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A study of Ki 67 immunostaining in breast carcinomas: Correlation with histopathological prognostic factors

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Abstract

Background: One of the most common causes of carcinoma deaths among women is breast cancer. In view of the above, early diagnosis and effective treatment of the disease are immensely important. The increasing number of options for the treatment of breast cancer has made the prognostic evaluation of the disease even more important. Proliferation plays an important role in the clinical behaviour of invasive breast cancer. Ki 67 binding, is an objective measurement of cell proliferation which significantly aids in the management of the breast cancer patients.

Objectives:

1. To study the proliferative activity using Ki 67 immunostaining in breast carcinoma.
2. To assess the relationship of Ki 67 scores with size, histological grade and lymph node status.

Methods: Seventy five cases of histologically proven breast carcinomas were studied**.** Histopathological grade was assessed using Bloom and Richardson's method, modified by Elston and Ellis. Immunohistochemistry (IHC) for Ki 67 was done on paraffin embedded wax sections.

Results: Ki 67 was positive in 73/75 cases (97.33%). The range of Ki 67 score was 0 to 90%. Mean value of Ki 67 was 31.86% and median was 30%. A statistically significant correlation was observed between size (P=0.037), grade (P < 0.0001) and Nottingham Prognostic Index (P < 0.0001) with Ki 67 scores.

No statistically significant correlation was seen with lymph node status (P=0.767), lymphovascular invasion, necrosis, presence of desmoplasia, ductal carcinoma in situ (DCIS), nipple and areola involvement.

Conclusion: Proliferation has been recognized as a distinct hallmark of cancer and acts as an important determinant of cancer outcome. As Ki 67 can be used to objectively measure this, it can be included in the pool of prognostic markers like tumor size, nodal status, histopathological grade and hormonal receptors.

Keywords: Breast Carcinoma, Ki 67, Size, Grade, Lymph node status

Introduction

Breast cancer is the most frequently diagnosed cancer and accounts for 23% (1.38 million) of the total cancer deaths. Breast cancer is also the leading cause of cancer deaths among females in economically developing countries like India.[(1)] In India, breast cancer accounts for 19% to 34% of all the malignancies.[(2)]

The advent of newer technologies and the realization that breast cancer is heterogeneous has shifted the focus to prognostication, with increased attention being paid to the identification of morphological features and immunohistochemical markers of prognostic relevance.[(3)]

Proliferation plays an important role in the clinical behavior of invasive breast cancer. Increased proliferation correlates strongly with poor prognosis. However, of the different methods to assess proliferation, mitosis counting has been shown most convincingly to provide reproducible and independent prognostic value in invasive breast cancer.[(4)] Ki 67 binding, as an objective measurement of cell proliferation aids significantly in the management of the breast cancer patient.[(5)]

In early breast cancer, measurement of proliferation can be used in conjunction with tumour size, grade, nodal status and steroid receptor status as a prognostic indicator. Proliferation rates can provide useful information on prognosis and aggressiveness of individual cancers and can be used to guide treatment protocols in clinical practice.[(6)]

The aim of the present study was to correlate Ki67 expression in breast carcinoma with known histopathological prognostic factors like size, tumor grade, lymph node status and Nottingham Prognostic Index (NPI).

Materials and Methods

In the present study, seventy five cases of mastectomy specimens were studied. Ethical clearance from the institutional ethical committee was obtained. A detailed gross examination was performed. Axillary fat was examined to isolate the lymph nodes.

Mastectomy specimens were fixed in formalin within one hour of resection followed by paraffin embedding and staining with haematoxylin and eosin. Sections were studied to evaluate histologic type, histologic grade, lymphnode status, tumor necrosis, stromal reaction, lymphovascular invasion, margin status and nipple-areola involvement. Histopathological grade was assessed using Bloom and Richardson's method, modified by Elston and Ellis. Pathologic tumor, node, metastasis (TNM) staging was done.

IHC for Ki 67 was done on 4 µm thick paraffin embedded wax sections on poly-l lysine coated slides. Antigen retrieval was done in tri sodium citrate buffer at pH 6. Monoclonal antibody Ki 67 (clone MM1) Novocastra code: NCL-L-Ki67-MM1 was used for Ki 67 antigen detection by one step horseradish peroxidase (HRP) polymer method. The dilution was 1:200. A section from a reactive lymph node was taken as positive control and sections treated with tris-buffer solution instead of primary antibody was used as negative control.

Brown granular nuclear reactivity was taken as positive. Ki 67 Labeling index (LI) was expressed as percentage of positively stained cells per 100 epithelial cells after counting at least 1000 cells using high power (40x10). An area with the maximum proliferation was chosen to evaluate the labeling index (LI).

