

Correlation of Ki-67 Proliferating Index with Histological Stage and Grade in Colorectal Carcinoma

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Colorectal carcinoma is the most common cancer of gastrointestinal tract. It is the 3rd most commonly diagnosed cancer and the 3rd leading cause of cancer death. The growth of tumor in colorectal carcinoma is highly variable and its histological grading and staging has important role in diagnosis, treatment and overall prognosis. To observe the Ki-67 expression in colorectal carcinoma and find out the possible correlation of Ki-67 proliferating index with histological grading and Duke's staging. This cross sectional study was conducted at Sir Salimullah Medical College from July 2014 to June 2016. 98 patients with colorectal carcinoma enrolled in this study by purposive sampling. The H&E staining was done on paraffin embedded tissue sample. Ki-67 expression by IHC method. Ki-67 is a proliferation associated nuclear antigen which can be recognize by MIB-1 monoclonal antibody, correlate with histological staging and grading in colorectal carcinoma. Then tumours were graded according to WHO grading criteria and pathological staging was done according to Duke's staging system and immunohistochemical staining for Ki-67 antigen expression. Results were subjected to statistical analysis. The results were considered to be significant when the $P < 0.05$. Ki-67 proliferative index was high in well and moderately differentiated adenocarcinoma but low in poorly differentiated which is statistically significant ($p < 0.05$). Ki-67 expression was high in early Duke's stage A and B but low expression in advanced Duke's stage C ($p > 0.05$). The result of this study will enlighten the clinician regarding the need for doing Ki-67 in colorectal carcinoma which would contribute to better understanding of the treatment as well as prognosis.

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Introduction

Colorectal carcinoma is the most common malignancy of gastrointestinal (GI) tract and is a major cause of morbidity and mortality worldwide.¹ Colorectal cancer accounts for 10% of all cancers and it is the 2nd leading cause of death from malignancy in the industrialized world.² There are nearly one million new cases of colorectal cancer diagnosed worldwide each year and half a million death.³ In 2013, there were an estimated 1,177,556 people living with colon and rectal cancer in the United States and the number of new cases of colon and rectal cancer was 41.0 per 100,000 men and women per year.⁴ Regarding age incidence of colorectal carcinoma, recent reports show that in the USA it was the most frequent form of cancer among the person aged between 60-70 years and fewer than 20% of cases occurs before the age 50.⁵ The incidence of colorectal cancer in Bangladesh is exactly not known but estimated population are approximately 15,10.1%.⁶ The distribution of colorectal carcinoma worldwide seems to be related to industrialization and socioeconomic standard and the incidence rate is higher in industrialized countries including Western Europe, Scandinavia and North America, whereas in the developing countries (sub-Saharan, Africa and Asia) the incidences appear to be lower.⁷

There are several staging system for colorectal carcinoma among these TNM and Duke's staging systems are the most common way of staging and grading of colorectal carcinoma.⁸ The American Joint Committee (AJC) and the Union for International Cancer control (UICC) joined to produce the TNM system, which attempt to record clinical and pathological data, guide therapy and forecast prognosis, all in one.⁹ Whereas the classification into Duke's stage A,B,C cases is the measurement of the boundaries reached and both methods

permit the grouping of cases into favorable and unfavorable outcome.¹⁰ Histologically the tumor is graded according to WHO grading criteria as well differentiated, moderately differentiated and poorly differentiated and the histological appearance of colorectal carcinoma may vary considerably with its major importance being related to prognosis.¹⁰

The use of monoclonal antibodies raised against specific antigens associated with the cell proliferation.¹¹ Ki-67 is a proliferation associated nuclear antigen expressed in all cycling cell except resting cell in the G0 phase and it reflects cell in the S/G2+M phases in particularly.¹² MIB-1 is a monoclonal antibody and it recognizes the Ki67 nuclear antigen in the formalin fixed paraffin embedded tissue section.¹³ Ki-67 expression is estimated as the percentage of tumors cells positively stained by the antibody with nuclear staining.¹² The importance of Ki-67 as an indicator of tumor behavior and in colorectal cancer this index may be used as a marker of prognosis.¹²

