Association of Ki67 expression with clinicopathological parameters and molecular classification in invasive breast carcinomas

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Abstract

Breast carcinoma is most common malignant tumour and leading cause of cancer death in women worldwide. Ki67 has a prediction of prognosis in breast carcinoma patients, and is also considered has an important biomarker in routine practice.

Aim: The aim of the present study was to investigate Association of Ki67 expression with clinicopathological parameters and molecular classification in invasive breast carcinomas.

Materials and Methods: A total of 100 patients diagnosed as invasive breast cancer were included in the present study. The clinical information of the patients was obtained from the institutional medical records. The expression levels of Ki67 were detected by immunohistochemical analysis. The associations between Ki67 scores and other clinicopathological parameters were evaluated.

Results: The value of the Ki67 scores in all the patients was, low in 57 cases (57%), intermediate in 11 cases (11%), high in 32 cases (32%) with a mean score of 33.33%. Ki67 expression was significantly associated with middle aged women, ranging from 31-50yrs (P<0.0107), ER negative status (P<0.0002), PR negative status (P<0.0038), Molecular subtype-triple negative (P<0.0024), higher Grade 3 (P<0.0106) and higher TNM Stage (P<0.0117). However association with left or right side affected, tumour size, menopausal status, lymphovascular invasion, lymph node metastasis and Her2/Neu status was not statistically significant in this study.

Conclusion: In conclusion, results revealed that there is presence of significant correlations between Ki67 and other clinicopathological parameters and molecular classification of breast cancer. Thus, Ki67 labeling index is an important prognostic and predictive member in management of breast carcinoma patients.

Keywords: Invasive breast carcinoma, Ki67 Labeling Index, Clinicopathological parameters, Molecular subtypes.

Introduction

Breast cancer (BC) is the most common type of cancer and the leading cause of cancer-associated mortalities.[1] Over 1,40,000 new breast cancer patients are estimated to be diagnosed annually in India with an age standardised incidence rate of 25.8 cases per 1,00,000 women per year, making it the most common cancer amongst Indian women.[2] Usually the three biomarkers, Estrogen receptor (ER), Progesterone receptor (PR), and HER-2/neu are routinely assessed and used to approximate the molecular category of breast cancer. Gene expression profiling, which can measure the relative quantities of mRNA for essentially every gene, has identified five major patterns of gene expression in breast cancer, they are Luminal A, Luminal B, Normal breast-like, Triple Negative, and HER2 positive. These molecular classes correlate with prognosis and response to therapy, and thus have taken on clinical importance.[3] Presence of both ER and PR is related to better prognosis and responsiveness to hormonal therapy.[4] A humanized monoclonal antibody, trastuzumab, targeting the HER-2/neu gene product is another example of targeted therapy in breast cancer.[5]

Ki67 is a proliferation marker that is expressed in all the phases of the cell cycle, with the exception of the G_0 phase.[6-7] Now, Ki67 is an important biomarker used in a routine clinical practice, with potential applications in its prognosis, and is also used to predict responses or resistance to chemotherapy and endocrine therapy, to estimate residual risks in the patients receiving standard therapies and also as an dynamic biomarker to measure treatment efficacies in samples obtained prior to, during and subsequent to the neoadjuvant therapy.[8] Ki67 levels are used for clinical research purposes, including as a primary efficacy during clinical trials and, in certain circumstances, for clinical management. The 2011 St Gallen Expert Panel indicated that a Ki67 level of ≥14% distinguished luminal B from luminal Atumors in Breast carcinoma molecular subtyping.[9-10] Thus, understanding the association of Ki67 with pathological parameters (including histological grade, tumor size and lymph node metastasis) and immunohistochemical markers [including the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status] is important for clinical evaluations and guiding treatment strategies. The aim of the present study was to analyze the correlation of the expression of Ki67 with clinicopathological parameters and molecular subtypes in invasive breast carcinomas cases.

