positivity increased with grade of tumor. The high p53 positive rate in high grade adenocarcinomas (100% in poorly differentiated adenocarcinoma) together with the high rate of p53 diffuse distribution in these tumors suggest that p53 is involved in cell dedifferentiation in colorectal adenocarcinomas. This is in accordance with study by Claudia *et al* [6].

Claudia *et al*[6] found that overexpression of p53 was associated with high mean Ki67 index. Azza *et al*[9] found that correlation between Ki67 and p53 was highly significant ($p<0.001$), whereas, Simona Gurzu *et al*[1] studied that p53 was not correlated with Ki67. In the present study correlation of p53 and Ki67 was not significant statistically ($p=0.05$). McNemar test was applied for agreement between p53 expression and Ki67.

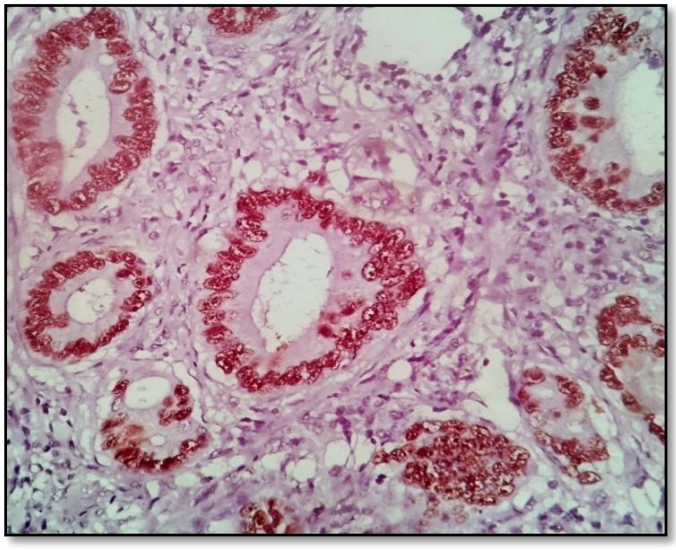

Figure 1: p53 positivity in well differentiated adenocarcinoma (x400)

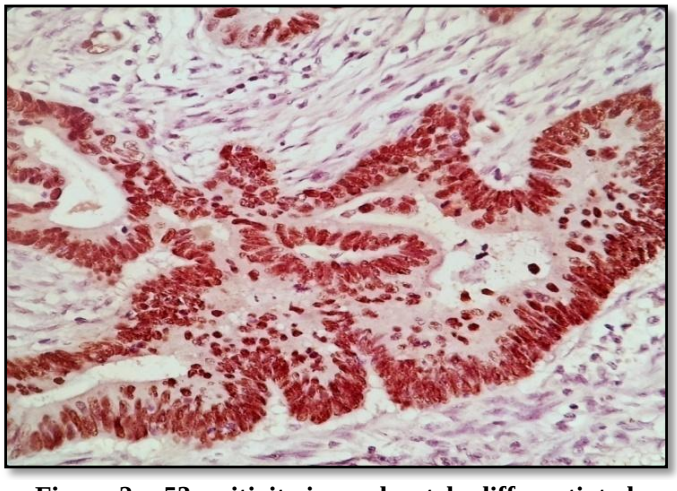

Figure 2: p53positivity in moderately differentiated adenocarcinoma(x400)

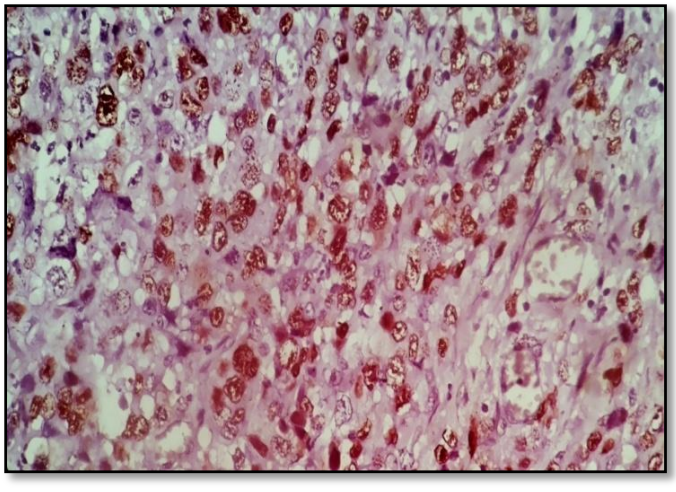

Figure 3: p53 positivity in poorly differentiated adenocarcinoma (x400)

