Expression of Ki-67 along with ER, PR in Relation with Ductal Carcinoma of Breast

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Abstract

Background: Breast carcinoma has become the most common malignancy in the female, affecting one in eight women and is one of the leading causes of mortality among women in Western countries. In Bangladesh, the incidence rate of breast cancer was about 22.5 per 100000 in females. The National Institute of Cancer Research and Hospital in Bangladesh (2010) showed that 21% of total number of death among women between 15 and 49 years of age was due to breast cancer. The most important prognostic factor of breast carcinoma is the tumor size, histological grade and lymph node status. Biomarker expression in breast cancer is used as a prognostic indicator and predictor of response to hormonal and chemotherapy. The leading parameters are steroid receptors estrogen receptor (ER), progesterone receptor (PR), Ki-76.

Methods: This cross sectional study was conducted at Sir Salimullah Medical College, Mitford. Specimens were collected from Sir Salimullah Medical College Hospital and also from different private laboratories of Dhaka city during the period from January'2014 – December'2015. Among 79 female patients with invasive ductal carcinoma of breast. They were divided into four groups according to age i.e. <30 years, 31-40 years, 41-50 years and >50 years among those 53.2% were premenopausal and 46.8% were postmenopausal. About 16.5% of women diagnosed had a first-degree relatives (mother, sister) with breast cancer.

Results: In this study Most of the patients 50 (59.5%) had tumor sizes 2-5 cm followed by 18 (22.8%) and 14 (17.7%) patients with tumor sizes >5 cm and < 2 cm respectively. Majority of the patients had their tumors fall in 54 (65.9%) grade II followed by 14 (17.1%) and 11 (13.4%) patients in grade I and grade III type respectively. Among 79 patients with invasive ductal carcinoma, 48 (60.8%) patients had positive Ki 67 and their mean Ki67 expression was 29.39 ± 23.80. The correlation of Ki-67 expression between different histological grades of invasive ductal carcinoma showed that with increasing grade of tumour, expression of Ki-67 was also increased. ER, PR and Ki 67 had predictive and prognostic factors and play a crucial role in the treatment of patients with breast cancer. Ki-67 is inversely correlate with ER and PR score. In this study, regarding prognosis of breast cancer by different prognostic markers 48 (60.8%), 44 (100%), 37 (100%) and 19 (24.1%) cases was found positive by Ki67, ER score, PR score respectively. In this study, among 44 ER- positive patients, 21 (47.7%) were Ki-67 positive and 27 (77.1%) were Ki 67 negative. Among 35 ER-negative patients, Ki-67 were positive in 23 (52.3%) and negative in 08 (22.9%). There was association between Ki-67 and ER status. ER positive cases were significantly higher in negative Ki 67 and ER negative cases were significantly high in Ki-67 positive cases. In this study, among 37 PR-positive cases 26 (61.9%) were Ki 67 negative and within 42 PR-negative cases Ki 67 was positive in 15(40.5%) cases.

Conclusion: Ki67 has emerged as a rapid and inexpensive method to detect proliferation of breast cancer and found as an excellent prognostic and predictive marker.

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Introduction

Breast cancer remains the most common cancer among women in Bangladesh. It has become a hidden burden which accounts for 69% of cancer deaths in women.[1] Breast cancer has been reported as the highest prevalence rate (19.3 per 100,000) among Bangladeshi women between 15 and 44 years of age when compared to other types of cancer.

The most important prognostic factor of breast carcinoma are the tumor size, histological grade and lymph node status. Several studies have shown that patients who have involved axillary lymph nodes have much poorer prognosis than those without nodal metastasis.[2] Since breast cancer is one of the hormone dependent tumor, much has been paid to the relationship between ER and PR and breast cancer. ER expression is strongly predictive of response to anti-estrogenic therapy. ER positive tumors have a good 10-years prognosis.

In terms of tumor biology, proliferation has been recognized as a distinct hallmark of cancer and act as an important determinant of cancer outcome.[3,4] Since tumors that exhibit increased proliferation tend to be more aggressive clinically, measures of proliferation are often incorporated into histological grading systems. The simplest and most widely used method is the mitotic count.

