Correlation of Ki-67 Proliferating Index with Histological Types and Characterization of Mucin in Colorectal Carcinoma

*Sultana S,[1] Islam N,[2] Kabir E[3]

Abstract

Background: Colorectal carcinoma is a major cause of cancer associated with a high rate of morbidity and mortality in the western world. One of the pathologic features considered to be important for prognosis is mucin production. Many authors confirmed that colon carcinomas with high mucin content tend to recur locally and carry a poor prognosis.

Aim*:* Correlation of Ki-67 proliferating index with different type of colorectal carcinoma as well as characterization of mucin.

Method: This cross sectional study was conducted at Sir Salimullah Medical College, Department of pathology from July 2014 to June 2016. Ninety eight patients with colorectal carcinoma was enrolled in this study who underwent surgical resection of colon, adenocarcinomas. For histological classification we used the WHO recommendation (2000) and to be more accurate we sub-classified mucinous adenocarcinomas by morphometrical analysis in three categories: pure mucinous, with extracellular mucin more than 80% of the tumoral volume; mixed type, with 50–80% extracellular mucin; and mixed type with less than 50% extracellular mucin and their correlation with Ki-67 proliferating index . For histochemical investigation, we used stains such as: D- PAS and Alcian Blue. A technique of manual tissue array was employed to see Ki-67 expression by IHC method. Ki-67 is a proliferation associated nuclear antigen which can be recognized by MIB-1 monoclonal antibody.

Result: It was observed that Ki-67 labeling index was high in nonmucinous tumor compared to mucinous adenocarcinoma and signet ring cell carcinoma which is statistically significant ($P<0.05$). Histochemical stain of mucin where both D-PAS and Alcian Blue positive cases(mixed type) are more than the Only D-PAS positive cases(pure type). Ki-67 proliferating index was also high in mixed type mucinous adenocarcinoma (<50%) compared to pure (>80%) and mixed type (50-80%). The result was statistically significant ($p<0.05$). Correlation of Ki-67 proliferating index with histologic type as well as mucin characterization and thereby provide information to clinician to better understanding of the treatment as well as prognosis.

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1. **Dr. Sahela Sultana, MBBS. MD, Assistant Professor, Department of Pathology, Ibrahim Medical College, Dhaka. sultana.sahela83@gmail.com*
2. *Prof. Dr. Nasimul Islam. MBBS. M. Phil., Professor & Head, Department of Pathology, Anwar Khan Modern Medical College & Hospital*
3. *Prof. Dr. Enamul Kabir. MBBS. M.Phil. MSc(Path), Professor, Department of Pathology, Popular Medical College, Dhaka*

**For Correspondence*

Introduction

Colorectal carcinoma is the most common malignancy of GI tract and is a major cause of morbidity and mortality worldwide.[1] It 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] 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.[5]

One of the histological feature of colorectal carcinoma with important impact on prognosis and therapeutic management of these patients are mucin production by the tumor.[6] Mucinous tumors and signet-ring cell tumors are associated with different clinicopathological features and outcomes compared to non-mucinous tumors, suggesting that biologic behavior differs in more than just the histological appearance.[7]

Most colorectal cancers are adenocarcinomas of which mucinous and signet ring adenocarcinoma constitute approximately 10%, with signet ring carcinoma comprising 1%–2.4%.[8] Development and progression of colonic cancers are well associated with abnormal expression of mucins.[9]In the normal colonictissues, the epithelium is covered by a mucous layer, which partly consists of secreted mucins and the function is to lubricant on surfaces to protect them from friction, erosion, harmful substances, unfavorable conditions and pathogens.[9,10] The mucosubstances secreted by the human colon and rectum appear to differ from the normal, both quantitatively and qualitatively, in various diseases.[11]

Mucinous cancers are defined histologically by the presence of abundant extracellular mucin, with more than 50% of the tumor mass being mucinous.[12] The impact of mucinous histology on prognosis is controversial, while it is associated with more advanced stage, metastases and recurrence.[13] Signet ring cell (SRC) carcinoma is a distinct clinical and histopathological subtype of colorectal cancer, with aggressive behaviour.[14] Signet ring cell carcinoma is rare, with a reported prevalence of 0.6–1.1%.[15,16,17,18]

