Immunohistochemical Evaluation of Ki67 Expression in Premalignant and Malignant Esophageal Lesions - Study of 54 Cases in a Tertiary Care Hospital

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Abstract

Background: In Bangladesh esophageal carcinoma is the leading malignant condition. Mortality rate of esophageal cancer is very high. Like other malignancies, early detection offers the best prognosis for esophageal carcinoma. Regular endoscopic and histologic surveillance have become standard procedure for follow up of patients with premalignant lesions. But sometimes histomorphological diagnosis on routine biopsies can be challenging for pathologist. Several studies showed that positive Ki67 expression is significantly associated with higher grade of esophageal lesions and is a potential prognostic marker.

Objectives: The aim of the present study was to evaluate Ki67 expression in each histopathological category and to investigate the relationship of Ki67 with premalignant and malignant esophageal lesions.

Methods: This study was a cross sectional study, carried out at the department of Pathology in Dhaka Medical College and Hospital from March 2018 to February 2020. Total 54 cases of esophageal biopsy were included in this study. They were divided in maligant and premalignant category. All obtained samples were selected for routine histopathological study and immunostaining for Ki67 was done in formalin fixed paraffin embedded tissue.

Results: Most of the patients were male and mean age was 55.04 (±15.48) years. Squamous cell carcinoma was the most common malignancy seen in (26 cases) 48.15 % of patients and Barrett's esophagus was common (13 cases, 24.07%) premalignant lesion. Ki67 expression was found statistically significant ($p<0.05$) when correlated with histomorphologic grading of premalignant and malignant lesions. Calculation result of malignant and premalignant category as a whole should be stated.

Conclusion: Ki67 expression positively correlates with histomorphologic grading of premalignant and malignant esophageal lesions.

[Journal of Histopathology and Cytopathology, 2023 Jan; 7 (1):3-11]

Keywords: Barrett's esophagus, esophageal carcinoma, Ki67

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Introduction

Esophageal carcinoma is in the seventh position among all the malignancies and there is an estimation of 572,000 new cases and 509,000 deaths in 2018 in the world.[1] It is the leading malignant condition in Bangladesh. In 2018 the incidence of esophageal carcinoma in Bangladesh was 20,906 (13.9%) and death rate was 19,357 (17.9%).[2] Esophageal carcinoma is more common in male and the male: female ratio is about 3:1.[3]

In esophagus, common premalignant lesions include Barrett's esophagus (BE) and squamous intraepithelial neoplasia. Squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma, small cell carcinoma, undifferentiated carcinoma are common among the malignant lesions. Among these, squamous cell carcinoma (SCC) is the most common type of esophageal cancer in the Indian subcontinent .[4]

Like other malignancies, early detection offers the best prognosis for esophageal carcinoma.[5] In majority of cases, due to advanced stage of the disease remedial treatment cannot be undertaken at the time of diagnosis.[6] In spite of recent advances in chemotherapy and surgery, the 5-year survival rate remains at the low level. It is due to the lack of early diagnosis, invasion and metastasis of the tumor, and over-reliant on confined drugs with some side effects and resistance, thus producing unsatisfactory results. Therefore, it is very important to discover a new agent to improve the life expectancy of patients with esophageal cancer.[7]

Esophageal squamous intraepithelial neoplasia (ESIN) has been widely recognized as a precursor lesion for esophageal squamous cell carcinoma (ESCC). According to 2000 World Health Organization, ESIN can be classified into two types based on the severity of cytologic and architectural atypia.[5] They are low grade (LGESIN) and high grade (HGESIN). Many studies have illustrated that there is a multistage tumor development from basal cell hyperplasia to ESIN and finally into an invasive ESCC.[8] As because Barrett's esophagus and esophageal adenocarcinoma sharing similar genetic mutations, so that Barrett's esophagus supposed to be a precursor lesion of esophageal adenocarcinoma.[9] Among patients with high grade dysplasia, the crude incidence of esophageal adenocarcinoma ranges from 5.6% to 6.6% per year.[10]

Regular endoscopic and histologic surveillance have become standard procedure for follow up of patients with premalignant lesions.[11] But the messages from histomorphology of biopsy specimen with Hematoxylin and Eosin (H&E) stain are sometime not sufficient for a conclusive result. So that the immunohistochemical analysis of tissue can give the clues about the cell proliferation and progression of the tumor.

