Original Research Article

Role of Ki67 in breast carcinoma and its association with clinicopathological parameters and luminal subtypes in a tertiary health care center

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A R T I C L E   I N F O

A B S T R A C T

Introduction & Objectives: In clinical practice, the evaluation of the breast tumour proliferative fraction is of important for the treatment decision, which is commonly performed by the immunohistochemical staining of a proliferation marker, the Ki67 antigen. The main objective of the current study was to analyze and evaluate the associations between Ki-67 and common histopathological parameters and luminal subtypes in the routine clinical setting.

Materials and Methods: A total of seventy cases were enrolled in the study. The surgical specimens were evaluated and histopathological reporting was done according to College of American Pathologists (CAP) guidelines. Suitable blocks were selected and immunostaining was performed with ER, PR, Her2/neu and Ki67 markers. The association between the Ki-67 and histopathological parameters [age, histological type, nuclear grade, tumor size, multifocality, an *in situ* component, lymphovascular invasion (LVI), estrogen and progesterone receptor (ER/PR) expression, human epidermal growth factor receptor (HER-2) status, axillary involvement and tumor stage] were evaluated in each group. The data thus obtained was analyzed by SPSS-20 software with chi square test.

Results: The intended age of presentation in the current study was 48.66±13.05 years and most common histological type was invasive ductal carcinoma of no special type (IDC-NST). Tumor size was found to have an association with Ki67 expression status with significant p value (0.003), were as tumor grade and pT category did not find any significant association with Ki67 expression. Luminal subtypes and ki67 expression status was seen to have an association with significant p value.

Conclusion: Ki67 was considered as important marker to predict the prognosis for better management of breast cancer patients.

1. Introduction

Breast cancer is the most common malignant tumor in women worldwide with over 1.7 million incidence every year.[1] It is a leading cause of increased mortality in women, especially in developing countries like India. Recent developments in science have determined that breast cancer scientifically presents as heterogeneous entity and manifesting with various biological subtypes, which can be described by immunohistochemical analyses or by genetic array testing.[2] Conventional clinicopathological parameters like age at presentation, tumor size, lymph node status, lymphovascular invasion, surgical margins, tumor grade and tumor staging have traditionally been used to determine prognosis in patients with breast cancer.[3] Uncontrolled proliferation of tumor cells is one of the crucial hallmark of cancer. Likewise, in addition to the traditional histomorphological parameters, the determination of procreation activity of a tumour cells is one of the essential element for the therapeutic choice in breast cancer patients.[4]

Various techniques are available to assess the proliferative activity of tumor cells, such as calculating mitotic figures in stained tissue segments, flow cytometric analysis to determine the proportion of cells being in the S phase of the cell cycle, examination of thymidine-labeling index,

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proliferating cell nuclear antigen (PCNA), or cyclins E and D.[5]

The most prevalent method for assessment of proliferative activity of different tumors is with immunohistochemical analysis of cell-cycle specific antigens. Ki- 67 has been extensively studied in the last decade. Ki-67 is present in all proliferating cells and its role as a proliferation marker attracts considerable interest. The Ki-67 antibody reacts with a nuclear non-histone protein of 395 KD expressed in all phases of the cell cycle such as Synthesis(S), G1,G2, and M(mitotic) phases, but is non existing in G0 phase.[6] Various studies have shown the importance of Ki67 expression as an autonomous predictive and prognostic biomarker in breast cancer,[6,7] which is meticulously affiliated with the growth and invasion of breast cancer. Ki-67 positive breast cancers are more active in growth, more aggressive in invasion, and more metastatic.[7]

Gallen Consensus Conferences in 2011 and 2013, proposed the screening of Ki-67 for the assay of cellular proliferation, and for categorizing the breast tumor into luminal A and B subtypes;[8] hence Ki67 proliferative activity is considered as one of the factor influencing molecular subtypes.[9] Several research have revealed that Luminal subtype B (ER and/or PR positive, HER-2 positive, >14% Ki67 positive cells) patients with positive axillary lymph had a poorer prognosis and survival rate when compared with Luminal A breast cancer patients (ER and/or PR positive, HER2 negative, <14% Ki67 positive cells).[10] Ki-67 a monoclonal antibody considered as a prognostic and predictive marker has been found to have an importance in selecting both the neo-adjuvant and adjuvant therapy for hormone receptor positive breast cancer because of sensitivity of cancer cells to endocrine therapy or chemotherapy.[11]

This study attempts to evaluate routine use and value of Ki-67 as a prognostic marker, to identify any correlations between Ki-67 expression and other clinical and histopathological parameters, in the breast cancer.