According to WHO digestive tract tumors classification mucinous adenocarcinoma is a subtype of colorectal carcinoma with more than 50% of the lesion composed of mucin and characterization of mucin by special stain (D-PAS and Alcian blue) helps in detecting either pure mucinous or mixed type.[19]

Clinically, pure mucinous forms are detected mainly in advanced stages. Tumor with high mucin content tends to recur locally and carry a poor prognosis.[19] Mucinous tumors and signet-ring cell tumors are associated with different clinicopathologic features and outcomes compared to non-mucinous tumors, suggesting that biologic behavior differs in more than just the histological appearance.[7]

However, there are controversies regarding expression of Ki-67 with histological type in colorectal carcinoma. The proliferative activity as measured by Ki-67 antibody is closely associated with histological type.[2]

In histological type of colorectal carcinoma, observed that proliferative index is high in non mucinous adenocarcinoma.[2]

But Ki67 proliferating activity is low in mucin secreting tumour.[20]It was correlated with the study reported by the Hideki Ishida et al.[20]

So, this study is aimed to find out the possible correlation of Ki-67 proliferating index with histologic type as well as characterizationof mucin and thereby provide information to clinician regarding its prognosis.

Methods

This cross sectional study was conducted among the 98 histopathologically diagnosed patients having colorectal carcinoma over a period of two yearsfrom July 2014 to June 2016, in the department of Pathology, Sir Salimullah Medical College. Study populations were the patients having colorectal cancer underwent surgical treatment in the department of surgery of Sir Salimullah Medical College. The representative sections were submitted for Immunohistochemical staining. The following prediluted primary antibody was used Ki 67. The Ki 67 immunostaining were performed according to manufacturer's recommendation, using the MIB-1 clone (DAKO, Carpenteria, CA & Ventena Medical System, Tucson, AZ). Ki-67 immunostainined slides were examined via light microcopy. Positive Ki 67 staining was observed brown granular nuclear staning. For Ki 67 scoring the most positive area of the tumor was selected avoiding foci of inflammation. The numbers 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.

For special staining tissues were predigested with diastase, because PAS (peroidic acid-Schiff) stains glycogen as well as mucins. So when tissues were pre-digested with diastase it removed glycogen.

D-PAS was used to detect the mucin and Alcianblue for characterization. Immunostaining was done by MIB-1 antibody for Ki-67 antigen in a paraffin embedded tissue section. We followed WHO classification of mucinous adenocarcinoma of digestive tract tumor. Colorectal adenocarcinoma with more than 50% of the lesion composed of mucin and to be more accurate sub-classification of mucinous adenocarcinomas was done in three categories:[19]

1. Pure mucinous, with extracellular mucin more than 80% of the tumoral volume
2. Mixed type, with 50-80% extracellular mucin
3. Mixed type with less than 50% extracellular mucin.

Alcian Blue and D-PAS staining were used to differentiate between acid and neutral mucins.After staining with D-PAS, mucin containing tumor that is neutral mucin stained into magenta red indicate positive staining and Alcian blue which stained acidic mucin into deep blue.

Result

Ki-67 proliferating index with mucin expression

Out of 98 cases, regarding mucin expression by the tumor it was observed that forty seven cases (47.9%) were non mucinous adenocarcinoma and their mean Ki67 was 48.77 ± 11.38. Mucinous cases were also forty seven (47.9%) with mean Ki-67 was 43.30 ±18.26. Only four cases (4.3%) were signet ring type with mean ± SD 15.00 ±10.00. There was significant difference in Ki-67 proliferating index in tumor with mucin

expressions and the result was statistically significant (P<0.05). It was also observed that Ki-67 proliferating index was high in non-mucinous adenocarcinoma when compared with mucinous and signet ring type adenocarcinoma. ANOVA test was done to measure the level of significance (Table I).

Table I: Correlation of Ki-67 proliferating index with histological types

Mucin expression	Frequency (n %)	Ki-67 expression (Mean ± SD)	P value
Mucinous	n=47(47.9%)	43.30 ± 18.26	0.001*
Signet ring cell	n=4(4.3%)	15.00 ± 10.00	
Non mucinous	n=47(47.9%)	48.77 ± 11.38	

ANOVA test was done to measure the level of significance.

Characterization of mucin with D-PAS and Alcian blue

In this study, mucin characterization of the tumor was determined by D-PAS and Alcian blue where maximum forty nine cases (83.05%) were both PAS and Alcian blue positive and two cases (3.92%) were only PAS positive. No alcian blue positive case was found (Table II).