The proliferative activity as measured by Ki-67 antibody is closely associated with histological grade and stage.² In 2008 Uzma Nabi, Nagi A H and Waqas Sami, Department of Pathology, University of Health Sciences, Lahore, Pakistan conducted a study on Ki-67 proliferating index and histological stage and grade of colorectal carcinoma and observed that proliferative index is high in well and moderately differentiated adenocarcinoma and in an early Duke's stage (A or B).² But Ki-67 proliferating activity is low in poorly differentiated tumour and in an advanced Duke's stage C.^{13,8} But there are some studies of Lanza, Cavazzinil in 1990 and Yokoyama N, Okomoto H in Japan in 2005 contradicting the above mentioned association of Ki-67 versus grading and staging of colorectal carcinoma, they concluded that proliferating

index of Ki-67 was increasing with increasing grade, stage.^{14, 15}

Methods

This cross sectional study was conducted among the 98 histopathologically diagnosed patients having colorectal carcinoma over a period of two years in the department of surgery, Sir Salimullah Medical College. Study population were the patients having colorectal cancer underwent surgical treatment in the department of surgery of Sir Salimullah Medical College. The proliferative activity as measured by Ki-67 antibody is closely associated with histological grading and staging of colorectal carcinoma. The representative sections were submitted for Immunohistochemical staining. The Ki-67 immunostaining were performed according to manufacture's recommendation, using the MIB-1 clone (DAKO, Carpinteria, CA & Ventena Medical System, Tucson, AZ). Ki-67 immunostained slides were examined via light microscopy. Positive Ki-67 staining was observed brown granular nuclear staining. For Ki-67 scoring the most positive area of the tumor was selected avoiding foci of inflammation. The number of positive nuclei were counted in 500 tumor cells in a high power field. The average of the counts over the same slides was taken and expressed as the percentage of Ki-67 positive cells in the tumor.

Statistical analysis were performed in SPSS statistical software program, Version 17.0. To correlate histological grading and staging of colorectal carcinoma with Ki-67 proliferating index were performed with Mann-Whitney U test. The result were consider to be significant when $P < 0.05$. One way ANOVA followed by Bonferroni test was performed to compare between groups.

Results

98 cases were included in the present study. Age incidence ranged from 28-78 years and their mean \pm SD 47.38 ± 10.37 . Maximum patients (30.6 %) were found in 41-50 years age group where M: F was 1.72: 1 (Table I). Regarding site of tumor more than 50% of patient had tumors in the left side of colon and in the rest of the cases tumors were present in caecum (26.5%; Table-II). 98 cases having different sizes of tumors and in most of the cases fifty nine cases (60.2%) tumors size were 3-4 cm and their mean \pm SD 4.8 ± 1.8 (Table-III). Different morphological types of tumor were observed in present study. In maximum forty cases (40.8%) the morphological types of tumors were ulcerative and only five cases (5.3%) tumors were infiltrative type. Rests were annular (33.5%) and polypoid (20.3%; Table IV).

In the present study, tumors were graded according to WHO grading system into well differentiated, moderately differentiated, poorly differentiated and grouped into A, B, C accordingly. The maximum cases 69 (70.4%) of colorectal carcinoma were well differentiated and their mean Ki-67 proliferating index was 47.83 ± 15.23 . There were significant differences among the groups (A vs B vs C), when mean proliferating index of Ki-67 were compared among the three groups. The result was found statistically significant ($P < 0.05$). However when compared in between groups only A vs C (between well differentiate & poorly differentiate) groups were found also statistically significant ($P < 0.05$). In the present study, it was also observed that with increasing grade, Ki-67 proliferating index decreases (Table V).

Regarding staging of the tumor (Duke's staging) where maximum cases (44.8%) were in stage B1 and their mean Ki-67 proliferating index was 46.25 ± 14.06 . There

was no significant differences among the stages and the result was not statistically significant ($P>0.05$). In the present study it

was also observed that with increasing stage of the tumors, there was decreasing Ki-67 proliferating index (Table VI).

Table I: Distribution of patients according to age group with male female ratio (n=98)

Age groups	Frequency			M: F	Percentage
	Total	Male	Female		
≤30	10	7	3	2.3:1	10.2
31-40	23	13	10	1.3: 1	23.5
41-50	30	19	11	1.72: 1	30.6
51-60	26	17	9	1.88: 1	26.5
≥60	9	9	0	9.00: 0	9.2
Total	98				
Mean ± SD				47.01± 10.99	
Range (Min-Max)			28 – 78		

Table II: Distribution of tumors according to site (n=98)

Site of tumor	Frequency (%)
Left side of colon	
Sigmoid colon	34 (34.7)
Transverse colon	24 (24.6)
Rectum	14 (14.7)
Right side of colon	
Caecum	26 (26.5)
Total	98 (100)

Table III: Distribution of patients according to tumor size (n=98)

Tumor size (cm)	Frequency (%)
1 – 2	14 (14.3)
3 – 4	59 (60.2)
5 – 6	19 (19.4)
Mean ± SD	4.8 ± 1.8
Total	98 (100)

Table IV: Distribution of patients according to morphological types of tumor (n=98)

