

KI-67 PROLIFERATING INDEX AND HISTOLOGICAL GRADE, TYPE AND STAGE OF COLORECTAL CARCINOMA

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Background: The objective of this study was to assess the relationship of histological type, grade and stage of colorectal carcinoma with proliferative activity as measured by Ki-67 LI. It was a descriptive study and conducted at the Department of Pathology, University of Health Sciences, Lahore. **Methods:** Ki-67 is a protein associated with cell proliferation and is expressed in all the phases of cell cycle except G0. In the present study, Ki-67 expression in 50 patients with colorectal adenocarcinomas was observed using immunohistochemistry with monoclonal antibody MIB-1. **Results:** Ki-67 LI was high in well and moderately differentiated adenocarcinomas (mean Ki-67 LI 392.50 ± 56.58 and 342.24 ± 96.84 respectively) as compared to poorly differentiated adenocarcinomas (mean Ki-67 LI 250.00 ± 113.46). Ki-67 LI was high in non mucinous adenocarcinomas than mucin secreting and signet ring cell adenocarcinomas (mean Ki-67 393.93 ± 55.91 vs 220.00 ± 49.72 and 200.00 ± 79.05 respectively). As regard the Dukes' staging, Ki-67 LI was high in colorectal carcinomas in Dukes' stage B than tumours in Dukes' stage C (Mean Ki-67 LI 360.13 ± 90.03 vs 241.66 ± 101.31 respectively). **Conclusion:** The proliferative activity as measured by Ki-67 antibody is related to histological type, grade and stage.

Keywords: Colorectal carcinoma, Grade, Proliferation index, Immunohistochemistry, Ki-67

INTRODUCTION

Colorectal cancer accounts for about 10 percent of all cancers (after exclusion of non-melanoma skin cancer) and it is fourth leading cause of cancer death globally.¹ It is the second leading cause of death from malignancy in the industrialised world, accounting for more than 10 percent of all cancer deaths.² Environmental and genetic factors play an important role in the pathogenesis of colorectal carcinoma. Major environmental risk factors for colorectal carcinoma include dietary factors. Diets high in red meat, fat and low in fruits and vegetables are associated with an increased risk of colorectal carcinomas.³

Colorectal cancer develops either sporadically (85%), or as a part of a hereditary cancer syndrome (less than 10 %) or against a background of inflammatory bowel disease.⁴ Accumulation of molecular alteration, including mutation in *Kirsten-ras* (*K-ras*), *p53* and *adenomatous polyposis coli* (*APC*), contribute to colorectal carcinogenesis.⁵ Adenoma carcinoma sequence, is a term that describes the stepwise progression from normal to dysplastic epithelium to carcinoma associated with the accumulation of multiple clonally selected genetic alterations.. It is accompanied by a series of molecular alterations that include the mutational activation of oncogenes and the inactivation of tumour-suppressor genes.⁶

The colorectal carcinomas grossly are polypoid, ulcerative, annular or diffusely infiltrating. Morphologically, 98% of colorectal carcinomas are adenocarcinoma. Its major subtypes are non-mucinous adenocarcinoma, mucinous or colloid adenocarcinoma and signet-ring cell type carcinoma.⁷

Dukes' staging system is the most common way of staging and grouping colorectal carcinomas

and is also used to determine patients who can be offered adjuvant therapies or entered into clinical trials.⁸ The histological appearance of colorectal carcinoma may vary considerably with its major importance being related to prognosis. The histological grade is essentially an estimate of the pace of growth, whereas the classification into Duke's A, B and C cases is a measurement of the boundaries reached. Both methods permit the grouping of cases into favourable and unfavourable outcomes.⁹

The growth of malignant tumours is highly variable and this probably reflects their clinical course, however proliferation is a key feature of progression of tumour. Ki-67 is a proliferation-associated nuclear antigen expressed in all cycling cells except resting cells in the G0 phase, and it reflects cells in the S/G2+M phase, in particular.¹⁰