Table II: Characterization of mucin with D-PAS and Alcian blue

Mucinous tumor	Frequency (n %)
BothD- PAS and Alcian Blue positive cases	n = 49 (83.05%)
OnlyD-PAS positive cases	n=2 (3.92%)
Only Alcian blue positive cases	n= 0

Correlation of Ki-67 proliferating index with characterization of mucin

In this study mucin secreting tumors were characterized as pure mucinous (>80% mucin), mixed type (50%-80% mucin) and mixed type (<50% mucin) and they were grouped into A, B, and C respectively. Only two cases (4.0%) were pure mucinous (>80.0% mucin) and their mean Ki-67 proliferating index was 25.00 ± 7.07. Thirty two cases (64.0%) were mixed having 50%-80% mucin and their mean Ki-67 proliferating index was 37.65 ± 16.50 and lastly 19 cases (38.0%) were mixed having less than 50% mucin whose mean Ki-67 proliferating index was 56.05 ± 15.86. There was significant differences among the groups (A vs B vs C) and the result was statistically significant (p<0.05). When compared in between groups pure mucinous (>80%) with mixed (<50%) (A vs C) and mixed (50%-80%) with mixed (<50%) (B vs C) also having significant differences and the result was statistically significant (P<0.05). In the present study it was also observed that highest proliferating index of Ki-67 was shown in mixed (<50% mucin) when compared with pure mucinous (>80% mucin). One way ANOVA followed by Bonferroni test was performed to compare between groups (Table III).

Table III: Correlation of Ki-67 proliferating index with characterization of mucin

Mucin type	Frequency N (%)	Ki-67 expression Mean SD	p value
Pure (>80% mucin) [A]	2 (4.0%)	25.00 ± 7.07	
Mixed (50%-80% mucin) [B]	32 (64.0%)	37.65 ± 16.50	
Mixed (<50% mucin) [C]	19 (38.0%)	56.05 ± 15.86	
Statistical analysis			
A vs B vs C			0.001*
A vs B			0.862ns
A vs C			0.038*
B vs C			0.001*

ANOVA followed by Bonferroni test was performed to compare between groups.

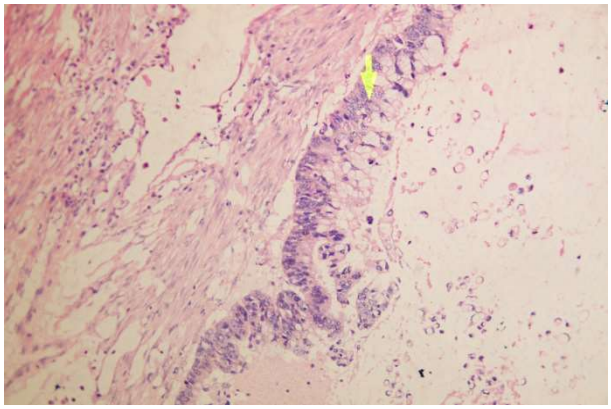


Figure 1. Photomicrograph of histopathological section of mucinous adenocarcinoma of colon (Signet ring type) stained by H& E method (x100).

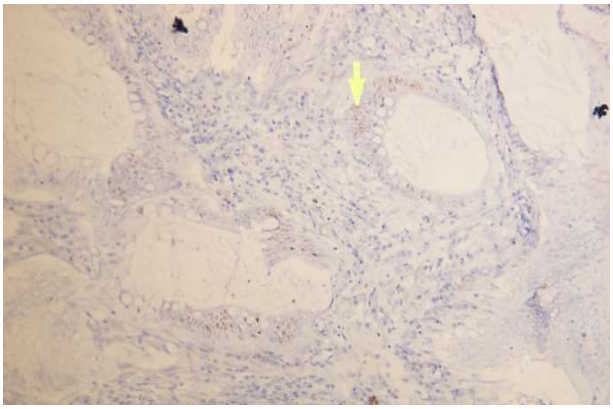


Figure 3. Photomicrograph of mucinous adenocarcinoma (Signet ring type) stained with Ki-67 immunostain showing low Proliferative Index (x100).