Ki67 is a marker of cell proliferation. It is present in all periods of the cell cycle G1, S, G2 and mitosis, and absent in the G0 phase. Thus, Ki67 is an excellent marker for proliferating cells except for G0.[5]Elevated Ki-67 expression is associated with a high mitotic index and high histological grade.[7] Ki67 can be an important biomarker to identify early stages of esophageal carcinogenesis.[12] Ki67 is a sensitive protein associated with cell proliferation thus is a potentially attractive therapeutic target in malignancy.[13] The main hallmarks of malignancy result from the uncontrolled cell cycle and evading apoptosis. Recently there are some clinical trials where some therapeutic drugs which can arrest the cell cycle and promote apoptosis in cancer cells

and also can reduce cell proliferation in a dose dependent manner. [7]
Limited studies have been done regarding Ki67 expression in premalignant and malignant esophageal lesions. The aim of the present study is to evaluate Ki67 expression in each histopathological category and investigated the relationship between Ki67 and premalignant and malignant esophageal lesions.

Methods
This cross sectional study was carried out at the department of Pathology in Dhaka Medical College and Hospital from March 2018 to February 2020 which enrolled 54 histopathologically diagnosed premalignant and malignant esophageal lesions. Cases of both sexes and all age groups were included in this study. The specimens were excluded if there any fragmented and tiny tissue unsuitable for immunohistochemistry or any history of oncological treatment or carcinoma on other sites.

Hematoxylin & eosin stained sections of each cases were reviewed to confirm the histological diagnosis and WHO histological grading. Then according to WHO defined criteria all malignant cases were categorized into well, moderate and poorly differentiated groups and all premalignant lesions categorized according to dysplasia.

For immunohistochemistry staining 4-micrometer thick tissue sections were taken on poly –L lysine coated slides from the paraffin blocks of tumor. Monoclonal mouse anti-Human Ki67 antigen (1:100, Clone: MIB-1, Dako®, Carpinteria, CA, USA) was used as primary antibody. Envision (ready to use DAKO) was used for Ki67 as secondary antibody.
Lymphoid follicle of tonsilar tissue used for positive control. Cells that displayed a brown nuclear stain were considered to be Ki67 positive. The final result was assessed by the proliferation index for every case, which is calculated from the average of stained cells in relation to the total analyzed cells, with a minimum count of 500 cells. Negative expression= 0% - 25%, Positive expression =26% - 100%.[5]

Results
A total of 54 cases were included in this study. Among them 40 cases were male (74.1%) and 14 cases were female (25.9%) and the male female ratio was 2.85:1. Age of the study patient range from 21 to 95 years. Most of the cases (25 cases, 46.3%) were found in 41-60 years of age. Mean age of the patients was found 55.04 (±15.48) years (Table I). Five histomorphological types were diagnosed in this study. It was observed that squamous cell carcinoma was found in 26 patients (48.15%) followed by adenocarcinoma in 11 patients (20.37%). Adenosquamous carcinoma was seen only in 1 patient (1.85%). Premalignant lesions include Barrett's esophagus (13 patients, 24.07%) followed by squamous intraepithelial neoplasia in 3 patients (5.56%) (Table II).The mean Ki67 labeling index was found 11.75±8.75 in negative for dysplasia, 22.5±5 in indefinite for dysplasia and 30±NA in low grade dysplasia. Ki67 expression was negative in 16.66% (2 cases) and positive in 25% (1 case) in squamous intraepithelial neoplasia cases. The difference was statistically significant (p=0.0487) among these groups (Table III). Mean Ki67 score of well differentiated was 28.17±3.71, 46.92±12.00 in moderately differentiated and 57.00±23.07 in poorly differentiated. The association between Ki67 expression and three grades of esophageal carcinoma were found statistically significant (p=0.006) (Table IV). The scatter diagram shows the relationship between Ki67 expression and premalignant & malignant lesions of esophagus. Ki67 percentage was higher for

malignant patients than that of pre-malignant patients. Pearson correlation coefficient test shows a positive correlation (r=0.367) between Ki67 expression and premalignant & malignant lesions of esophagus (Figure 1).