The *Ki-67* gene is present on the long arm of the human chromosome 10 (10q25). The half life of Ki-67 protein has been estimated about 60-90 minutes. The Ki-67 antigen is detected in G1, S, G2 and M phases of cell cycle but not in G0 phase. The Ki-67 is a protein phosphorylated via serine and threonine with a critical roll in cell division. This has been observed from the arrest of cell proliferation when Ki-67 is blocked either by microinjection of blocking antibodies or by inhibition of dephosphorylation. The Ki-67 expression is estimated as the percentage of the tumour cells positively stained by the antibody, with nuclear staining being most common criterion of positivity. MIB-1 is a monoclonal antibody and it recognizes the Ki-67 nuclear antigen in the formalin fixed paraffin embedded tissue sections and its reactivity is not affected if there is a delay in fixation.¹¹

In the present study, we evaluated the Ki-67 proliferation index (PI) in colorectal carcinomas. The aim of this study was to assess the proliferation index (PI) in formalin-fixed, paraffin-embedded tissue sections of colorectal carcinomas and to investigate the relationship between the proliferative activity of colorectal carcinoma with histological type, grade and stage of the tumour.

MATERIALS AND METHODS

This descriptive study was conducted at the Department of Pathology, University of Health Sciences, Lahore. The study consisted of 50 colectomy/hemicolectomy specimens of colorectal carcinomas. Patients of all ages and both sexes were included. Multiple 3-5 mm thick representative slices were taken from the tumour including the junction of the tumour with adjacent uninvolved tissue. In addition the proximal and distal resection margins, with at least one random block from the uninvolved bowel, and necessary blocks from the background abnormalities, including polyps or if any other disease process. The mesenteric fat was thoroughly examined for lymph nodes. These tissues were processed for paraffin embedding and subsequent staining by Haematoxylin and Eosin. For demonstrating the mucin, Alcian blue stain was performed.¹² The tumours were graded as, well, moderately and poorly differentiated according to WHO grading criteria. The pathological stage was determined according to Dukes' staging system.

Immunohistochemical staining for the Ki-67 antigen was performed using the MIB-1 antibody and the avidin-biotin complex method using paraffin embedded blocks cut into 3-4 μ m thick sections.¹³ 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. After MIB1 staining, the number of tumour cells with distinct nuclear staining was recorded after counting 500 tumour cells in consecutive high power fields in the most reactive areas of the slides. Cells with questionable nuclear staining were discounted. The percentages of positive tumour cells were then calculated as MIB1 labelling index (MIB1 LI) or proliferating index (PI). Necrotic or thick areas and severely overlapping tumour cells were avoided during evaluation.¹⁴

The data was entered and analysed using SPSS version 16.0. Mean \pm SD are given for quantitative variables. Frequencies and percentages are given for qualitative variables. One way analysis of variance was applied to observe group difference and Post Hoc Tukey test was applied to observe which means difference. Pearson chi-square and Fisher exact test were applied to observe associations.

A *p*-value of < 0.05 was considered statistically significant.

RESULTS

A total of 50 formalin fixed colectomy/hemicolectomy specimens from patients with colorectal carcinoma were included in this study. Among these patients, 10% (n=5) were below the age of 40 years, 2% (n=1) above the age of 70 years and 88% (n=44) were between 41 and 69 years of age.

All the 50 cases were adenocarcinoma among these, 66% (n=33) were reported as non-mucinous adenocarcinoma, 20% (n=10) as mucinous adenocarcinoma and 14% (n=7) as signet-ring cell type carcinoma. Regarding histological grades, 20% (n=10) were reported as grade I, 58% (n=29) as grade II and 22% (n=11) as grade III adenocarcinomas. According to modified Dukes' staging, 2% (n=1) was in Dukes' stage A, 74% (n=37) were in Dukes' Stage B, and 24% (n=12) were in Dukes' stage C.