2. Materials and Methods

A total of seventy cases of breast cancer were enrolled retrospectively from Yenepoya University, Mangalore during the study period. Clinical details were recorded from the case file. The Hematoxylin & Eosin slides were fetched from the repository of the Department of Pathology and morphological features like age, laterality, morphological, tumor size, grade, stage and lymph nodal involvement were analyzed. CAP protocol was followed for Histopathological diagnosis and all invasive breast carcinoma were graded from grade I to grade III according to Nottingham Histologic Score system. Suitable sections were further supplemented for immunohistochemical (IHC) study. Primary antibody Ki-67 (code-GM001, Mouse Monoclonal Antibody, Pathnsitu) and ER, PR and Her2/neu

(code-EP3, Rabbit Monoclonal Antibody, Pathnsitu) were used. The Polyexcel HRP (non-biotin, micro-polymer based) DAB Detection system was used with adequate positive and negative controls.

2.1. Interpretation of IHC

Scoring of IHC for ER, PR, Her2/neu was done according to the 2014 ASCO/CAP guidelines.

For ER, PR staining score was considered Positive if ≥1% Immunoreactive tumor cells present; Negative if <1% Immunoreactive tumor cells present

Her2/neu staining was scored as

0= no reactivity or membranous reactivity in < 10% of the tumour cells;

1+ = faint or barely perceptible membrane staining of > 10% of tumour cells;

2+= incomplete and weak-to-moderate circumferential membrane staining of > 10% of tumour cells or complete, intense, circumferential membrane staining in <10% of tumor cells ;

3+ = complete, intense, circumferential staining of > 10% of tumour cells.

Scores of 0 or 1+ was considered tumour negative for Her2/neu expression, score of 2+ considered as equivocal required further confirmation with FISH and 3+ was regarded as positive expression of Her2/neu[7.]

Ki-67 was recorded as positive in tumour cells as identified by the presence of brown-coloured product in the nucleus. Ten high power magnification fields (HPF) were checked for relatively 100 tumor cells in each field. The average of positive cells in 10 HPF was ascertained as percentage of positive cells. In biopsies the percentage of cells positive for 100 tumor cells was estimated and expressed as percentage. The percentage of positive cells were scored as <15% and >15%.

The slides were observed and scored by two independent observers with consensus reached in case of any discrepancy.

2.2. Statistical analysis

The data was entered and analyzed in SPSS. Frequencies and percentages of all the variables were computed. The Chi-square test was used to compare the association of expression of ER, PR and HER-2/neu, Ki67 and the macroscopic and microscopic characteristics of the tumors. The results were considered statistically significant if the *P*-value was <0.05.

3. Results

3.1. Clinic-pathological characteristic of patients

A total 70 (54-MRM & 16- biopsy) cases of breast carcinomas were included in the current study. All

seventy cases of breast carcinoma were found only in females with mean age being 48.66±13.05 years. Most of patients presented with complaints of lump in the breast, the left breast being the most frequently involved (44 cases) as compared to right (26 cases) with single focus of involvement in all. Based on the tumour size breast carcinoma cases were divided into two categories, those with an average diameter of >5cm (21cases) and <5cm (49 cases).

Among the histological types, IDC-NST (84.2%) (Figure 1) cases was the most prevalent, followed by 8.6% cases of medullary carcinoma, and 7.2% cases of other category (3 cases of mucinous and 1 cases of papillary (Figure 2) and 2 cases poorly differentiated carcinoma. IDC-NST (58 cases) had an associated component in some cases: DCIS (15 cases) and Paget's disease (2 cases).