Statistics: Data was analysed using statistical software package SPSS ver.17. One way Anova was employed to examine the correlation of Ki67 as a continuous variable with other prognostic markers (tumor size, tumor grade, lymph node status) and correlation of Ki67 as a categorical variable was determined by chi square test. Data was expressed as Mean. *P* value <0.05 was considered statistically significant.

Results

In the present study, the age group of patients ranged from 31 to 84 years, with a mean age of 52.81 years. Left sided breast carcinoma was more common accounting to 58.7%. Infiltrating ductal carcinoma- not otherwise specified (IDC-NOS) type was the most common histological subtype. Histologic grade was assessed with the maximum number of cases belonging to grade 3. The size of the tumors ranged from two to eleven cm, with a mean size of 4.33cm. Axillary node involvement was noted in 36/75 cases accounting for 48%. NPI was calculated by - [Size (cm) x 0.2] + [lymph node stage (1-3)] + [grade (1-3)]. Three prognostic groups were identified; A good group with score of less than 3.4; a moderate group with score of 3.4-5.4; a poor group with score of over 5.4.

Ki 67 was studied as a continuous variable as well as a categorical variable. Ki 67 was positive in 73/75 cases (97.33%). The range of Ki 67 score was 0 to 90% with a mean value of 31.86% and median of 30%. The mean value of Ki 67 in IDC- NOS type was 31.6%.

Ki 67 scores were grouped according to recommendations from St Gallen Consensus - 2009 as Group 1- Low (<15%), Group 2 - Intermediate (16-30%) and Group 3 - High (>30%) (Fig. 1, 2 and 3). Sixteen (21.3%) cases showed low proliferation, twenty five (32%) cases showed intermediate and thirty four (46.7%) cases showed high proliferation. Both as continuous and categorical variable, a statistically significant correlation was noted between tumor type, size, grade & NPI with Ki 67 scores (Tables 1, 2 and 3). However no significant association was found between Ki67 index and lymph node status (Table 4).

Table 1: Summary of the characteristics of the study population with Mean Ki 67 (continuous variable)

Characteristics	No of cases	Mean Ki 67 index	*P* value
1.Primary tumour type			
Invasive ductal carcinoma	68(90.7%)	31.60%	**0.025**
Invasive lobular carcinoma	2(2.7%)	11.5%	
Infiltrating lobular carcinoma-Pleomorphic type	1(1.3%)	81%	
Mixed ductal and lobular	2(2.7%)	22%	
Mucinous carcinoma	1(1.3%)	70%	
Metaplastic carcinoma	1(1.3%)	23%	
2.Primary tumour grade			
1	5(7.1%)	7.6%	**<0.0001**
2	22(31.4%)	18.5%	
3	43(61.42%)	40.8%	
3.Primary tumour size (cm)			
T1(<2cm)	7(9.3%)	13.14%	
T2(2-5cm)	50(66.7%)	34.56%	**0.037**
T3(>5cm)	10(13.3%)	27.50%	
T4(Any size with extension to skin & chest wall)	8(10.7%)	38.12%	
4.Lymph node status			
Positive	39 (52%)	32.66%	**0.767**
Negative	36 (48%)	31.27%	
N0	39(52%)	32.66%	**0.099**
N1	13(17.3%)	21.53%	
N2	12(16%)	32.16%	
N3	11(14.7%)	41.81%	

5.Nottingham prognostic index			
<3.4	20(28.6%)	20.1%	
3.4 – 5.4	30(42.85%)	33.12%	**0.006**
>5.4	20(28.6%)	38.10%	

Table 2: Correlation of Ki 67 scores (categorical variable) with size of the tumor

Tumor size(T)	Group 1 <15%	Group 2 16-30%	Group 3 >30%	Total	*P* value
T1	4(57.1%)	3(42.9%)	0(0%)	7(100%)	
T2	8(16.0%)	17(34.0%)	25(50%)	50(100%)	
T3	4(40%)	2(20.0%)	4(40.0%)	10(100%)	**0.043**
T4	0(0%)	3(37.5%)	5(62.5%)	8(100%)	
Total	16(21.3%)	25(33.33%)	34(45.3%)	75(100%)	

Table 3: Correlation of Ki 67 with grade of the tumor

Grade	Group 1 <15%	Group 2 16-30%	Group 3 >30%	Total	*P* value
1	5(100%)	0(0%)	0(0%)	5(100%)	
2	7(31.8%)	12(54.5%)	3(13.6%)	22(100%)	**<0.0001**
3	2(4.7%)	11(25.6%)	30(69.8%)	43(100%)	
Total	15(21.1%)	24(33.8%)	32(45.1%)	70(100%)	