As regards the relationship between mean Ki-67 proliferating index and grades of colorectal carcinoma, it was observed that the 10 cases of well differentiated (Grade I) colorectal adenocarcinomas had mean Ki-67 proliferating index 392.500 ± 56.58 , 29 moderately differentiated (grade II) adenocarcinomas revealed mean Ki-67 PI 342.24 ± 96.84 and 11 poorly differentiated (grade III) adenocarcinomas had mean Ki-67 PI 250.00 ± 113.46 . Significant difference was observed between Ki-67 PI and grades of colorectal carcinomas ($p=0.004$). After applying Post Hoc Tukey test, significant difference was observed between grade I and grade III (392.50 ± 56.5 vs 250.0 ± 113.46) carcinomas ($p=0.003$). However, no significant difference of mean Ki-67 PI was found between Grade I and II (392.50 ± 56.58 vs 342.24 ± 96.84) ($p=0.324$). Significant difference was found (342.24 ± 96.84 vs 250.00 ± 113.46) ($p=0.02$) between grade II and grade III.

The association between mean Ki-67 PI and various histological types of colorectal carcinomas showed that the mean Ki-67 PI was 393.93 ± 55.91 in 33 non mucinous adenocarcinomas, 220.00 ± 49.72 in 10 cases of mucinous adenocarcinomas and 200.00 ± 79.05 in 7 cases of signet ring cell carcinomas. Significant difference was observed between Ki-67 PI and histological types of colorectal carcinomas ($p=0.000$). After applying Post Hoc Tukey test, significant difference of mean Ki-67 PI was observed between non mucinous and mucinous adenocarcinomas (393.93 ± 55.91 vs 220.00 ± 49.72) ($p=0.000$). A significant difference of Ki-67 PI was found between non mucinous and signet ring cell carcinomas (393.93 ± 55.91 vs 200.00 ± 79.05) ($p=0.000$). No significant difference of mean Ki-67 PI was observed

between mucinous and signet ring cell adenocarcinomas (220.00±49.72 vs 200.00±79.056) ($p=0.767$).

As regard the association between mean Ki-67 PI and Dukes' stage of colorectal carcinomas, it is calculated that mean Ki-67 PI was 360.13±90.03 and 241.66±101.31 in Dukes' stage B and C respectively. Significant difference was observed between Ki-67 PI and Dukes' stage B and C of colorectal carcinomas after applying Post Hoc Tukey test ($p=0.001$). Only 1 case was in Dukes' stage A, so mean of Ki-67 could not be calculated (Table-1).

Table-1: Immunohistochemical expression of Ki-67 (MIB-1) antibodies in colorectal carcinomas (n=50)

Histological Grade, Type & Stage	No	Mean Ki-67 PI±S,D
Histological grade*		
well	10	392.50±56.58
Moderate	29	342.24±96.84
Poor	11	250.00±113.46
Histological Type**		
Non Mucinous	33	393.93±55.91
Mucinous	10	220.00±49.72
Signet ring cell	7	200.00±79.05
Pathological stage***		
Dukes' A	1	375.00±0.00
Dukes' B	37	360.13±90.03
Dukes' C	12	241.66±101.31

* $p=0.004$, ** $p=0.000$, *** $p=0.001$

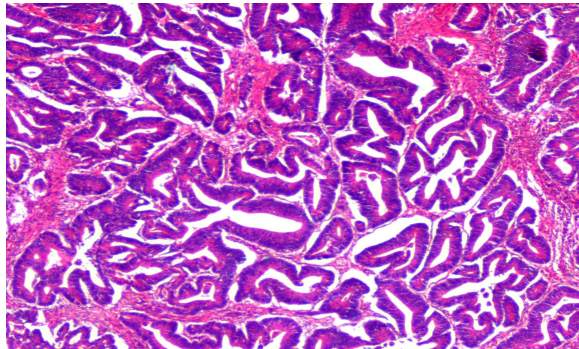


Figure-1: Well to moderately differentiated adenocarcinoma of colon in Dukes' Stage B.