All the carcinomas were graded from I- III based on glandular differentiation, pleomorphism and mitotic figures, with 17 in grade I, 35 in grade II and 18 cases in grade III with a significant correlation between grade and histological type (p value 0.003).

Pathologic Staging (pTNM) of primary tumour, lympho - vascular invasion and lymph node assessment was available in mastectomy/excision specimens (54 cases). The pathological assessment of primary tumors was categorized into T1 to T4 based on tumor size and direct extension into adjacent areas. The most prevalent primary tumour categories (T) was T 2 category (24 cases); while least prevalent was T4, with a significant correlation with tumour type and pT category with p value 0.015. Lympho-vascular invasion was noted in 24 (44%) of breast carcinoma, the lymph node positive status was observed in 26(48.1%)cases of which 15(12 -IDC, 2- medullary and 1-others) cases presented in N1 category; while N2(6 cases) and N3 (2 cases) category had only IDC as histological type.

3.2. Quantitative assessment of immuno staining and stratification

All 70 cases were subjected to Immunostaining with ER, PR, HER2 and Ki 67 antibody. The intensity of the staining was recorded and graded according to the standard protocol (Table 1).

Correlation of receptor expression status with histological types are summarized in the table (1) and their correlation found a significant association between histological types with PR and Ki67 status with P value being 0.051 and 0.018 respectively.

Based on their receptor expression status, molecular subtyping was done. The proportion of various molecular subtypes was as follows : luminal A 18(25.7%) cases, luminal B 16(22.9%), while HER2/neu enriched were 14(20.0%) and basal like 22(31.4%)cases. (Table 2)
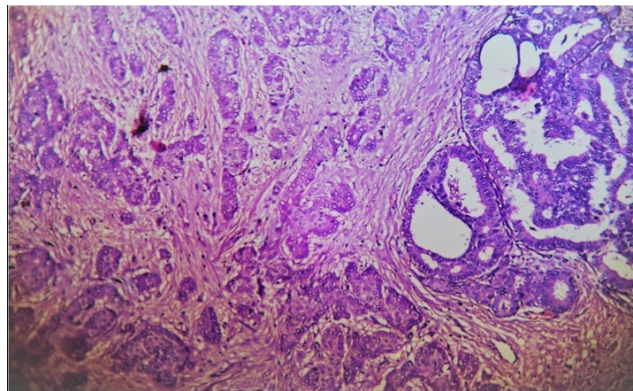
Fig. 1: Photomicrographic image of Infiltrating ductal carcinoma breast. (H&E, X10)
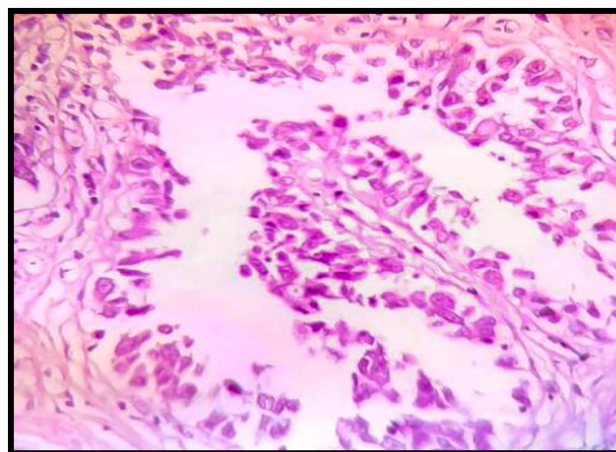
Fig. 2: Photomicrographic image of infiltrating papillary carcinoma breast (H&E,X40)
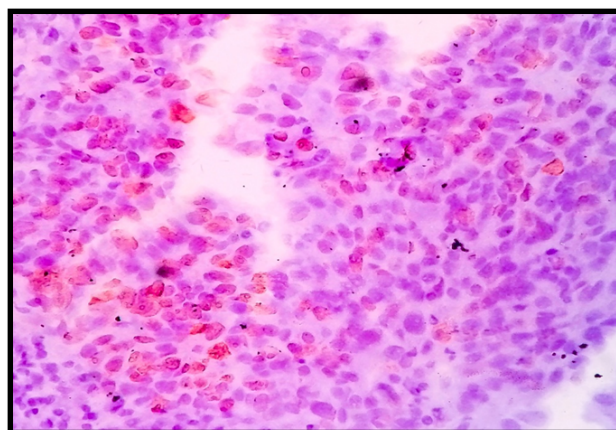
Fig. 3: Photomicrographic image of Ki 67 immunostain <15% in IDC(Ki 67x 40)