Table 4: Correlation between Ki 67 and nodal status of the tumor

Nodal stage	Group 1	Group 2	Group 3	Total	*P* value
N0	8(20.5%)	14(35.5%)	17(43.6%)	39(100%)	
N1	5(38.1%)	5(38.1%)	3(23.1%)	13(100%)	**0.151**
N2	2(16.7)	4(33.3%)	6(50.0%)	12(100%)	
N3	1(9.1%)	1(9.1%)	9(81.8%)	11(100%)	
Total	16(21.3%)	24(32.0%)	35(46.7%)	75(100%)	

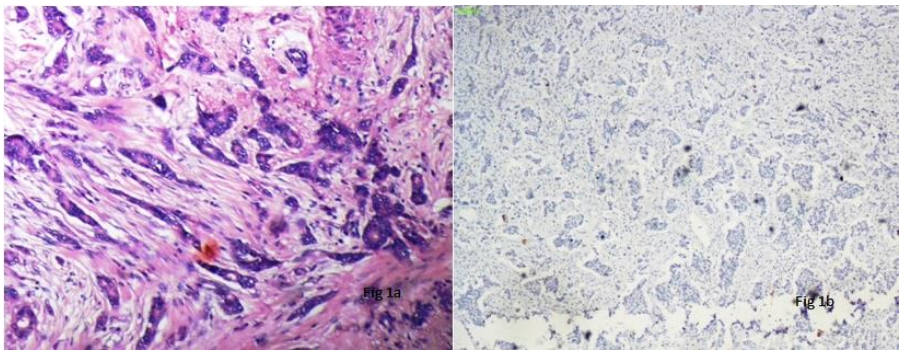

Fig. 1 (1a and 1b): IDC-NOS type Grade 1 (H&E X100); Tumor cells are negative for Ki 67 immunostain (Ki 67 X100)

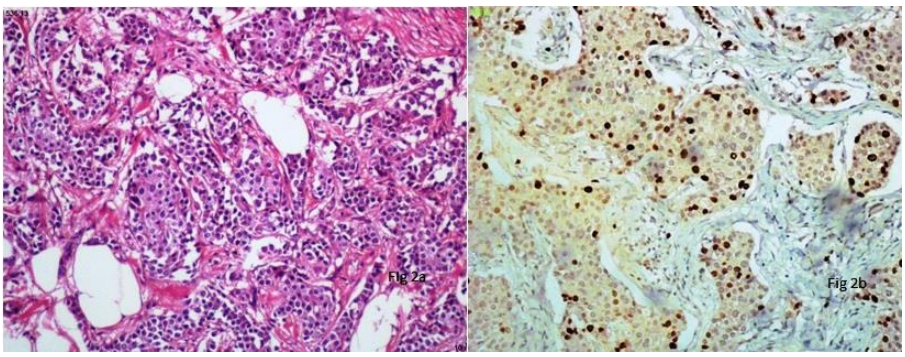

Fig. 2 (2a and 2b): IDC- NOS type Grade 2 (H&E X200); Tumor cells showing intermediate proliferation, Ki 67 index - 22% (Ki 67 X200)

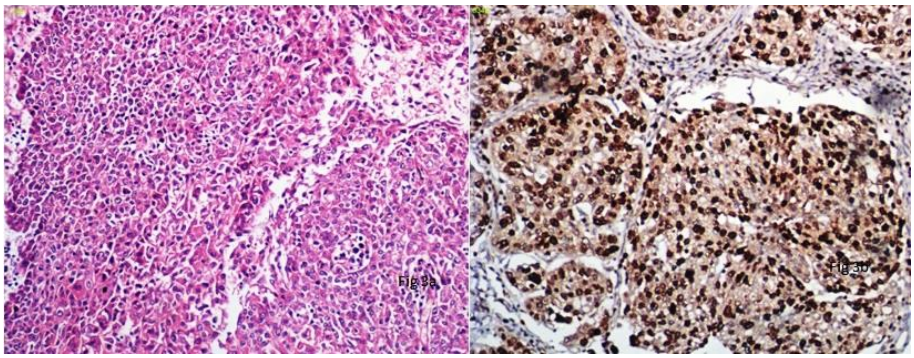

Fig. 3 (3a and 3b): IDC - NOS Type Grade 3 (H&E X200); Tumor cells showing high proliferation, Ki 67 index-71% (Ki 67 X200)

