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A study on the role of proliferative index marker Ki67 in breast carcinoma

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ABSTRACT

Background. Breast cancer is the most common malignancy and the leading cause of cancer death among women worldwide. This study was conducted to establish early diagnosis and therapy, thus improving prognosis and overall survival rate. *Objectives*. To assess the levels of proliferation index marker Ki67 and hormone receptor status in patients with breast carcinoma and to correlate Ki67 with ER, PR, Her2neu status, as well as to correlate Ki67 and hormone receptor status with onco-pathological parameters. *Materials and Methods*. 392 patients diagnosed with breast carcinoma over a period of four years were included in the study. Data regarding patient's identification, history, clinical finding, investigations and histopathological report were recorded in a specific proforma. *Result*. Breast cancer was seen to affect people in the older age group, and invasive ductal carcinoma of nonspecific type was the most common type. Most patients had unifocal tumor with grade 2 disease. Stage II disease was most common stage among the patients studied and Luminal B subtype was the predominant type of molecular subtype. Most of the patients were Ki67 positive, i.e., Ki67 >14%. The correlation of Ki67 with estrogen receptor, progesterone receptor, tumor size, mitotic count, lymph node ratio, lymphovascular invasion and extra-nodal extension was found to be statistically significant. *Conclusions*. There exists a statistically significant, positive correlation between breast cancers, its onco-pathological parameters, proliferation index marker Ki67 and hormone receptor status. Therefore, the judicious use of these tests (Ki67 is an old marker of proliferation but not routinely used) will help in the early appropriate treatment of patients and contribute to the assessment of treatment response and prognosis.

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Introduction

Breast cancer is the most common malignancy among women and is the leading cause of death from cancer among women worldwide. The number of patients with breast cancer is expected to increase in the developing countries in the coming years [1]. Breast carcinoma is a heterogeneous disease with different clinical behavior in different people. It has a wide spectrum of clinical, pathological and molecular features with different prognostic and therapeutic implications [2-4].

The management of breast cancer depends on the knowledge of prognostic factors [5]. Prognosis depends on the patient's age, various clinical parameters, size of the tumor, histological type, grade, lymph node metastasis, Estrogen Receptor (ER), Progesterone Receptor (PR), Her2neu Receptors [6-8]. Tumor size, grade, lymph node metastasis, ER, PR status are first generation prognostic factors while Her2neu growth receptor factor is a second-generation prognostic factor [5]. Nuclear proliferation index marker Ki67 is a marker of the rate of proliferation of malignant tumors. It is found to be a predictive as well as a prognostic marker for breast carcinoma [1]. Hormone receptors like ER, PR and Her2neu are known to be good predictive factors of breast cancer response to hormone therapy. Tumors expressing estrogen and progesterone receptors are generally well differentiated and are low grade tumors [2].

The objective of this study was to assess the role of proliferation marker Ki67 as a prognostic marker in Breast Cancer and correlate with the hormone receptors (ER, PR) and Her2neu status as well as various oncopathological parameters like tumor size, grade, histologic type to predict the prognosis and response to treatment.

Materials and Methods

A hospital based cross sectional study was conducted in the Department of General Surgery at A. J. Institute of Medical Sciences and Research Centre, Mangalore, from July 2018 to June 2022.

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The study was approved by the Ethical Committee of the Institute. The patients were explained about the study protocol and written informed consent was obtained.

Inclusion criteria. Female patients more than 18 years of age diagnosed with breast cancer and giving informed consent to the study were included.

Exclusion criteria. Patients less than 18 years of age and those not willing to participate in the study were excluded.

After obtaining clinical history from the patients and thorough clinical examination, tissue sample was obtained. This was fixed with formalin, embedded with paraffin and stained with hematoxylin and eosin for histopathological typing and grading. Histopathological grading was done according to the Nottingham modification of the Bloom Richardson grading system which is based on three morphological features – tubule formation, mitotic count and nuclear size. Patients diagnosed with breast cancer on histopathological examination were further evaluated with immunohistochemistry by using antibodies against estrogen receptors, progesterone receptors, her2neu status and Ki67. Breast cancers were further classified based on ER, PR and Her2neu as Luminal type A (ER, PR positive, Her2neu negative), Luminal type B (ER, PR positive, Her2neu positive), Her2neu enriched (ER/PR negative, Her2neu positive) and Basal like (ER, PR, Her2neu negative). Ki67 was further classified as negative and positive. Further ER, PR Her2neu status was correlated with other oncopathological factors like tumor size, grade and histologic type. Hormone receptor status was also correlated with Ki67 levels.

Baseline data regarding the patient's clinical presentation, histopathological reports, Ki67, Hormone receptors was represented in percentage and diagnosed. Also, Chi square test was used to test the significant association between Ki67 with the Breast cancer outcome and hormone receptor status.