Table 1: Correlation of receptor expression status with histological types.

Estrogen receptor status	IDC-NST(58)	Medullary (6)	Others (6)	X^2_{test},P value
Positive	25(43.1%)	1(16.7%)	4(66.7%)	
Negative	33(56.9%)	5(83.3%)	2(33.3%)	P= .215
Progesterone receptor status				
Positive	15(25.9%)	1(16.7%)	3(50%)	
Negative	43(74.1%)	5(83.3%)	3(50%)	P= .051*
HER2/neu receptor status				
Positive	23(39.70%)	1(16.7%)	3(50%)	
Negative	35(60.3%)	5(83.3%)	3(50%)	P= .455
Ki67 receptor status				
<15%	44(57.9%)	3(50%)	6(100%)	
>15%	14(24.1%)	3(50%)	0(0%)	P= .019*

Table 2: Correlation of Ki 67 with grade, tumour size, pT category and luminal status

| Variables	Ki 67(<15%) (n=53)	Ki67(>15%) (n=17)	P value
Grade			
Grade I(n=17)	14(26.4%)	3(17.6%)	
Grade II(n=35)	25(47.2%)	10(58.8%)	P=0.720
Grade III(n=18)	14(26.4%)	4(23.6%)	
Tumour size			
≥5cm(n=21)	11(52.4%)	10(47.6%)	P=0.003*
<5cm(n=49)	41(85.7%)	07(14.3%)	
pT category(n=54)	(n=38)	(n=16)	
pT1(n=14)	10(26.3%)	4(25.0%)	
pT2(n=24)	18(47.4%)	6(37.5%)	
pT3(n=15)	09(23.7%)	6(37.5%)	P=0.694
pT4(n=1)	01(2.6%)	0(0%)	
Lymph node status (n=54)	(n=38)	(n=16)	
pN0 (n=28)	19(50.0%)	09(56.2%)	
pN1 (n=15)	11(28.9%)	04(25.0%)	
pN2 (n=06)	04(10.5%)	02(12.5%)	P=0.902
pN3 (n=02)	02(05.3%)	00(0%)	
pN4 (n=03)	02(05.3%)	01(6.2%)	
Luminal types	(n=53)	(n=17)	
Luminal A	17(32.1%)	1(05.9%)	
Luminal B	08(15.1%)	8(47.1%)	
HER2/neu enriched	13(24.5%)	1(5.9%)	P=0.007*
Basal like	15(28.3%)	7(41.2%)	

3.3. Association of Ki 67 expression with histopathological parameter

Correlation of Ki 67 expression with grade, tumour size, pT category and luminal status are summarized in table. The most prevalent Tumor grade was grade II and most of the cases (25) had <15% of expression while 10 cases had >15% expression of Ki67 without significant p value.

Size of the tumor; majority of cases observed in the study were of average tumor diameter < 5cm ; 14.3% of cases from <5cm tumour size and 47.6% cases with >5cm had high ki67 expression status with significant p valve (0.003 (<0.05)). Regarding pT category; majority of them (18 of 54 mastectomy cases) presented in pT2 category and showed<15% ki67 expression while 6 cases

from both pT2 and pT3 had >15% of ki 67 expression. Under pT4 there was only 1 cases which showed <15%. There was no significant association with pT category and Ki 67 expression with p value >0.05 (Table 2)