Materials and Methods

The present study is an Institutional study conducted during the period of 5 years (August 2012 to July 2017)

All the surgical specimens from patients operated for breast carcinoma of all ages received for routine histopathological evaluation were included in the study.

A total of 100 cases were studied of which 76 were modified radical mastectomies, 18 were simple

mastectomies and 6 lumpectomies. For all the cases lymph nodes were received. In modified radical mastectomy cases, axillary lymph node dissection was done in all cases and also lymph nodes were identified in the axillary tail in 48 cases. In simple mastectomy cases, lymph nodes were identified in the axillary tail while lumpectomy cases were followed by sentinel lymph node biopsy.

Following clinicopathological parameters like Age, Sex, Menstrual status, Site, Tumour size on gross examination were noted when surgical specimens from patients operated for breast carcinoma were received in the Institutional department of Pathology.

Specimens were subsequently formalin fixed, paraffin embedded and stained by Haematoxylin and eosin (H & E) for histopathological study to assess.

1. Histological subtype of breast cancer.
2. Axillary nodal status.
3. Lymphovascular invasion.
4. Any other significant features.

For retrospective cases, H&E sections were retrieved from the records and screened for confirmation of diagnosis and selection of representative tumour paraffin blocks for immunohistochemistry.

Histological grading of tumour was done according to Modified Bloom Richardson grading and staging according to TNM Classification designated by AJCC.

Immunohistochemistry Procedure:

1. 4µm sections were cut from representative neoplastic tissue blocks and taken on 4 glass slides coated with adhesive (poly-L-lysine) for immunohistochemistry (IHC) to detect ER, PR, HER2/neu overexpression and Ki-67 proliferative index.
2. 4 µm thick sections from the tissue blocks were deparaffinized and brought to water. Thereafter, the sections were rinsed in distilled water and heated in microwave oven for 20 minutes in 0.01 M citrate buffer (pH-6.0). After microwave oven heating, the slides were rinsed in PBS buffer (pH-7.2) for 15 minutes after being brought to room temperature. Endogenous

peroxide was blocked by 4% H_2O_2 for 30 minutes followed by 3 washings of 5 minutes each with PBS buffer. The sections were incubated with the primary mouse monoclonal antibody (BIOGENEX). The antibody was used in 1:50 dilution. The slides were then incubated overnight at 4°C in a refrigerator. After bringing the slides to room temperature the next morning, the sections were washed thrice for 5 min with PBS. They were treated with the Polyscan HRP label for 30 minutes and then were given 3 washes with PBS buffer, each for 5 minutes. Colour was developed using diaminobenzidinetetra hydrochloride as a substrate, counterstaining was done with Harris Haematoxylin followed by washing in distilled water. The sections were blotted, air dried, and mounted with DPX.

The immunostained slides were examined for nuclear staining in case of ER, PR and Ki-67, and membrane staining in case of HER2/neu. For hormone receptors, the proportion of positive staining tumour cells (expressed in percentage) and the average intensity of staining were evaluated based on Allred score method.[11] HER2/neu staining was scored from 0 to 3+.[12] Ki-67 was scored as percentage of positively stained cells among the total number of malignant cells and divided into 3 groups- low (≤15%), intermediate (15- 30%) and high (>30%).[13]

The relationship between various parameters such as age, menopausal status, tumour size, tumour extent, histologic type, histologic grade, lymph node status, and Ki-67 index were studied and based on their expression classified into Luminal A, Luminal B, Triple Negative and HER2 positive. The statistical analysis for correlation among these parameters was determined using the Pearson chi-square test. Significance was assumed at p-value less than 0.05.