In recent years immunohistochemistry for Ki-67 has also been used to determine tumor proliferation. Ki-67 is a nuclear non-histone protein which was first identified after immunizing mice with nuclei of the Hodgkin Lymphoma cell line L428. The name was derived from the city of origin (Kiel, Germany) and the number of the original clone in the 96-well plate.[5,6] The murine monoclonal antibody Ki-67 reacts with a human nuclear antigen that is expressed in G1, S, G2, and mitosis, but not in G0.[5] In breast cancer, a strong correlation has been found between the percentage of cells positive for Ki-67 and nuclear grade and mitotic rate.[7]

Ki-67 is one of the most widely used IHC proliferation antigen and has been confirmed as an independent predictive and prognostic factor in early breast cancer.[8] Many studies have demonstrated the prognostic and predictive value of Ki67 in adjuvant therapy.[9,10]

In Bangladesh, there are a few data on the biological characteristics of breast tumour. In this study it is aimed to find out the possible association between Ki-67 expression with estrogen receptor (ER), progesterone receptor (PR) tumor markers in breast cancer with a view to add valuable information to predict the post operative prognosis of the patient.

Methods

This Cross sectional study was conducted among the 74 histopathologicallydiagnosed patients having invasive ductal carcinoma of breast. The ER, PR immunohistochemistry reports of those patients along with their diagnosed tumour blocks with histopathological reports were collectedin the department of pathology, Sir Salimullah Medical College, Mitford.The representative sections were submitted for Ki-67 immunohistochemicalstaining.

Ki-67 immunostaining were performed according to manufacturer's recommendation, using MIB-1 clone (1:100, DAKO Envision, Glostrup, Denmark). Ki-67 immunostained slides were examined via light microscope. Positive Ki-67 staining was observed as brown, granular nuclear staining. The number of positive nuclei were counted in 1000 tumour cells in a high power field . A Ki-67

cut-off point of 15 % was defined according to previous study.[11]

Results

The totals of patients were 79. They were divided into four groups according to age. Most of the patients 26(32.9%) were within age group of 41-50 years. Over half 53.2% of the patients were premenopausal and rest of the patients were in postmenopausal state. Only 13 (16.5%) patients had positive family history of breast cancer. Rest of the patients did not have an affected family member with breast carcinoma. 50 (59.5%) patients had tumor 2-5 cm in size followed by 18 (22.8%) and 14 (17.7%) patients had tumor size > 5cm and <2 cm respectively.

In this study, most of the cases 54 (65.9%) cases were histopathologically categorized as grade II followed by 14 (17.1%) and 11 (13.4%) cases were categorized as grade I and grade III type respectively (Table II). Among total 79 cases 48 (60.8%) cases showed Ki-67 positivity with mean Ki-67 expression 29.39 ± 23.80. Most of the cases grade II (54) expressed Ki-67 median value 30 followed by grade I (14) and grade III (11) expressed Ki-67 median value 10 and 15 respectively. To measure the correlation of Ki-67 expression between different histological grades of invasive ductal carcinoma Spearman's correlation co-efficient test was performed. Here r_s= 0.160 and p = 0.158. It was seen that with increasing grade of tumour, expression of Ki-67 was also increased (Figure 1).
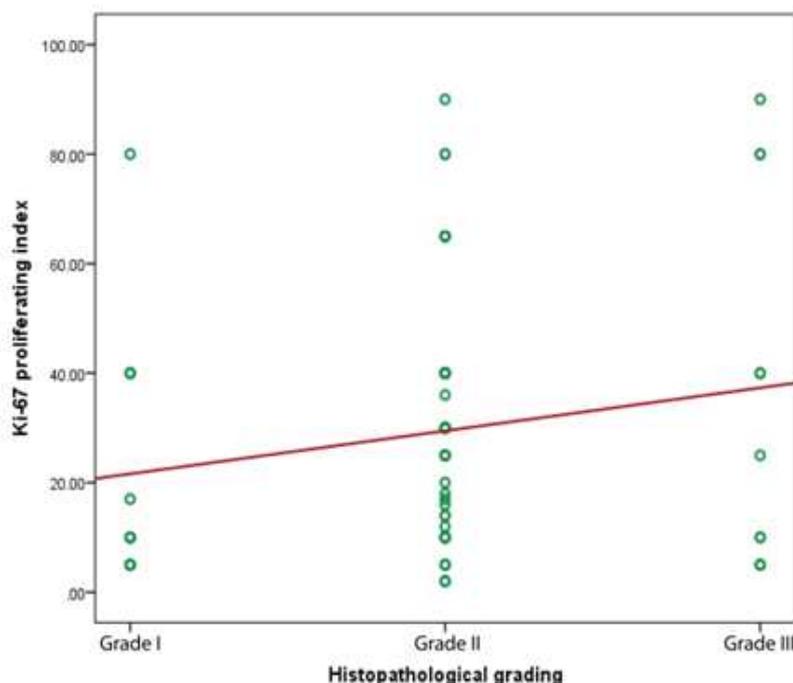