Table I: Distribution of study patients according to age (n=54)

Age	Number	Percentage
21-40	11	20.40
41-60	25	46.30
61-80	16	29.60
>80	2	3.70

Table II: Distribution of study patients according to histomorphology (n=54)

Histomorphological type	Number	Percentage
Barrett's esophagus	13	24.07
Squamousintraepithelial neoplasia	3	5.56
Squamous cell carcinoma	26	48.15
Adenocarcinoma	11	20.37
Adenosquamous carcinoma	1	1.85

Table III: Comparison between Ki67 expression and premalignant lesions (n=16)

Premalignant lesions	Ki67		Mean±SD	p-value
	Negative (%)	Positive (%)		
Premalignant lesion for adenocarcinoma (Barrett's esophagus)				
Negative for dysplasia	7 (58.33)	1(25)	11.75± 8.75	
Indefinite for dysplasia	3 (25.00)	1(25)	22.50± 5.00	0.0487[s]
Low grade dysplasia	0(0.00)	1(25)	30.00± NA	
High grade dysplasia	--	--	--	
Premalignant lesion for squamous cell carcinoma (Squamous intraepithelial neoplasia)				
Low grade dysplasia	2 (16.66)	1 (25)	16.66±6.07	
High grade dysplasia	--	--		
Total	12(100)	4(100)		

NA= Not Applicable
p-value= 0.0487 (s=significant) (p value reached from ANOVA test)

Table IV: Ki67 expression according to histomorphologic grading in malignant cases (n=38)

Grading of esophageal carcinoma	Ki67			Mean±SD	p-value
	Negative (%)	Positive (%)	Total (%)		
Well differentiated	5 (41.66)	7 (58.33)	12(100)	28.17±3.71	
Moderately differentiated	6 (31.58)	13 (68.42)	19(100)	46.92±12.00	0.006[s]
Poorly differentiated	2(28.57)	5 (71.43)	7(100)	57.00±23.07	

p-value = 0.006 (s=significant)
(p value reached from ANOVA test)

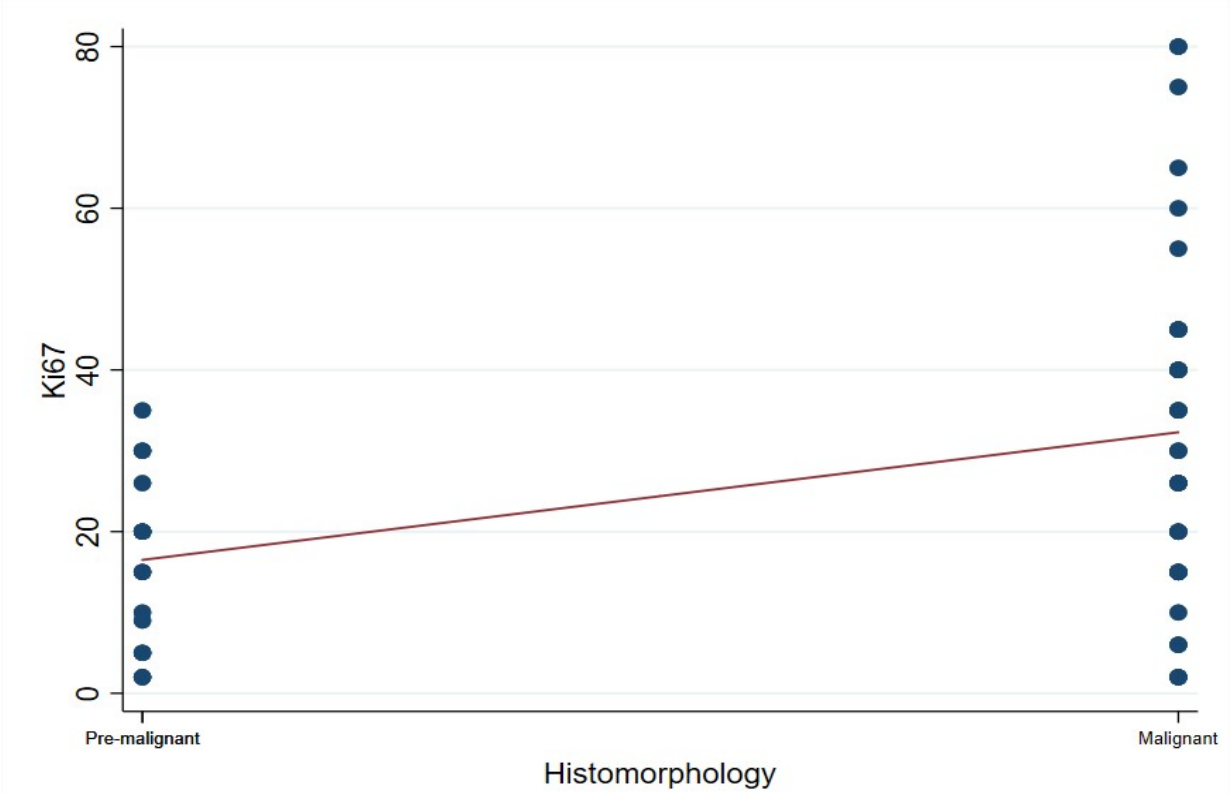
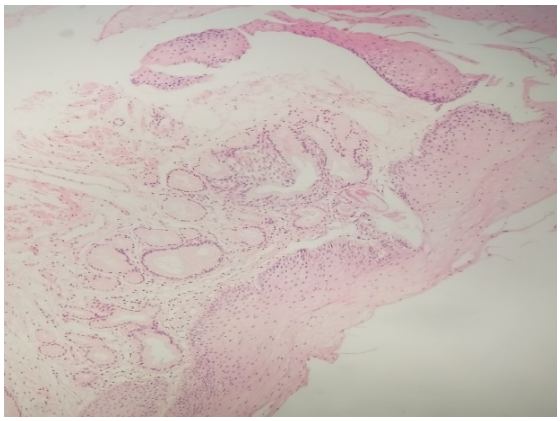
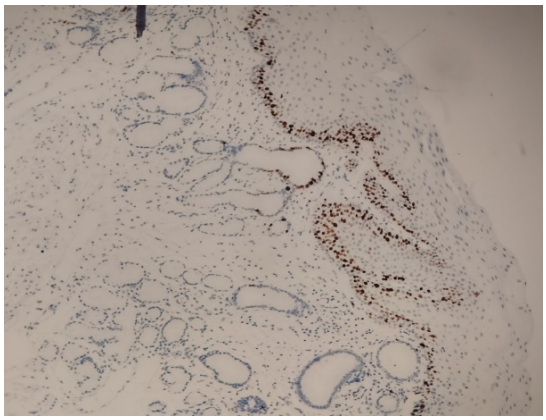