Morphology	Frequency (%)
Ulcerative	40 (40.8)
Annular	33 (33.5)
Polypoid	20 (20.3)
Infiltrating	5 (5.3)
Total	98 (100)

Table V: Relation of Ki-67 proliferating index with histological grading (n=98)

Grading	Frequency n(%)	Ki-67 expression (Mean \pm SD)	P
Well differentiated (A)	69 (70.4)	47.83 \pm 15.23	
Moderate differentiate (B)	15(15.3)	46.33 \pm 18.07	
Poor differentiated (C)	14(14.3)	35.35 \pm 11.17	
Statistical analysis			
A vs B vs C			0.023*
A vs B			1.000 ^{ns}
A vs C			0.019*
B vs C			0.162 ^{ns}

ANOVA followed by Bonferroni test was performed to compare between groups

Table VI: Relation of Ki-67 proliferating index with histological stage (n=98)

Duke's staging	Frequency n (%)	Ki-67 expression (Mean \pm SD)	p
Stage A	3 (3.3%)	53.33 \pm 20.81	0.727 ^{ns}
Stage B1	44 (44.8)	46.25 \pm 14.06	
Stage B2	20(20.5)	43.25 \pm 14.06	
Stage C1	14(14.8)	44.64 \pm 21.07	
Stage C2	17(17.5)	42.05 \pm 13.69	

ANOVA test was done to measure the level of significance.

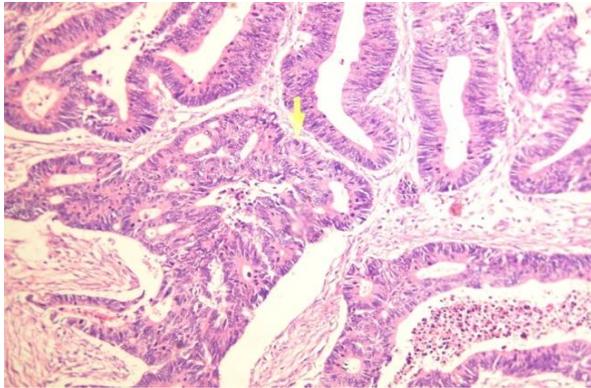


Figure 1. Photomicrograph of histopathological section of well differentiated adenocarcinoma of colon (H&E method x100)

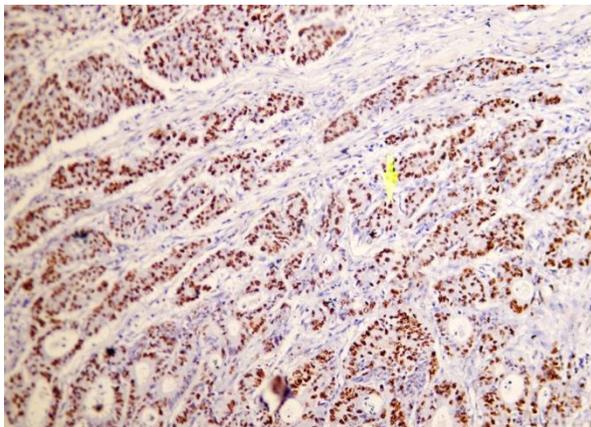


Figure 2. Photomicrograph of well differentiated adenocarcinoma stained with Ki-67 immunostain showing high proliferative Index(x100).

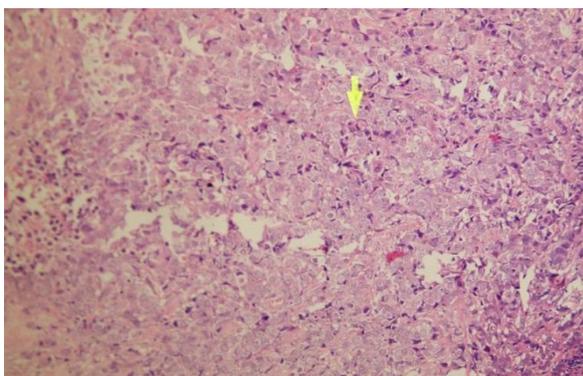


Figure 3. Photomicrograph of histopathological section of poorly differentiated adenocarcinoma of colon (H& E method x400).

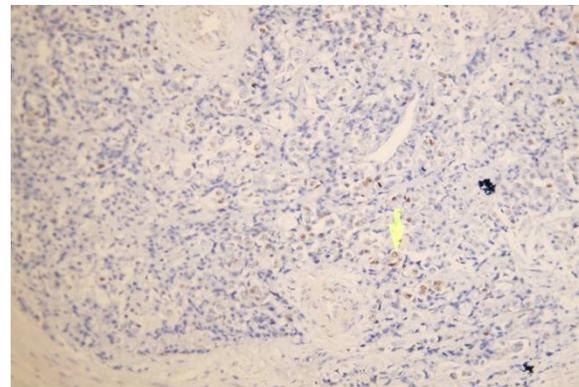


Figure 4. Photomicrograph of poorly differentiated adenocarcinoma stained with Ki-67 immunostain showing low Proliferative Index (x400).