Results

392 patients were included in the study in the age group of 31 to 80 years. The maximum number of patients was seen to be in the age group of 41 – 50 years. 196 (50%) were right side malignancies and 196 (50%) were left side malignancies. 93.9% were unifocal tumors while 6.1% were multifocal tumors. 356 patients underwent Modified Radical Mastectomy while 36 patients underwent Breast Conservation Surgery.

98% of the patients had Invasive ductal carcinoma and 2% of the patients had Ductal carcinoma in situ. 71.4% were T2 tumors, i.e., >2cm but <5cm in size, followed by patients with T3 tumors 16.3%. T1 and T4 tumors accounted for 7.1% and 5.1% of the disease respectively. 28.6% patients were Grade I tumors, 66.3% were grade II tumors and 5.1% were grade III tumors. 4.1% patients were in stage I of the disease, 66.3% in stage II disease, 28.6% in stage III disease and 1% in stage IV disease.

Lymphovascular invasion was present in 39.8% patients and extranodal extension in 21.4% patients. Ductal carcinoma in situ component was present in 40.8% patients. Necrosis was present in 21.4% patients. Deep margins were seen to be positive in 5.1% patients (Figure 1).

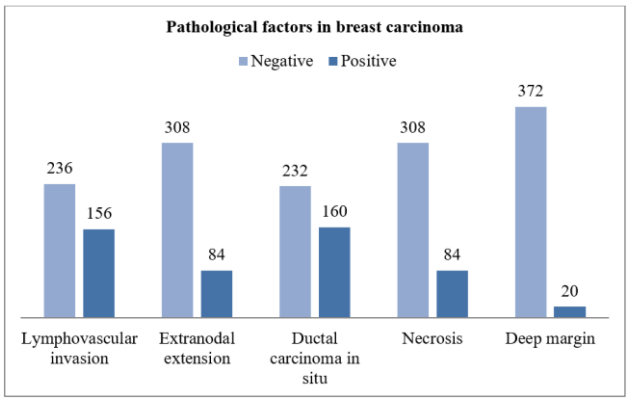

Figure 1. Pathological factors in breast carcinoma

53.1 % patients had Estrogen receptor positive, 48% had progesterone receptor positive and 26.5% had Her 2 neu growth factor. Based on molecular subtype - 18.4% were of Luminal A type, 37.8% were Luminal B type, 17.3% were Her 2 enriched and 26.5% were Basal like (Figure 2).

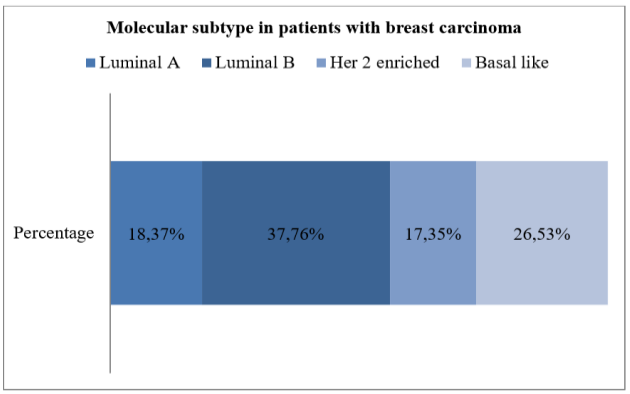

Figure 2. Molecular subtype in patients with breast carcinoma

Ki67 was seen to be positive in 316 (80.6%) of the patients and was negative in 76 (19.4%) of the patients (Table 1). No statistically significant association was seen on correlating the type of carcinoma with Ki67 levels.

Table 1. Ki67 positivity in patients with breast cancer

	Positive	Negative
Ki67	316	76

The Ki67 positivity increased with the size of the tumor being 42.8% positive in T1 tumors, 81.4% positive in T2 tumors, 87.5% positive in T3 tumors and 100% positive in T4 tumors. The p value of correlation of tumor size with Ki67 was 0.044 which was statistically significant (Table 2). The correlation of Ki67 with the stage and grade of the tumor was not found to be statistically significant.

Table 2. Correlation of Ki67 with tumour size

	Ki67 +	Ki67 -
T1	12(42.8%)	16
T2	228(81.4%)	52
T3	56(87.5%)	8
T4	20(100%)	0

There was a statistically significant correlation of ER (p=0.002) and PR (p=0.047) with Ki67 but not Her 2 neu (p=0.238). There was no statistically significant association of factors like age, side of malignancy, focality, tumor grade and pathological factors like lymphovascular invasion, extranodal extension, ductal carcinoma in situ, necrosis and deep margin involvement with Ki67 levels (Table 3).

Table 3. Correlation of Ki67 with age, side, focality, grade, lymphovascular invasion, extranodal extension, ductal carcinoma in situ, necrosis and deep margin

	P value (Chi square / Fishers exact test)
Age	0.507
Side	0.798
Focality	0.215
Grade	0.488
Lymphovascular invasion	0.819
Extranodal extension	0.965
Ductal carcinoma in situ	0.695
Necrosis	0.505
Deep margin	0.260

There was a significant association present between the stage of the tumor and mitotic count in the cells, lymph node ratio, lymphovascular invasion, extranodal extension and the type of surgery the patient underwent but no association was found with the histologic type of the tumor, grade, DCIS component, necrosis, deep margin positivity and the molecular subtype (Table 4).