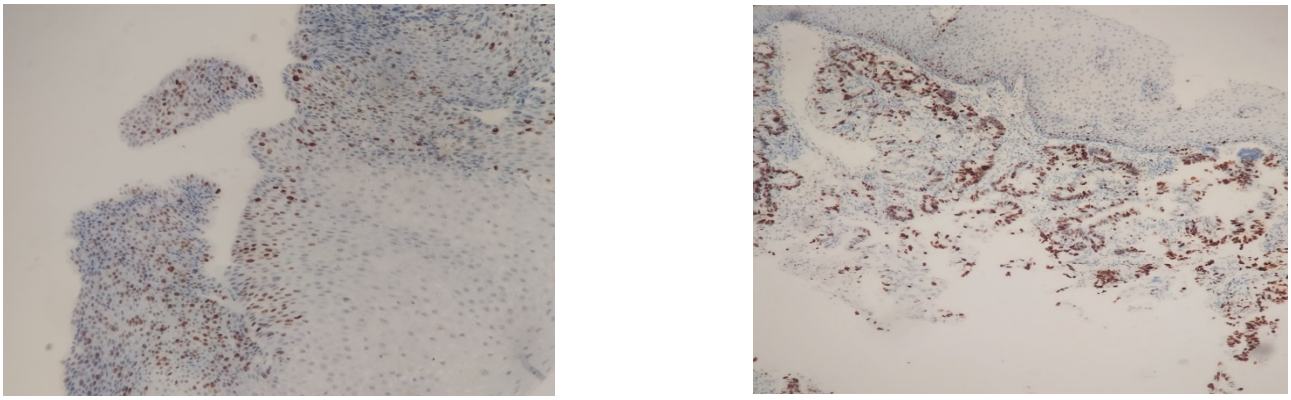
Figure 1. Correlation between Ki67 expression and premalignant & malignant lesions of esophagus (n=54).
r= 0.367 (positive correlation)
r-value reached from Pearson correlation coefficient





A B

Figure 2. A. Photomicrograph showing Barrett's esophagus with negative for dysplasia (Case No:04, H&E X10). B. Photomicrograph showing negative Ki67 in same case(IHC for Ki67 X10)



A B

Figure 3. A. Photomicrograph showing positive Ki67 in squamous dysplasia, low grade (Case No:18, IHC for Ki67, X20). B. Photomicrograph showing positive Ki67 in well differentiated adenocarcinoma (Case No: 1, IHC for Ki67, X10)

Discussion

Esophageal carcinoma is one of the most deadly form of malignant condition in gastrointestinal tract. Despite of recent advances in chemotherapy and surgery, this condition is extremely aggressive in nature and shows low survival rate, mostly 5-years. Lack of early diagnosis, invasion and metastasis of the tumor at the time of diagnosis and over-reliant on confined drugs with some side effects and resistance are said to be the causes of producing unsatisfactory results.[7]More than half of the patientshaving dysplasia progress to carcinoma and a cancer-associated lesion.[14]