Results

Table 1: Clinicopathological parameters of invasive breast carcinomas

Parameters	Total N=100 cases (%)
Age (years)	
<30	12(12%)
31-50	74(74%)
>50	14(14%)
Side affected	
Right	47(47%)
Left	53(53%)
Tumour size (in cms)	
<2	28(28%)
2-5	42(42%)
>5	30(30%)
Menopausal status	
Premenopausal	53(53%)
Postmenopausal	47(47%)
Histological type	

IDC	90(90%)
Medullary	07(7%)
ILC	02(2%)
Mucinous	01(1%)
Tumour grade	
I	36 (36%)
II	27(27%)
III	37(37%)
Tumour stage	
I	12(12%)
II A	30(30%)
II B	19(19%)
III A	16(16%)
III B	16(16%)
III C	03(3%)
IV	04(4%)
Lymphovascular invasion	
Positive	56(56%)
Negative	44(44%)
Lymphnode metastasis	
Positive	61(61%)
Negative	39(39%)
Estrogen receptor status	
ER Positive	43(43%)
ER Negative	57(57%)
Progesterone receptor status	
PR Positive	40(40%)
PR Negative	60(60%)
Her2/Neu status	
Her2/Neu Positive	39(39%)
Her2/Neu Negative	61(61%)
Molecular subtypes	
Luminal A	25(25%)
Luminal B	24(24%)
Basal like	36(36%)
Her2/Neu	15(15%)

Table 2: Correlation of Ki 67 labelling index with clinicopathological parameters and molecular classification of Invasive breast carcinomas

Parameters	Low Ki67 (<15%)	Intermediate Ki-67 (16-30%)	High Ki-67 (>30%)	P-value
Age (yrs)				
<30	2	2	8	
31-50	43	6	23	
>50	12	1	1	0.0107
Side affected				
Right	27	7	13	
Left	28	4	21	0.3061
Tumour size (in cms)				
<2	15	3	10	
2-5	27	6	9	
>5	13	2	15	0.1602
Menopausal status				
Premenopausal	28	5	20	
Postmenopausal	27	5	15	0.8295
Histological type				

|				
IDC	54	07	29	
Medullary	02	02	03	
ILC	00	02	00	
Mucinous	01	00	00	0.1130
Tumour grade				
I	27	2	7	
II	13	1	13	
III	15	7	15	0.0106
Tumour stage				
I				
II A	10	1	1	
II B	13	1	16	
III A	11	2	6	
III B	11	2	3	
III C	10	2	4	
IV	0	1	2	
	0	2	2	0.0117
Lymphovascular invasion				
Positive	29	6	21	
Negative	27	4	13	0.6290
Lymphnode metastasis				
Positive	33	7	21	
Negative	24	3	12	0.7160
Estrogen receptor status				
Positive	34	2	7	
Negative	22	7	28	0.0002
Progesterone receptor status				
Positive	30	3	7	
Negative	25	7	28	0.0038
Her2/Neu status				
Positive	19	4	16	
Negative	36	7	18	0.4918
Molecular subtypes				
Luminal A	25	0	0	
Luminal B	13	3	8	
Basal like	14	5	17	
Her2/Neu	06	1	8	0.0024
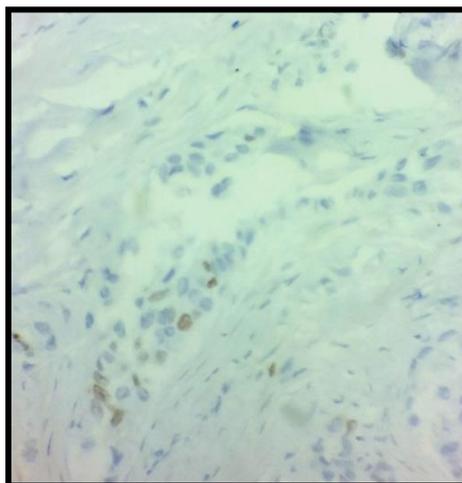
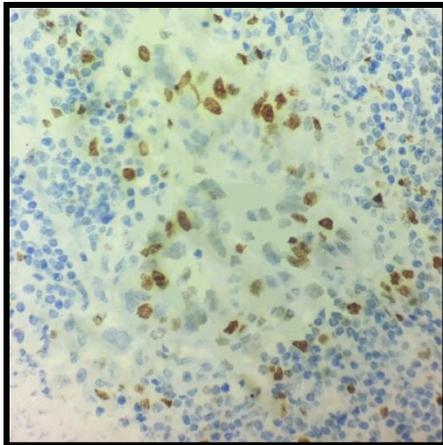**Fig. 1: Ki-67 Expression in carcinoma of male breast case showing 1% positivity[400x]**