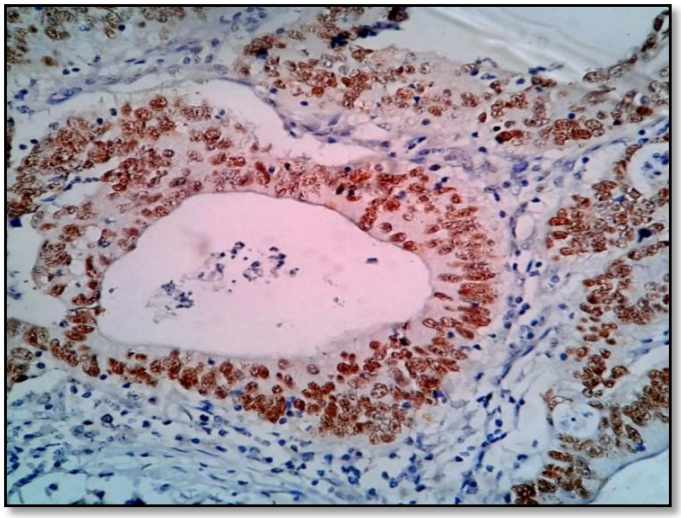

Figure 4: Ki67 positivity in moderately differentiated adenocarcinoma (x400)

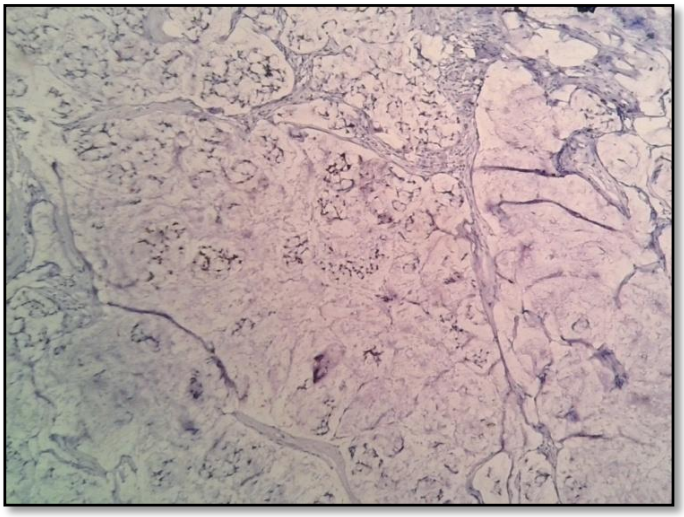

Figure 7: Ki67 positivity in mucinous adenocarcinoma(x100)

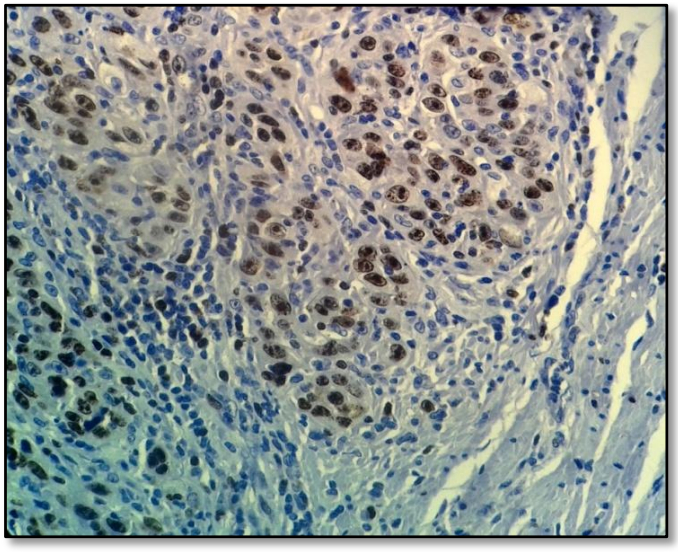

Figure 5:Ki67 positivity in poorly differentiated adenocarcinoma(x400)