In this present study, it was observed that the age of majority cases (46.3%) was between 41-60 years. The mean age of the study population was 55.04 (±15.48) years, ranging from 21 to 95 years (Table I) and almost similar to the study of Elkareem et al. (2015) where age ranged between 14 to 89 years with mean age 55.9 years.[20] Mean age 53.7 years was found by Samarasam (2017) which is close to our study.[3]

In this study, 40 cases were male (74.1%) and 14 cases were female (25.9%) and the male female ratio was 2.85: 1. Study done by Khattab et al. in 2013 observed that male-to-female ratio was 3:1 which is almost similar to our study.[15] Samarasam (2017) also observed in their study that, there were 99 male patients and 39 female patients with the ratio of 3:1.[3]

In this study, five histomorphological types were diagnosed in premalignant and malignant esophageal lesions. It was observed that out of three types of malignant lesions, squamous cell carcinoma was found in 26 patients (48.15%) followed by adenocarcinoma in 11 patients (20.37%). Premalignant lesions including cases of Barrett's esophagus were found in 13 patients (24.07%) followed by squamous intraepithelial neoplasia in 3 patients (5.56%) (Table II).Study done by Shil et al. (2010) in Bangladesh, stated that squamous cell carcinoma was the most common malignancy followed by adenocarcinoma.[6] A similar finding of common cases of squamous cell carcinoma in esophagus was observed by Samarasam (2017).[3] A study done by Xu et al. (2002) were found 59 cases of premalignant lesions and 218 cases of esophageal cancer from the Cancer Hospital, China.[12] Among

the premalignant lesions, there were 28 cases of mild dysplastic lesions and 31 cases of severe dysplastic lesions. Of these esophageal cancers, there were 169 cases of squamous cell carcinoma (SCC), 29 cases of adenocarcinoma (AC) and 20 cases of adenosquamous carcinoma.

In current study, the mean value of Ki67 expression varied among different histomorphologic grading for dysplasia in BE. The mean Ki67 labeling index was found 11.75±8.75 in cases with negative for dysplasia, 22.5±5 in cases with indefinite for dysplasia and 30±NA in low grade dysplasia. Ki67 expression was negative in 2 cases (16.66%) and positive in 1 case (25%) in squamous dysplasia cases. The difference was statistically significant ($p<0.05$) among these groups (Table III). These results were supported by some other studies done by Trakal et al. (2010) and Kerkhof et al. (2008).[17,18]In a study by Feith et al. (2004) and Hong et al. (1995) also reported Ki67 proliferation fraction significantly increased from normal squamous epithelium to columnar metaplasia to dysplasia.[19,11]

In the current study, Ki67 expression was compared among three grades of esophageal carcinoma. Mean Ki67 score of well differentiated esophageal carcinoma was 28.17±3.71, 46.92±12.00 in moderately differentiated carcinoma and 57.00±23.07 in poorly differentiated carcinoma. The association between Ki67 expression and three grades of esophageal carcinoma were found statistically significant (p=0.006)n(Table IV). A similar study was done by Elkareem et al. in 2015, they found that Ki67 expression increases with grade of tumor.[20] This result was also consistent with the study of Huang et al. (2005).[16] They reported that there was significant correlation between Ki67 index and the histological grade of tumor.

In this study Ki67 expression was found higher for malignant patients than that of pre-malignant patients. The mean Ki67 expression of premalignant lesions were 16±10.44 and malignant lesions were 34.58±22.29. The Pearson correlation coefficient (r=0.367) showed a positive correlation between Ki67 expression and premalignant & malignant lesions of esophagus. This finding was similar to the observation noted by Khattab et al. (2013).[15] They showed that mean Ki67 expression was higher in malignant lesions and lower in premalignant lesions. Another study done by Feith et al. in 2004 showed that mean Ki67 expression was more in malignant lesions.[19]

Although this study showed that Ki67 expression was higher in malignant cases then premalignant cases, it faced some limitations. It was not possible to follow up the patients and it would give more appropriate information if larger number of patients from multiple centers all over the country were included.

Conclusion

Ki67 expression was significantly raised in malignant cases then premalignant cases and showed a positive correlation between Ki67 expression and premalignant & malignant lesions of esophagus. Immunohistochemistry of Ki67 in esophageal biopsy may be included in some selected cases where histopathological assessment cannot give a conclusive result. However, further studies with expression of Ki67 in premalignant and malignant esophageal lesions may improve the result.

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