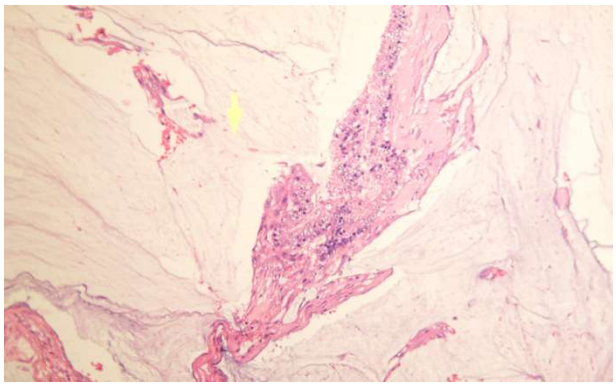
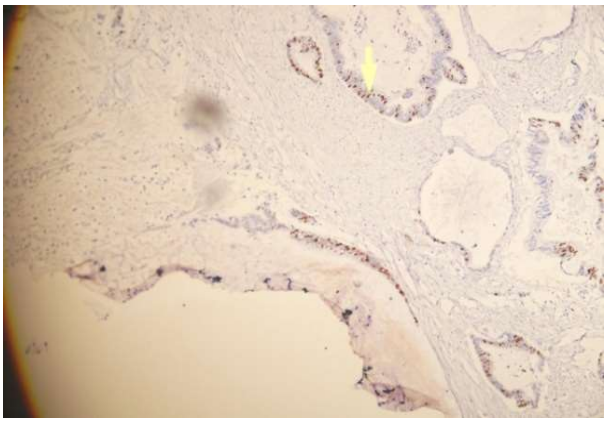


Figure 2. Photomicrograph of histopathological section of mucinous adenocarcinoma of colon stained by H& E method (x100).



Figurre 4. Photomicrograph of mucinous adenocarcinoma Stained with Ki-67 immunostain showing low Proliferative Index (x100).

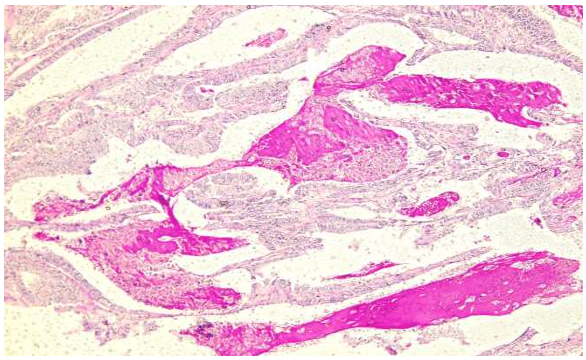


Figure 5. Photomicrograph of well differentiated mucinous adenocarcinoma stained with D-PAS and Alcian blue showing mucin.(X100) (Pure mucinous >80% mucin).

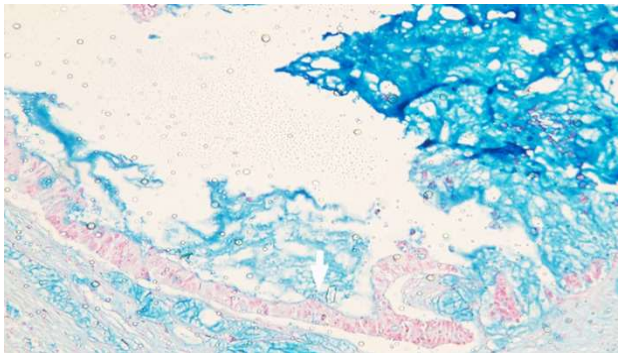


Figure 6. Photomicrograph of well differentiated mucinous adenocarcinoma stained with D-PAS &Alcian blue (PH 2.5) showing mucin (X400) (Mixed type 50-80% mucin).

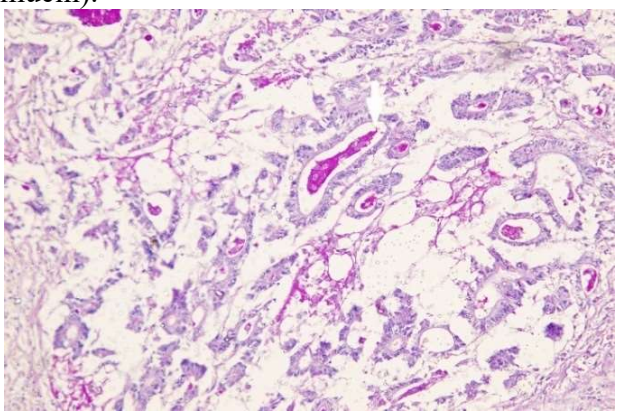


Figure 7. Photomicrograph of well differentiated mucinous adenocarcinoma stained with
D-PAS & Alcian blueshowing mucin (X100) (Mixed type less than 50%).