Discussion

Colorectal cancer (CRC) is one of the most common malignancies and a leading cause of cancer death worldwide.¹⁶ The incidence of cancer colon and rectum was 41.0 per 100,000 men and women per year and the number of deaths was 15.1 per 100,000 men and women per year in Bangladesh.¹⁷ Management of colorectal carcinoma depends on a number of morphological and biological factors which include the pathological tumor stage (including involvement of lymph nodes, breach of serosa, distant spread etc.), primary tumor characteristics (including depth of tumor penetration in the bowel wall, histological subtype, histological grade and differentiation, venous and lymphatic invasion, perineural invasion and lymphocytic infiltration), status of surgical resection margins (free or involved).¹⁸ With assessment of tumor cell proliferation may predict tumor behavior.¹⁹ The aim of this study was to evaluate the proliferating index (PI) in formalin fixed, paraffin embedded tissue section of colorectal carcinoma, using monoclonal MIB-1 antibody (Ki-67) and to assess the relationship between proliferative index (PI) and various pathological findings in colorectal carcinoma including histological grade, and stage.

In the present study, the mean age of the patients was 47.01 ± 10.99 years and the highest number of malignant cases were seen in the 4th and 5th decades. Male female ratio was 1.72:1 and 1.88:1 in 4th and 5th decades accordingly. So, in present study, male were predominant than female. These findings were similar to other studies.^{20,21}

In 34.7% patients the tumors were located in sigmoid colon followed by caecum (26.5%), transverse colon (24.5%) and rectum (14.3%). As per gross morphological type of cancer, maximum 40.8% were ulcerative and 33.5% were annular type and rest were polypoid (20.3%) and infiltrating type (5.3%). In one study, colon was more commonly affected site compared to rectum and most of the lesions were ulcerative.²² In another study rectum was the most common affected site and predominant lesions were annular.²³

In present study, 60.2% of malignant cases had tumors size between 3-4 cm and 19.4% malignant cases had tumors size 5-6 cm. In a study in 2011 by Kornprat showed that maximum size of the tumor in colon cancer were in between 4.5 to 6.5 cm in majority of cases.²⁴ In 2013 ASCO annual meeting showed a report where a study conducted on tumor size in colorectal cancer found that in majority of the cases the tumors sizes were in between 4-6 cm in colorectal carcinoma.²⁵

In this present study it was observed that the Ki-67 labelling index was high in grade I and grade II compared to grade III. These results showed that proliferating index was low in poorly differentiated tumor compared to well and moderately differentiated tumor. When Ki-67 proliferating index was compared among three groups a statistically significant correlation was found in present study ($p < 0.05$). These findings are similar to studies in Japan² Pakistan¹² and Finland.²⁶ On the other hand, some of the studies showed

contradictory result that Ki-67 proliferating index increased with increasing histological grade.^{26,27}

In the present study, it was observed that Ki 67 labeling index was high in Duke's stage A and B and tumor in advanced stage (Duke's C) have a low proliferating index compared to tumors in an early invasive stage (Duke's A and B). Regarding the correlation of Ki-67 LI with staging, no statistically significant correlation was found. This result was similar with other studies in which they concluded that Ki-67 proliferating index was significantly lower in carcinoma in subserosa or deeper invasion compared to carcinoma with submucosa or muscularis mucosa invasion.^{2,8,26} In another study contradictory results have been reported on associations of Ki-67 with prognosis and survival of colorectal tumors with a low proliferation index in Duke's A and B tumors to be associated with survival impairment compared to those with high values.²⁸ Tumors with high proliferative activity are known to be most responsive to radiotherapy and Willett and collaborators showed that radiation eradicates preferentially rapidly dividing cells in rectal cancer, whereas populations with slow proliferation show greater radioresistance.²⁹

Conclusion

It is concluded that Ki-67 labeling index is high in well to moderately differentiated adenocarcinomas in an early Duke's stage A or B compared to poorly differentiated adenocarcinomas, and in an advanced Duke's stage C. Thus Ki-67 proliferating index can be useful in a patient with colorectal carcinoma as a ancillary diagnostic support. Moreover, it may help in the prognostic evaluation of patient, survival as well as in considering them for post surgical treatment.

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