In relation to the lymph node status, Ki67 showed expression irrespective of positive or negative lymph node involvement, with no meaningful association noted between these parameters according to statistical analysis. Among luminal subtypes Basal like was more prevalent followed by Luminal A and luminal B. High expression of Ki67 was seen with mainly in Luminal A (32.1%) followed by Basal like (28.3%), Her2/neu enriched (25.5%) and Luminal B (15.1%) and found a significant association between Luminal subtypes and Ki67 expression status with p value
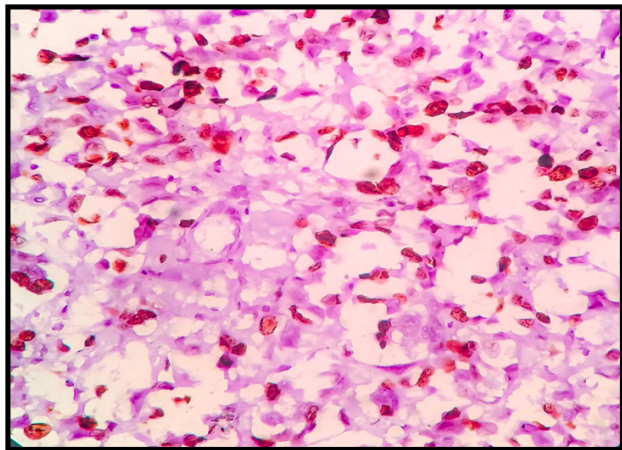
Fig. 4: Photomicrographic image of Ki 67 immunostain >15% in IDC(Ki 67x 40)

of 0.007 (Table 2).

4. Discussion

The overall incidence of breast cancers has decreased in the developed countries because of the awareness and early screening. [12,13] However, the incidence of biologically intrusive tumors, with high-proliferative index subtypes has increased in developing countries, and also in African-American populations in developed countries. [13,14] So, it is important to assess the predictive markers like ki67 expression apart from traditional prognostic parameters to identify the patients who would benefit the most from chemotherapy. [13] Therefore, Ki67 is routinely used as surrogate marker to assess the response.

The current study focused on assessment of ki67 expression association with other clinicopathological parameters. The mean age our study was 48 years, which was concordant with other studies (Yadav *et al.* and Zineb Bouchbika et al.) [15,16] The most common morphological type of breast carcinoma in this study was infiltrating ductal carcinoma NST which was found to have similar findings by Thiyagarajan M *et al.* and others (Sharathkumar). [17,18] With regards to tumor grading majority of our cases were in grade II followed by III and I; same results was observed in various studies Thiyagarajan M *et al.* and Geetamala *et* al. [17,19] Tumor size in other studies [18,20] have categorized size as <2cm and >2cm where as in our study we considered as mean diameter of 5cm as cut off for tumor size.

Nodal status in our study was prevalent in N0 (28 case) followed by N1, N2, N4 and N3 with similar observation in Gulalco et al study. [8] Based on the prognostic receptor expression status luminal stratification was done and observed in the following order Luminal A followed by Basal like, Her2/neu enriched and luminal B and found to be concordant with Gulalco et al study. [8] We found significant association between ki67 expression status with histological type, tumor size and PR receptor expression; were as other studies by (Mohammad A *et al* and Sharathkumar et al) [18,19] found a significant association with tumor size, lymph node, stage and NSBR grading. We also noted a significant association between Ki67 and luminal subtypes and found to be concordant with studies in the literature. [2] Thus, Ki 67 is expressed autonomously in highly metastasizing tumors, with high grade tumours and proportionately in larger tumour sizes based on current and other studies in the literature. This probably is due to the high proliferative ability of tumor cells and also carries dismal prognosis. [21] This explains that Ki 67 expression status can be a good prognostic marker; as it reflects high proliferative potential in breast cancer.

5. Conclusion

The Ki67 being a cellular marker for proliferation can be predictive and prognostic marker exclusively in managing breast cancer patients and asses their treatment response. As it is easily available in the laboratory, it can be used routinely in all breast cancer cases to stratify further and determine the treatment.

6. Acknowledgment

7. Funding

8. Conflict of Interest

None.

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