Figure 1. Correlation of Ki67 proliferating index with histological grade.
Spearman's correlation co-efficient test was performed ($r_{s=}$ 0.160 and p= 0.158).
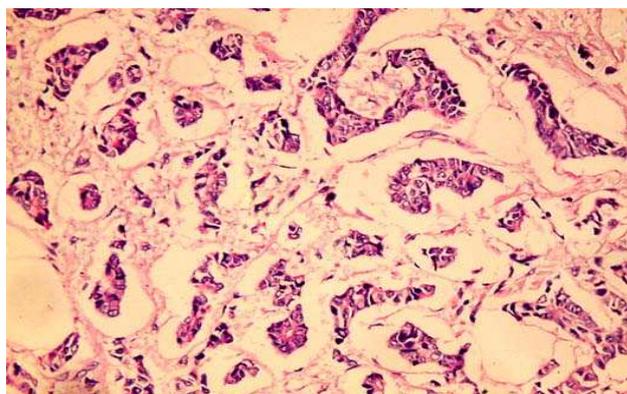
Figure 2. Photomicrograph of histopathological section of invasive ductal carcinoma of breast (Grade I) stained by H and E (x 400), Case 2.
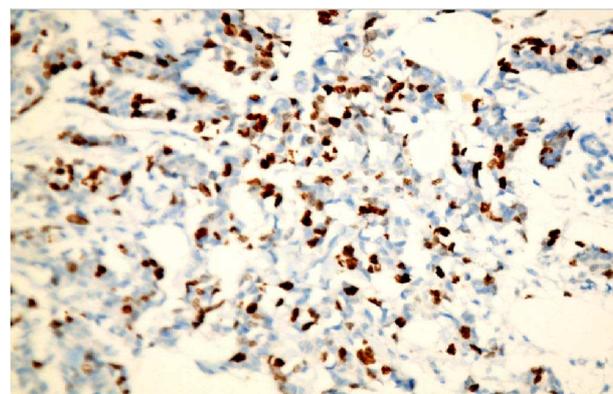

Figure 3. Photomicrograph of invasive ductal carcinoma (Grade I) stained by Ki-67 showing intermediate proliferative index (x 400), Case 02.

The association between Ki-67 and ER status was done. Among 44 ER- positive patients was observed where 21 (47.7%) were Ki-67 positive and 27 (77.1%) were Ki 67 negative. Among 35 ER-negative patients, Ki-67 were positive in 23 (52.3%) and negative in 08 (22.9%) (Table I). Chi-square test was done to measure the level of significance. ER positive cases were significantly higher in negative Ki 67 and ER negative cases were significantly high in Ki-67 positive cases. 0n the other hand 37 PR-positive patients, 22 (59.5%) were Ki 67 positive and 26 (61.9%) were Ki 67 negative. Among 42 PR-negative patients, Ki 67 was positive in 15 (40.5%) and negative in 16 (38.1%) (Table II).
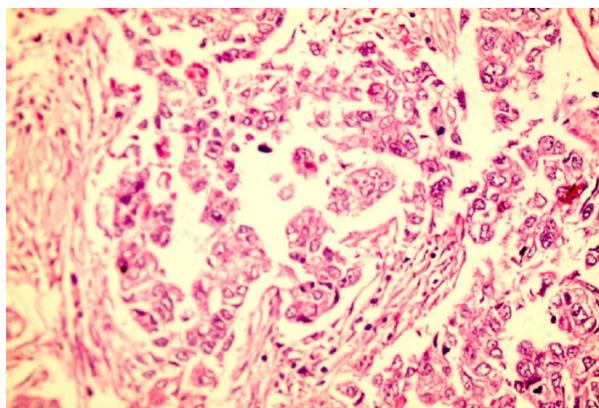