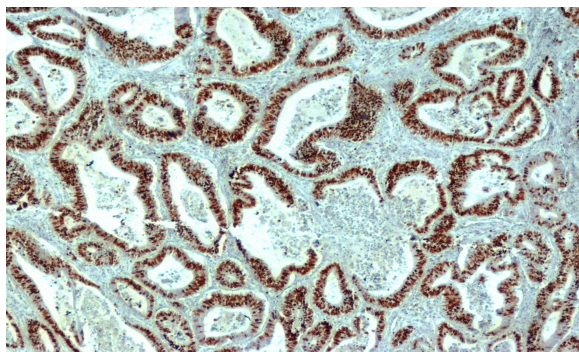


Figure-2: Ki 67 nuclear staining in well to moderately differentiated adenocarcinoma of colon in Dukes' stage B

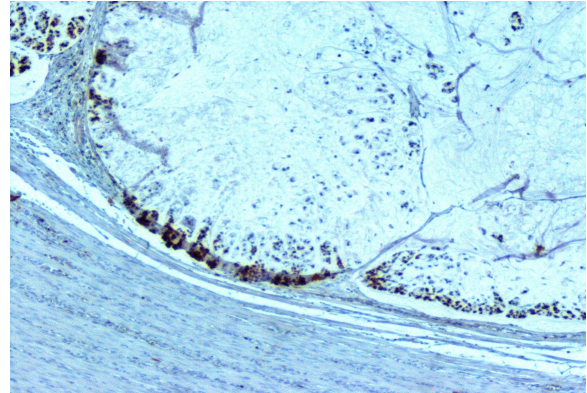


Figure 3: Ki 67 nuclear staining in signet ring cell adenocarcinoma of colon in Dukes' Stage C

DISCUSSION

Colorectal carcinoma is fourth leading cause of cancer death in the world.¹ Cellular proliferation is fundamental to maintain tissue homeostasis and is important in oncogenesis. Assessment of tumour cell proliferation may predict tumour behaviour.¹⁵ Quantification of cell proliferative activity in neoplasia is currently the subject of considerable investigation. The Ki-67 is nuclear antigen expressed in highest concentration in all stages of the cell cycle but not in resting cells. MIB-1 is a monoclonal antibody that recognises a fixation resistant epitope of Ki-67 antigen and it is used to estimate the proliferative fraction of neoplasia.¹⁴ The aim of this study was to evaluate the proliferation index (PI) in formalin fixed, paraffin embedded tissue sections of colorectal carcinomas using monoclonal MIB-1 (Ki-67) antibody and to assess the relationship between proliferative index (PI) and various pathological findings in colorectal carcinoma including histological grade, type and Dukes' stage.

It was observed that Ki-67 LI/PI was high in non-mucinous tumours (Ki-67 LI 393.93±55.91) than in mucinous or signet ring cell carcinoma (mean Ki-67 LI was 220.00±49.72 and 200.00±79.05 respectively). The finding that mucinous carcinomas have low proliferating activity is matched with the study reported by Hideki Ishida *et al.*¹⁶ The result in the present study is different from those of Lanza *et al* where immunostaining with monoclonal antibody Ki-67 had been employed to determine the growth fraction in series of 139 primary adenocarcinomas of large bowel. Mucinous carcinomas showed higher levels of Ki-67 reactivity than non-mucinous adenocarcinomas ($p=0.0003$).¹⁷

As regard the association between grade of colorectal carcinomas with mean Ki-67 labelling index, it was observed, that Ki-67 LI was high in Grade I and Grade II as compared to the Grade III carcinomas (mean Ki-67 was 392.50±56.58,

342.24±96.84 and 250.00±113.46 in grade I, II, III, respectively). These results show that proliferating index was low in poorly differentiated tumours than the well or moderately differentiated. These findings are similar to a study in Japan. They reported 207 invasive colorectal carcinoma without metastases, and 82 invasive colorectal carcinomas with metastases. They concluded that among 207 invasive carcinomas, the positive rate of Ki-67 antibody in poorly differentiated adenocarcinoma (46.6±24.5) and mucinous carcinomas (43.9±10.3) was significantly lower than in well differentiated (57.7±23.24) and moderately differentiated adenocarcinomas (60.9±23.8), suggesting that proliferative activity is low in cancers with poor differentiation (16). On the other hand, Saleh *et al* reported a study on 52 cases of colorectal carcinomas. Among them, well and moderately differentiated carcinomas were grouped together (n=42) whereas poorly differentiated cases (n=10) were grouped separately. They performed Ki-67 immunostaining and concluded that Ki-67 PI appeared to increase with decreasing degree of differentiation of carcinoma: 35.7±9.5 in well/moderately differentiated vs. 48.3±11.7 in poorly differentiated carcinomas ($p=0.0007$).¹⁴