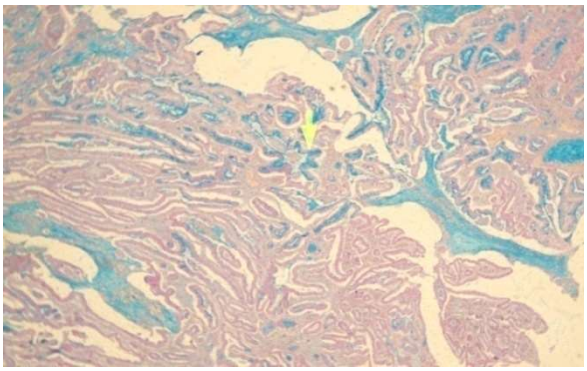


Figure 8. Photomicrograph of well differentiated mucinous adenocarcinoma stained with D-PAS & Alcian blue (PH 2.5) showing mucin (Mixed type 50-80% mucin) (X100) .

Discussion

Regarding the histological type of colorectal carcinoma in this study, it was observed that Ki-67 labeling index was high in nonmucinous tumor (48.77 ± 11.38) compared to mucinous adenocarcinoma (43.30 ± 18.26) and signet ring cell carcinoma (15.00 ± 10.00).

The findings that mucinous carcinoma and signet ring cell mucinous adenocarcinoma showed low proliferative activity was in agreement with the study by Ishida et al. (2003) and Uzma et al. (2008).[2,20] They found high expression of Ki-67 in non-mucinous adenocarcinoma than mucinous adenocarcinoma and signet ring cell carcinoma.[2,20] The result in present study was in contrast with that of Lanza et al. (1990) where Ki-67 expression was found higher in mucinous adenocarcinoma compared to non-mucinous.[21]

Regarding characterization of mucin in this study, mucin expression of the tumor was determined by special stain D-PAS and Alcian blue. Maximum forty nine cases (83.05%) were both D-PAS and Alcian blue positive and two cases (3.92%) were only D-PAS positive (Table-II). These findings were similar to study conducting by Ionila et al.

where predominant cases were both D-PAS and Alcian blue positive 67% (Mixed type) in relation to either D-PAS or Alcian blue positive 32 % (pure type).[19]

Our study revealed a significant relation between Ki-67 PI and characterization of mucin expression ($P<0.05$). As far knowledge goes, no study has been carried out in past in Bangladesh regarding the correlation between Ki-67 expression and characterization of mucin.

It is concluded that Ki-67 labeling index is high in non-mucinous adenocarcinomas compare to mucinous and signet ring cell adenocarcinomas. Ki-67 proliferating index was high in mixed type mucinous adenocarcinoma (<50%) compared to pure (>80%) and mixed type (50-80%). It is also observed that Ki-67 PI is low in pure mucinous type of adenocarcinoma.
Clinical and histological features suggestive of aggressive behavior and poor prognosis were more frequently observed in mucinous adenocarcinoma.[22] Clinical pure mucinous forms were detected mainly in advanced stages, but in terms of lymph node metastasis rate, they were secondary after mixed type with 50–80% extracellular mucin.[19] The beneficial role of characterization of mucin in colon carcinomas is that it helps in prognosis and survival and the tumor with high mucin content and pure mucinous form tend to recur locally, more aggressive and carry a poor prognosiss.[19] Signet-ring cell type CRC are rare with an incidence ranging between 0.9% to 4%, associated with a poorer outcome compared to adenocarcinoma and mucinous CRC.[23]

Conclusion

Ki-67 proliferative index is high in non-mucinous adenocarcinomas. It is also high in mixed type with mucin content less than 50%. On the other hand, Ki-67 proliferating activity is low in mucin secreting signet ring tumours, mixed type with mucin content 50-80% and pure mucinous more than 80% mucin. Thus Ki-67 proliferating index and characterization of mucin can be useful in a patient with colorectal carcinoma as an 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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