Figure 4. Photomicrograph of histopathological section of invasive ductal carcinoma of breast (Grade II) stained by H and E (x 400), Case 21.
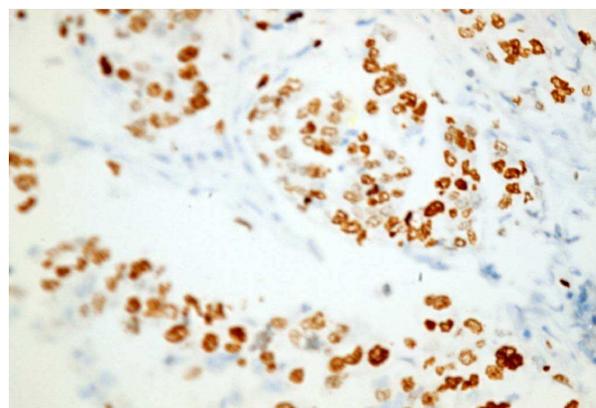

Figure 5. Photomicrograph of invasive ductal carcinoma (Grade II) stained by Ki-67 showing intermediate proliferative index (x 400), Case 21.

Table I: Association of Ki-67 with ER (n=79)

Ki 67	ER status		Total	p value
	Positive n (%)	Negative n (%)	n (%)	
Positive	21 (47.7)	27 (77.1)	48 (60.8)	0.008^s
Negative	23 (52.3)	8 (22.9)	31 (39.2)	
Total	44 (100.0)	35 (100.0)	79 (100.0)	

Chi-square test was done to measure the level of significance. s=significant

Table II: Association of Ki 67 with PR (n=79)

Ki 67	PR status		Total	p value
	Positive n (%)	Negative n (%)	n (%)	
Positive	22 (59.5)	26 (61.9)	48 (60.8)	0.824^{ns}
Negative	15 (40.5)	16 (38.1)	31 (39.2)	
Total	37 (100.0)	42 (100.0)	79 (100.0)	

Chi-square test was done to measure the level of significance.
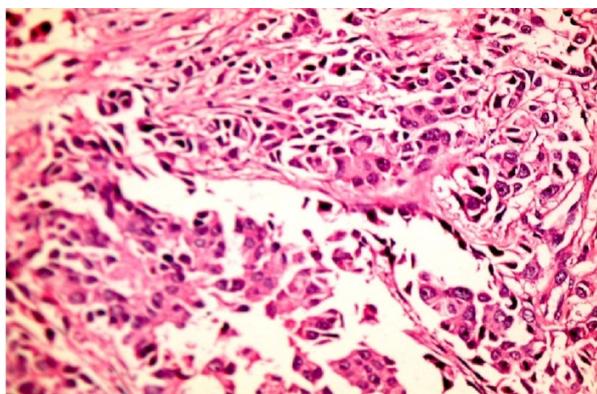ns= not significant.