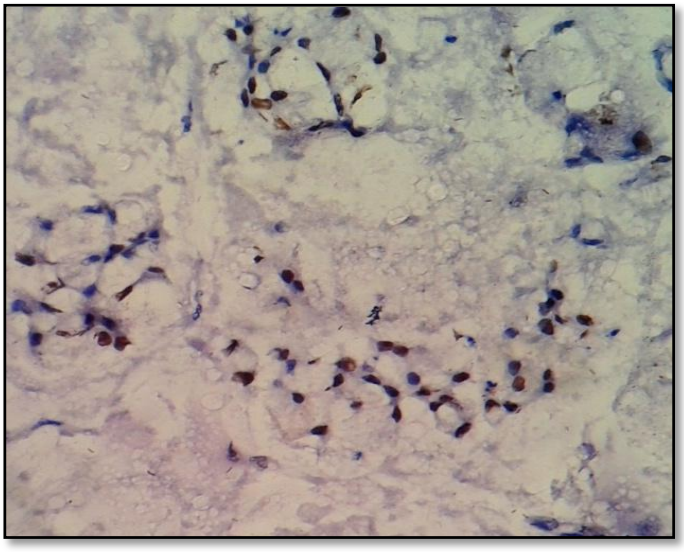

Figure 7a: Ki67 positivity in mucinous adenocarcinoma, same as above(x400)

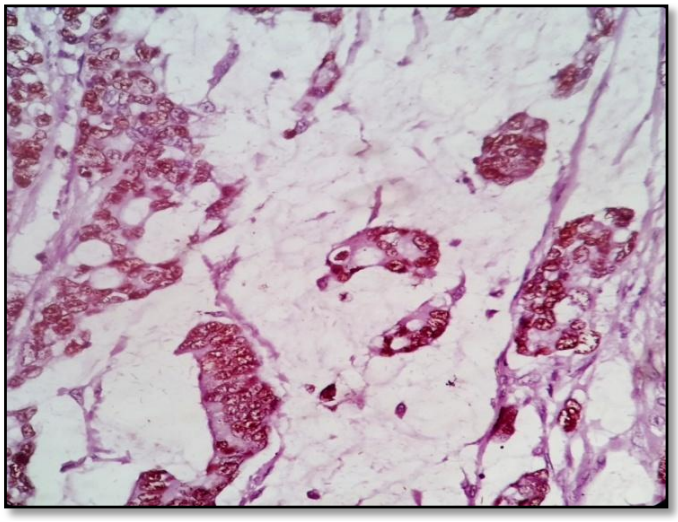

Figure 6: p53 positivity in mucinous adenocarcinoma; malignant epithelial cells floating in mucin are positive for p53(x400)

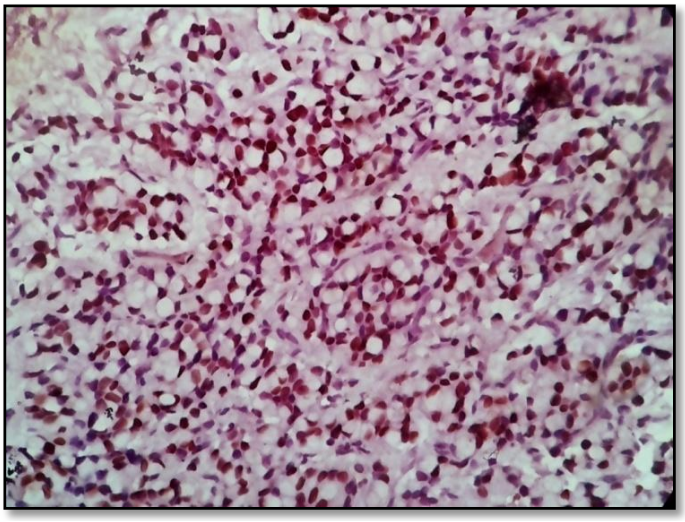

Figure 8: p53 positivity in signet ring cell carcinoma (x400)