Discussion

Biomarker expression in breast cancer is used as a prognostic indicator and predictor of response to hormonal and chemotherapy. To date, the leading parameters that guide adjuvant therapy in breast cancer are estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2/neu). In recent years, gene expression analysis studies have demonstrated the vitality of proliferation signatures not only in the prognosis of breast cancer but also as a predictive response to subsequent therapy. IHC for Ki-67 has been routinely used to determine tumor proliferation.[7] Ki 67 is an easily available, rapid and a more reproducible biomarker compared with other markers like proliferating cell nuclear antigen (PCNA) and bromodeoxyuridine (BrdU).[8]

Potential uses of Ki 67 include prognosis, prediction of relative responsiveness or resistance to chemotherapy or endocrine therapy, estimation of residual risk in patients on standard therapy and as a dynamic biomarker of treatment efficacy in samples taken before, during and after neoadjuvant therapy, particularly neoadjuvant endocrine therapy.[9]

Several studies have investigated the prognostic significance of Ki 67 in breast cancer. The present study was also done to evaluate the prognostic significance of Ki 67 in breast cancer by correlating with traditional well known histopathological prognostic factors like size, grade and lymph node status.

In the present study, the range, mean and median value of Ki 67 were comparable with other studies.[5,7,8,10,11,12,13]

Of the commonly used pathological features of breast cancer probably the most robustly related to Ki 67 is histological grade with virtually no studies refuting this positive correlation. This is to be expected given that mitotic index is one of the three components of the grading system.[14] High Ki-67 scores are associated with high mitotic counts and histologic grade while no correlation is noted with nuclear pleomorphism, tubule formation.[15] Histologic grade of breast cancer has been recognized for a long period of time and its prognostic value has been validated in multiple independent studies.[16] Histologic grade has been associated with poor prognosis and it is found that Ki-67 labeling rates increase with nuclear and histologic grade.[13] This supports the well-established view, that proliferating cells or those with deranged proliferation regulation, usually do not differentiate and conversely, cells when differentiating, usually cease dividing.[12] A definite correlation between the grade of the tumour and Ki 67 has been documented in several studies.[5,7,11,13,15,17,18] Hence, our observation was comparable with them.

Various studies have shown that the gross size of tumor is one of the most significant prognostic factors in breast carcinoma and there is increased incidence of axillary lymph node metastasis and decreased survival with increasing size of the tumor.[19,20]

Hence we correlated the size of the tumor with Ki 67, to find an association between the two. A positive association between Ki-67 staining and tumor size, with smaller tumors having lower Ki-67 values has been noted in other studies [8,10,11] though some have not confirmed this relationship.[2,5,7,21,22] However, in the present study we saw a positive correlation between size and Ki 67 scores.

The strongest prognostic factor in breast cancer, lymph node status, has been intensively studied with regard to its correlation with Ki 67, in an attempt to find an easy marker of nodal involvement that would avoid unnecessary axillary surgery. Lymph node involvement is a valuable indicator of short-term survival. Node-positive patients have about a four to eight times higher mortality than those without nodal involvement. Prognosis for patients with ten or more involved axillary nodes showed 70% more deaths at ten years than for those with one to three involved nodes. Ki-67 values have been found to be directly associated with nodal status in some studies,[5,7,10,11,18,23,24] while no significant association was observed in others.[5,13,15,21,25] Hence, we assessed the correlation between nodal status and Ki 67 expression and found no statistically significant correlation.

In studies with more than 200 patients, there seems to be more evidence in favour, than against a positive correlation. In smaller studies there are some favouring a lack of correlation. This may reflect the size of studies and/or the relative weakness in the relationship.[14]

Thus, growth kinetics and metastatic capability may be different tumor properties.[15]

Owing to the fact that in the present study, there was a positive correlation between size and grade of the tumor with Ki 67 scores, a statistically significant correlation between Nottingham Prognostic Index and Ki 67 was noted. An interesting feature was an increase in the number of lymphocytes in the stroma of tumors, which showed low proliferation.

There was no statistically significant correlation between other prognostic indicators like pre and post-menopausal status, lymphovascular invasion, necrosis, desmoplasia, presence of in situ carcinoma components, nipple and areola involvements with Ki 67 scores.

Conclusion

In addition to the conventional histopathological prognostic parameters, the assessment of proliferation is one of the major factors for the treatment decisions in breast cancer patients. Ki-67 is a convenient method for assessing the proliferation as it is rapid and more reproducible than other proliferative markers.

Proliferative activity determined by Ki-67 may reflect the aggressive behavior of breast cancer and predict the time of recurrence and the appropriate therapy. It is therefore important to take the Ki-67 index into consideration in the treatment and follow-up of breast cancer patients.

The level of Ki 67 appears to be a good independent marker of patient outcome in breast cancer and can be included in the pool of prognostic markers like tumor size, nodal status, histopathological grade and hormonal receptors.

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