Figure 6. Photomicrograph of histopathological section of invasive ductal carcinoma of breast (Grade III) stained by H and E (x 400), Case 74.
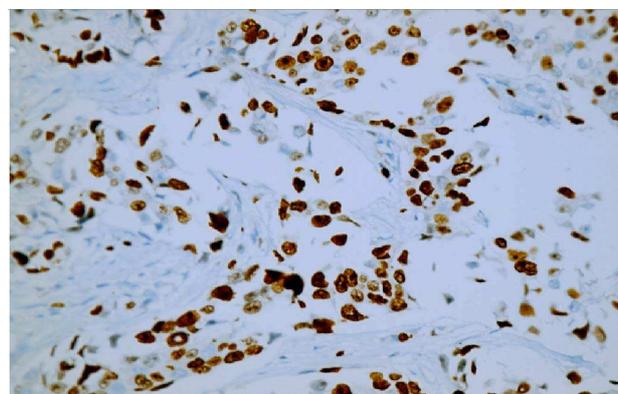

Figure 7. Photomicrograph of invasive ductal carcinoma (Grade III) stained by Ki-67 showing low proliferative index (x 400), Case 74

In this study, among 79 patients correlation of Ki67 was done with other biomarkers such as ER, PR. Spearman's correlation co-efficient test was done to find the level of significance. Ki67 is inversely significantly correlated with ER scores with negative r value (-0.253). Ki67 is inversely but not significantly correlated with PR scores with negative r value (-0.017) (Table III).

Table III: Correlation of Ki67 with different type of markers (n=79)

Markers	r value	p value
ER score	-0.253	0.025[s]
PR score	-0.017	0.882[ns]

Spearman's correlation co-efficient test was done to measure the level of significance.

s= significant

ns= not significant

Discussion

Breast cancer is the top cancer in women both in the developed and the developing world. The incidence of breast cancer is increasing in the developing world due to increase life expectancy, increase urbanization and adoption of western lifestyles.

The study comprised of 79 cases of breast cancers with mean age of 43.59 years with standard deviation 10.40 which is similar to Gogoi et al.[12] Majority of the patients 53.2% were in premenstrual state and 46.3% were in post menstrual state which is similar to Gogoi et al.[12]

Thirteen (16.5%) patients had family history of breast cancer in this study. Gogoi et al.[12] found family history of breast cancer in 2.68% cases. About 13.0% of women diagnosed have a first-degree female relative (mother, sister or daughter) with breast cancer.[13] A woman who has one first-degree female relative with breast cancer has almost twice the risk of a woman without a family history. If she has more than one first-degree female relative with a history of breast cancer, her risk is about 3-4 times higher.[14]

Majority of the patients 50 (59.5%) had tumor sizes 2-5 cm followed by 18 (22.8%) and 14 (17.7%) patients with tumor sizes >5 cm and < 2 cm respectively. Mean size of tumor was 3.52 ± 1.87 cm. Similar result was seen in the study of Gogoi et al.[12] found <2 cm in 13.39%

of cases, 2-5cm in 75.89% of cases and >5 cm in 10.71% of cases.

In this study, 79 patients had invasive ductal carcinoma and their mean Ki67 expression was 29.39 ± 23.80. Among invasive ductal carcinoma, 48 (60.8%) patients had positive Ki 67.

Majority of the patients had their tumors fall in 54 (65.9%) grade II followed by 14 (17.1%) and 11 (13.4%) patients in grade I and grade III type respectively. Gogoi et al.[12] found grade I constituted 16.96%, Grade II was 59.82%, and Grade III was 23.21% respectively. Among 14 grade I patients 5 (35.7%) were Ki 67 positive, among 54 grade II patients 37 (68.5%) were Ki67 positive and among 11 grade III patients 6 (54.5%) were Ki 67 positive patients.

Ki67 is inversely correlated with ER and PR score. Gogoi et al.[15] found that patients with higher level of Ki-67 expression have lower level of ER and PR expression. ER, PR and Ki 67 had predictive and prognostic factors and play a crucial role in the treatment of patients with breast cancer.[15,16]

Regarding prognosis of breast cancer by different markers, Prognostic markers of breast cancer was found positive in 48 (60.8%), 44 (100%) and 37 (100%) cases by Ki67, ER score, PR score respectively. ER positivity was significantly high in negative Ki 67 patients. Similar findings were seen in the study of Liu et al.[17]

This high Ki67 was found to be significantly associated with bad prognostic clinicopathological parameters, such as old age, high grade tumors, and lymph node metastasis. In this study, a significant relationship was also found between Ki67 and tumor grade, as tumors with higher grades have higher levels of cell proliferation and are

consequently more invasive than the other grades; these findings were consistent with the findings of other studies.[18]

Conclusion

Ki67 (anti-MIB1) has emerged as a rapid and inexpensive method to detect proliferation rate in breast cancer. There is a high Ki67 count in malignant breast lesions and it is higher in higher grade DCC. With increasing grade of the tumour Ki67 expression was also increased. As a result, it can be concluded that Ki67 expression is directly correlate with tumour aggressiveness. Ki67 is inversely correlated with ER and PR score.