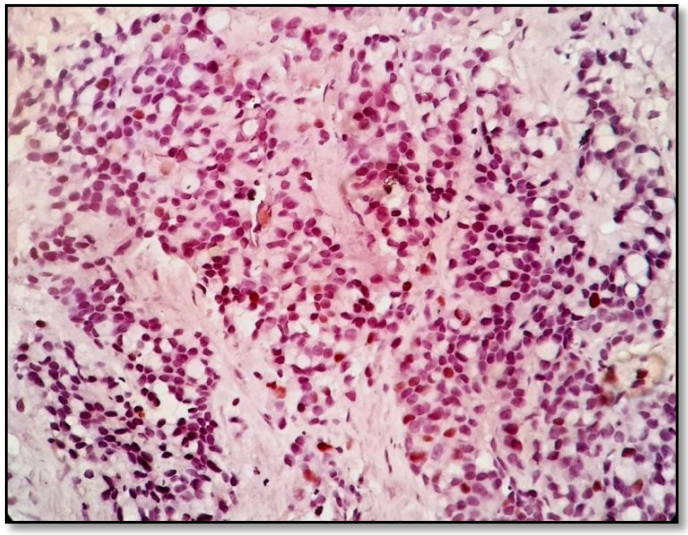

Figure 9: Ki67 positivity in signet ring cell carcinoma (x400)

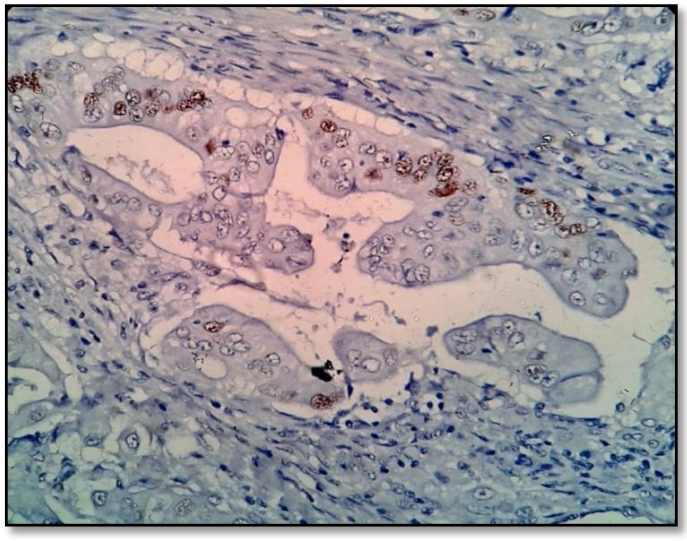

Figure 10: Focal p53 positivity in moderately differentiated adenocarcinoma (5-50% malignant epithelial cells show nuclear positivity) (x400)

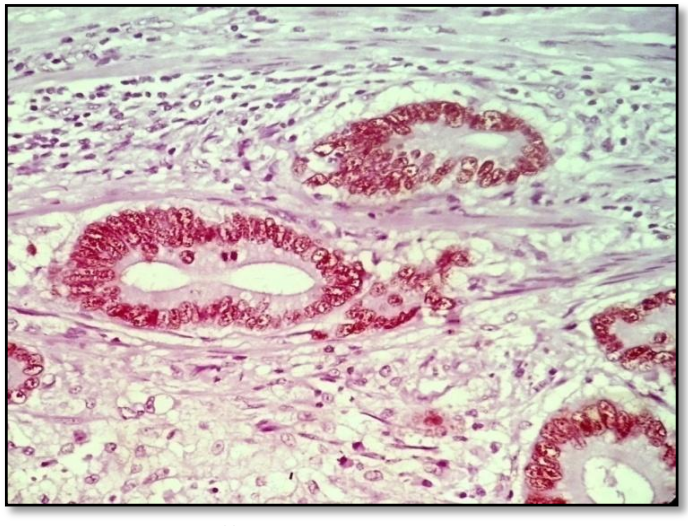

Figure 11: Diffuse p53 positivity in moderately differentitiated adenocarcinoma (>50% malignant epithelial cells show nuclear positivity) (x400)

4. Conclusion

Mean Ki67 labelling index correlated with histological grade of colorectal carcinoma but not the histological type of colorectal carcinoma. p53 positivity rate did not correlate with histological grade nor the histological type. The combined expression of Ki67 and p53 in colorectal carcinomas did not correlate in the present study.

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