Fig. 2: Ki-67 Expression in medullary carcinoma with lymphoplasmacytic infiltrate showing 30% positivity [400x]
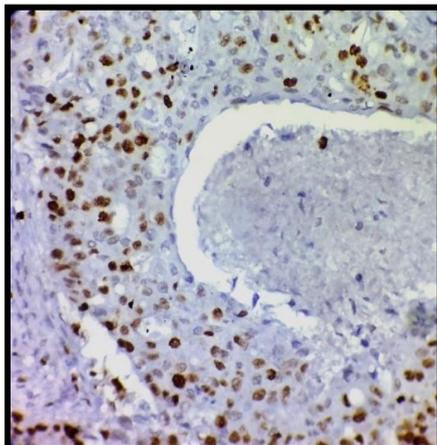

Fig. 3: Ki-67 Expression in IDC case with central foci of necrosis showing 75% positivity [400x]

The clinicipathological characteristics of 100 breast cancer patients are shown in Table 1. Out of 100cases 99 were females and 1 case was male patient with age ranging from 22 to 85yrs with mean age of 47.65 yrs. In 53 cases (53%) left breast was affected and in 47 cases (47%) right breast was affected. The tumours size ranged between 1-12cms with mean size of 4.52cms. Most of the patients were premenopausal state (53 cases; 53%) and rest were postmenopausal state (47 cases; 47%). The pathological examination showed IDC-NOS in 90 cases (90%), medullary carcinoma in 7 cases (7%), infiltrating lobular carcinoma in 2 cases (2%) and mucinous carcinoma in 1 case (1%). Histological grading was done by Nottingham modification of the Bloom Richardson system.[12] Most of the tumours were grade Ⅲ-37 cases (37%), followed by grade-Ⅰ 36 cases (36%) and grade Ⅱ-27 cases (27%). Staging was done according to TNM classification which showed stage Ⅰ-12 cases (12%), stage Ⅱ A-30 cases (30%), stage Ⅱ B-19 cases (19%), stage Ⅲ A-16 cases (16%), stage Ⅲ B-16 cases (16%), stage Ⅲ C-03 cases (3%) and stage Ⅳ-04 cases (4%). 61 cases (61%) had positive lymph node metastasis and 56 cases (56%) had lymphovascular invasion.

Molecular Classification of Breast Cancers

The number of ER positive breast cancers were 43 cases (43%) followed by PR positive breast cancers in 40 cases (40%) and Her2/Neu positive breast cancers in 39 cases (39%). The most frequent breast cancer subtype in the studied patients was Basal like in 36 cases (36%) followed by Luminal A in 25 cases (25%), Luminal B in 24 cases (24%) and Her2/Neu in 15 cases (15%).

Assessment of Ki67 Immunostaining

The Ki67 expression in individual clinicopathological parameters of 100 breast cancer cases shown in Table-2.The mean value of the Ki67 scores of all patients was, low in 57 cases (57%), intermediate in 11 cases (11%), high in 32 cases (32%) with a mean score of 33.33%. Ki67 expression was significantly associated with middle aged women, ranging from 31-50yrs ($P<0.0107$), ER negative status ($P<0.0002$), PR negative status ($P<0.003$), Molecular subtypes-triple negative ($P<0.0024$), Higher Grade 3 ($P<0.0106$) and higher TNM Stage ($P<0.0117$). However association with side affected, tumour size, menopausal status, Her2/Neu status, lymphovascular invasion and lymph node metastasis was not statistically significant. Fig. 1 showing Low Ki 67 index <15%, Fig. 2 showing Intermediate Ki67 index 15-30% and Fig. 3 showing High Ki67 index >30% in breast carcinoma patients.