Acknowledgement

Authors are greatful to all the laboratory technologists who were involved in tissue processing of supplied samples and staining of the slides during the study period.

References

1. GLOBOCAN 2012 (IARC) , Section of Cancer Surveillance (13/10/2017), Breast Cancer
   Estimated Incidence, Mortality and Prevalence Worldwide in 2012.
2. Swenson KK, Decher L, Haselow R, Farrell JB and Sperduto PW: Prognostic factors after conservative surgery and radiation therapy for early stage breast cancer. Am J Clin Oncol 21: 111-116, 1998.
3. Desmedt C, Sotiriou C (2006). Proliferation: the most prominent predictor of clinical outcome in breast cancer. Cell Cycle. 2006; 6(35):2198-202.
4. Van Diest PJ, van der Wall E, Baak. Prognostic value of proliferation in invasive breast cancer: a review. J Clin Pathol. 2004; 57:675-81.
5. Gerdes J, Lemke H, Baisch H, et. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol. 1984; 133:1710-5.
6. Gerdes J, Li L, Schlueter C et al. Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. Am J Pathol.1991; 138:867-873
7. Keshgegian AA, Cnaan A. Proliferation markers in breast carcinoma. Mitotic figure count, S-phase fraction, proliferating cell nuclear antigen, Ki-67 and MIB-1. Am J Clin Pathol. 1995; 104:42-9.
8. Viale G, Regan MM, Mastropasqua MG, et al. Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. J Natl Cancer Inst. 2008;100(3):207-212
9. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol. 2010;11(2):174-183
10. Penault-Llorca F, Andre F, Sagan C, et al. Ki67 expression and docetaxel efficacy in patients with estrogen receptor-positive breast cancer. J Clin Oncol. 2009; 27(17):2809-2815
11. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn HJ, Panel members Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013; 24:2206–2223
12. Gogoi G, Borgohain M and Saikia H. Study of Her2/Neu Expression in Breast Carcinoma and Correlation with Various Prognostic Parameters. Indian Journal of Pathology and Oncology. 2015; 2(4):265-276.

13. Collaborative Group on Hormonal Factors in Breast Cancer. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet. 2001;358:1389-99.

14. Kharazmi E, Chen T, Narod S, Sundquist K, Hemminki K. Effect of multiplicity, laterality, and age at onset of breast cancer on familial risk of breast cancer: a nationwide prospective cohort study. Breast Cancer Res Treat. 2014; 144(1):185-92.

15. Salmen J, Neugebauer J, Fasching PA, Haeberle L, Huober J, Wöckel A et al.. Pooled analysis of the prognostic relevance of progesterone receptor status in five German cohort studies. Breast cancer research and treatment. 2014; 148(1):143-151.

16. Akbar M, Akbar K and Naveed D. Frequency and correlation of molecular subtypes of breast cancer with clinicopathological features. J Ayub Med Coll Abbottabad, 2014; 26(3):290-293.

17. Liu Z, Zhang C, Zhuo P, He K, Wang X, Yu Q, Huo Z, Wang F and Yu Z. Characteristic of ER+/PR-and Ki67 value with breast cancer. Int J Clin Exp Med. 2017; 10(2):3533-3539.

18. Shokouh TZ, Ezatollah A and Barand P. Interrelationships Between Ki67, HER2/neu, p53, ER, and PR Status and Their Associations With Tumor Grade and Lymph Node Involvement in Breast Carcinoma Subtypes: Retrospective-Observational Analytical Study. Medicine. 2015;94:32