Another study conducted by Valentina *et al* determined the expression of PCNA and Ki-67 in 41 cases of colorectal carcinomas and concluded that in adenocarcinomas tumour proliferative activity, detected with PCNA and Ki-67 antibodies, increased with the histological grade.¹⁸

In the present study, it was observed that Ki-67 labelling index was high in Dukes' stage B (Mean Ki-67 LI 360.13±90.03) than in Dukes' stage C (Mean Ki-67-LI 241.66±101.31), showing that tumours in advanced stage (Dukes' C) have a low proliferating index than tumours in an early invasive stage (Dukes' B). This result is the same with the study reported by Ishida *et al*, in which they concluded that Ki-67 proliferating index was significantly lower in carcinomas with subserosa or deeper invasion (55.2±21.4) than in carcinomas with submucosa (63.8±26.0) or muscularis propria (65.8±23.6) invasion (16). On the other hand the results of the study by Saleh *et al* concluded that as regards the pathological stage, mean Ki-67 proliferating index increased with advancing tumour stage, i.e., 34.7±7.8 in Dukes' stage A, 37.3±10.9 in Dukes' stage B and 42.8±12.8 in Dukes' stage C.¹⁴

The use of this MIB-1 antibody has allowed retrospective examination for Ki-67 previously. Jansson *et al* examined expression of Ki-67 in 255 colorectal carcinomas by using immunohistochemistry with the monoclonal antibody MIB-1. One hundred and 57 (62%) cases had more than 50% positive

tumour cell nuclei and 98 (38%) cases had less than 50% positive tumour cell nuclei. The tumour showed a wide range of Ki-67 expression, from 13% to 90% that indicated a variation in proliferative activity. There was no significant relationship between Ki-67 expression and sex, age, tumour location, Dukes' stage, growth pattern, differentiation, DNA content, S-phase fraction or survival ($p>0.05$). Therefore it was concluded that the proliferative activity as measured by Ki-67 antibody was not related to clinicopathology and prognosis in colorectal cancer.¹⁹ This is supported by the reports from Shepherd *et al*²⁰, Kyzer *et al*²¹ and Sahin *et al*²² who reported no relation between Ki-67 immunoreactivity and various clinicopathological and prognostic variables in cases with colorectal carcinomas. Explanation for these discrepancies could be a difference in epitope preservation, in staining procedures, in methods of evaluation and quantification of Ki-67 immunostaining and in study population. Investigators have suggested that this lack of correlation is due to the considerable heterogeneity in colorectal carcinomas.^{23,24}

As a result of this work it is proven that Ki-67 labelling index is high in well to moderately differentiated, non-mucinous adenocarcinomas in an early Dukes' stage (A or B) as compare to poorly differentiated, mucinous adenocarcinomas or signet-ring cell colorectal carcinomas in an advanced Dukes' stage (C).

CONCLUSION

As the result of this study it is concluded that in colorectal carcinoma, immunohistochemical technique employing the Ki-67 antigen is simple and applicable to surgical specimens; and reproducibility with MIB-1 antibody staining is an excellent even when paraffin-embedded tissue sections are used. Ki-67 proliferative index is high in well and moderately differentiated non-mucinous adenocarcinomas in an early Dukes' stage (A or B). On the other hand, Ki-67 proliferating activity is low in poorly differentiated, mucin secreting tumours in an advanced Duke's stage. Our results revealed a close association between histological grade, type and stage of colorectal carcinomas.

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