Discussion

Ki 67 (anti MIB1) has emerged as a rapid and inexpensive method to detect proliferation in breast cancer. Ki-67 is present in all the proliferating cells, and there is potential interest in its role as a proliferation marker. The Ki-67 antibody reacts with the 395 kDa antigen, which is a nuclear non-histone protein which is present in all the active phases of the cell cycle, except the G0 phase.[14] Ki-67 levels are low in the G1 and S phases and rise to their peak level in mitosis. Later in the mitotic phase (anaphase and telophase), a sharp decrease in Ki-67 levels occur. Mostly, Ki-67 is measured on paraffin sections by an immunohistochemical method, using the MIB-1 antibody.[15] The International Ki-67 in Breast Cancer Working Group recommendations for Ki-67 interpretation in breast cancer includes that only nuclear staining be considered positive, staining intensity is not relevant and scoring should involve the counting of at least 500 malignant invasive cells. The Ki-67 score has been expressed as the percentage of positively staining cells among the total number of invasive cells in the area scored.[16] The panel of experts at the St Gallen Consensus in 2011 considered the Ki-67 labelling index important for selecting the addition of chemotherapy to endocrine therapy in the hormone receptor positive breast carcinomas and classified these tumours as low, intermediate, and highly proliferating according to the value of Ki-67 labelling index of <15%, 15%−30%, and >30%, respectively.[17] Despite the controversies regarding the value of Ki67, there is a strong

data in the literature to show the Ki67 is a good prognostic and predictive marker. One of the previous study done by Nishimura et al who studied use of Ki-67 as a prognostic marker in 3652 breast carcinoma cases and found there is a higher Ki-67 index (≥20%) which correlated significantly with young age, large tumours, positive lymph nodes, negative ER/PR, p53 overexpression, positive HER2/neu and with a poorer prognosis and early recurrence (<2 years).[14] Extensive review of literature in this regard by Luporsi et al showed that Ki-67 provides useful information for therapeutic decisions in breast cancer patients. It is an independent prognostic factor for overall survival and disease free survival in breast carcinoma patients, and the greatest benefits from Ki-67 assessment was observed in patients with ER positive breast carcinomass. It is not predictive for chemotherapy, but high Ki-67 was found to be associated with immediate pathological complete response in the neoadjuvant therapy.[18] Study done by Nahed A. Soleman et al in 2016 showed patients with Ki67 less than 15% displayed better overall survival than those with Ki67 higher than 15%.[19] Study done by Marwah N et al on 76 invasive primary breast ca in 2018 revealed higher tumor size, ER negative, PR negative, grade 3 tumours had higher Ki 67 expression which was statistically significant and has similar findings with our study.[20]

In this study the Ki67 PI in 100 breast cancer patients was assessed using immunohistochemical staining. The standardized guidelines of the breast cancer working group for assessment of Ki67 has been followed. The study demonstrated high Ki67 index in most of the clinicopathological parameters which included middle aged women, ranging from 31-50yrs (P<0.0107), ER negative status (P<0.0002), PR negative status (P<0.003), Molecular subtypes- Triple negative (P<0.0024), Higher Grade 3 (P<0.0106) and Higher TNM Stage (P<0.0117) which were statistically significant.

In conclusion, in present study there is significant correlation between Ki67 and various clinicopathological parameters and molecular classification of breast carcinoma patients. Thus, assessment of Ki67 LI can be useful in clinical practice as an important predictive and prognostic marker in managing breast cancer patients.

Conflict of Interest: None.

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