Table 4. Correlation of stage of the tumour with onco-pathological parameters

	P value
Histologic type of the tumour	0.890
Grade	0.633
Mitotic count	**0.007**
Lymph node ratio	**0.000**
Lymphovascular invasion	**0.000**
Extranodal extension	**0.044**
Ductal carcinoma insitu	0.685
Necrosis	0.164
Deep margin	0.907
Luminal type	0.487
Type of surgery	**0.027**

Discussion

Marker of proliferation Ki67 (MKi67) also known as KIA, MIB, MIB-1, PPP1R105, Antigen Ki67, Proliferation related Ki67 Antigen encodes a nuclear protein associated with and necessary for cellular proliferation. It is seen to increase as the cells prepare to divide into new cells.

With a half-life of about 1 to 1.5 hours, Ki67 is present during all active phases of the cell cycle (G1, S, G2 and M), but is absent in resting cells (G0). Thus, the expression of protein Ki67 activity is associated with the proliferative activity of intrinsic cell population in malignant tumors. It can be determined by mitotic counting, flow-cytometric determination of synthetic phase function and immunohistochemistry [9-11]. Estimation of Ki67 is done by counting the number of positive and negative cells. Based on the percentage of cells that stain positive, the tumors are further classified as low, intermediate and highly proliferating according to the Ki67 labelling index of <15%, 16%-30% and >30% respectively. The tissue used for the evaluation of grade is the Formalin Fixed Paraffin Embedded (FFPE) tissue sample.

Ki67 is considered to be a proliferative marker which describes the proliferation of the tumor cell thus providing information and prediction of the response to chemotherapy. It also gives information on the prognosis for breast cancer patient who have received neoadjuvant chemotherapy. A significant correlation exists between the grade of the tumor and Ki67 score. Triple negative tumors have shown to have higher positive levels of Ki67 and ER, PR positive cases have shown to have low Ki67 levels. But it has not shown to have any relation with the mitotic count, lymph node status and lymphovascular invasion of the tumor [12,13].

Ki67 expressed immunohistochemically has both predictive as well as prognostic value for breast cancer [14-16]. It is included as a part of Oncotype DX which is a clinically validated set of 21 prospectively selected genes including sixteen cancer related genes and five reference genes. It is used to predict the risk of recurrence and chemotherapy benefit in node negative, ER positive women [17,18].

The advantages of using Ki67 include high sensitivity as compared to mitotic figure count as it identifies cells in the active phase of the cell cycle, cost effectiveness [19,20]. Also, it can be used in early-stage breast cancer to plan adjuvant treatment, i.e., chemotherapy in patients who are hormone responsive [21,22].

Similar results have been obtained in other studies. A study done by Dirican et al. at Mustafa Kernal University, Turkey on 224 patients, the results obtained were similar with 149 (66.8%) of the patients being Ki67 positive and 74 (33.2%) of the patients being Ki67 negative. As in our study it showed a significant correlation of Ki67 and hormone receptor status with tumor size [23]. Also, a

retrospective study done by Kaur et al. on a sample of 50 patients showed similar results with 42 (84%) patients being positive for Ki67 and 8 (16%) being negative for Ki67. It showed that Ki67 positivity increased with the mitotic count as in our study [13]. In a study conducted by Azamris et al. at University of Andala, Indonesia on a sample of 96 patients, similar results were obtained with Luminal B type (40.8%) being the most common molecular subtype [12]. Soliman et al. in their study found lymphovascular invasion (p=0.05) and extranodal extension (p=0.00) correlating with Ki67 and hormone receptor status [24].

The results of this study showed that breast cancer was seen to affect people in the older age group and invasive ductal carcinoma of nonspecific type was the most common type. Most of the patients had unifocal tumor with grade 2 disease. Stage II disease was most common stage among the patients studied and Luminal B subtype was the predominant type of molecular subtype. Most of the patients were Ki67 positive, i.e., Ki67 >14%. The correlation of Ki67 with Estrogen receptor, progesterone receptor, the tumor size, mitotic count, lymph node ratio, lymphovascular invasion and extra-nodal extension was found to be statistically significant.

Conclusions

In the ever-developing world with ever increasing number of patients with breast cancer, the knowledge of prognosis of the disease and its response to treatment plays a vital role in both – management of the patient and their counselling. Ki67 though an old marker of proliferation is still not being used in common by surgeons and oncologists as a routine test. As there is a statistically significant, positive correlation between breast cancers, its oncopathological parameters, proliferation index marker Ki67 and hormone receptor status the judicious use of these tests will not only help in the early appropriate treatment of the patients but will also help assess their prognosis and response to treatment.

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Department of General Surgery, A. J. Institute of Medical Sciences and Research Centre, Mangalore.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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