Original Article

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Expression of Ki67 as a Prognostic Marker in Invasive Breast Carcinoma

Pathology Section

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ABSTRACT

Introduction: Breast cancer is the most common cancer in women of developed countries. It accounts for 16% and 22.9% of cancers and invasive cancers, respectively. About 18.2% of deaths related to cancer amongst men and women have been attributed to breast cancer. Every year, more than 10,000 incipient breast cancer patients reckon to be diagnosed in India.

Aim: To evaluate the role of Ki67 as a predictive biomarker in invasive breast cancer patients and to study its correlation with various molecular subtypes of breast cancer.

Materials and Methods: A cross-sectional and descriptive study, of 100 cases of breast carcinoma coming to Histopathology section in Department of Pathology, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, India, was carried out over a period of one year, from 2013 to 2014. Patients were randomly selected for the study.

Results: Sixty nine patients showed high proliferating index of Ki67 (>30%), followed by 20% patients that showed low proliferating index (≤15%) and 11% patients showed intermediate proliferating index (16-30%). Maximum patients were of Luminal A subtype, of which 50% showed high proliferating index. In the Luminal B subtype, 64% patients showed high proliferating index and in the Her-2 subtype, 73.9% showed high proliferating index. Of Triple Negative Breast Cancer (TNBC) subtype 86.3% showed high proliferating index. Majority of patients were of IDC (n=94). Out of these, 64(68%) patients showed high proliferating index for Ki67 immunostaining. Out of 5 patients of ILC, 3 (60%) showed high proliferating index for Ki67 immunostaining. One case of mucinous carcinoma showed low proliferating index for Ki67 immunostaining.

Conclusion: High proliferating index tumours were mostly large in size. We could not find any correlation with various molecular subtypes of breast carcinoma. Though, not statistically significant, we observed that TNBC were most aggressive and showed highest rate of proliferation and Ki67 expression.

High levels of Ki67 were associated with TNBC, Her2/neu and Luminal B while low and medium levels with Luminal A subtype. Ki67 immunostaining can be used as an important biomarker for proliferation.

Keywords: Molecular markers, Proliferating index, Receptors

INTRODUCTION

Breast cancer is the most common cancer in women of developed countries [1]. About 16% cancers and 22.9% invasive cancers of breast occur in women and additionally, 18.2% of deaths cognate to breast cancer amongst men and women [2]. Every year, more than 10,000 incipient breast cancer patients reckon to be diagnosed in India [3].

The extent of breast cancer involves a series of processes starting with epithelial hyperplasia, which is then followed by subsequent evolution to carcinoma in situ, invasive carcinoma, and finally into a metastatic disease. Ductal tumours represent 80% of tumours and lobular tumours account for 10 to 15% of cases.The major invasive tumour types include infiltrating ductal, invasive lobular, ductal/lobular, mucinous (colloid), tubular, medullary and papillary carcinomas. Patients with invasive ductal carcinoma present higher lymphatic involvement and worse prognosis than less common types of breast carcinoma [4].

The importance of several molecular markers in, especially the steroid receptors [Estrogen Receptor (ER), Progesterone Receptor (PR)], Her2/neu, Ki67 and p53 have gained considerable interest during recent years, not only as prognostic markers, but also as predictors of response to therapy [5]. The Ki-67 antigen is not expressed in quiescent or resting cells of G0 and early G1 phases, but is expressed only in mid-G1,S,G2, and M phases of proliferating cells [4]. The role of Ki-67 has been of great interest as a potential marker of cell proliferation. This has mostly been attributed to its absence in quiescent cells and its universal expression in proliferating tissues [6].

The prediction of recurrence free survival for breast cancer in patients may be improved by measuring the tumour Ki67 expression after short term endocrine treatment. Therefore, before undergoing surgery, efficient markers of tumour cell

proliferation such as Ki-67, can be assessed after a short duration, for instance after 2 weeks of primary breast cancer treatment [7].

MATERIALS AND METHODS

A cross-sectional and descriptive study was carried out in Department of Pathology, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, India, over a period of one year, from 2013 to 2014. Patients were randomly selected for the study. Signed informed consent, was taken prior to the procedure.

Total 100 cases of breast carcinoma coming to histopathology section of Department of Pathology were consecutively selected for the study purpose. Patients with benign breast disease were excluded from the study.

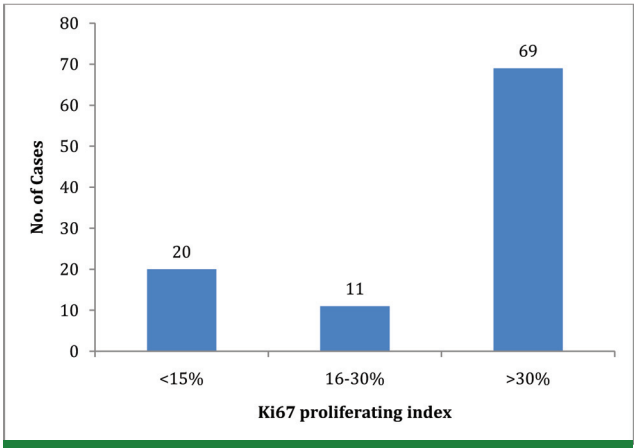
Histopathological analysis was done using Harris Hematoxylin and Eosin (H & E) stain. Immunohistochemistry was done using Ki67 antibody (BioGenex Super Sensitive Detection Systems). This antibody kit was intended for invitro diagnostic use for the chromogenic detection of antigen-antibody binding reactions in tissue specimens.

STATISTICAL ANALYSIS

Structured study instrument (case reporting format) was developed and used to generate data. Data analysis was done using SPSS and Microsoft Excel software.

RESULTS

The age wise distribution of breast carcinoma cases (n=100) showed that maximum number of cases (n=27; 27%) were reported in the third decade, followed by 22 cases (22%) in the fourth decade. Patients presented with their right breast fairly more involved (52%) than the left breast (48%). Maximum number of patients presented with lump in breast [n=57(57%)]. Out of which 30(30%) patients presented with a painful lump, 12(12%) patients presented with lump along with nipple discharge, and one patient presented with nipple discharge only. 95(95%) patients were married and 5(5%) were unmarried. Seven (7%) patients had a positive family history in their first degree relatives. In 54% of the patients (n=54) tumour was present in the Upper outer quadrant, followed by and 26% patients in Upper inner quadrant of breast. The lower outer quadrant was involved by 11% and subareolar regions were involved in 6% (n=6) of the patients. The least involved quadrant [n=3(3%)] was the lower inner quadrant. Distribution of breast carcinoma was done according to the histological diagnosis. Infiltrating Ductal Carcinoma (IDC) was the most frequent occurring tumour [n=94(94%)] followed by Infiltrating Lobular Carcinoma (ILC) comprising of 5(5%) cases and 1 case (1%) of Mucinous carcinoma. Grading of IDC according to modified Bloom Richardson scoring system showed that out of 100 cases, 69% cases of IDC were categorized in Grade II, 20% cases were of Grade III, and 11% cases of Grade I type [Table/Fig-1].

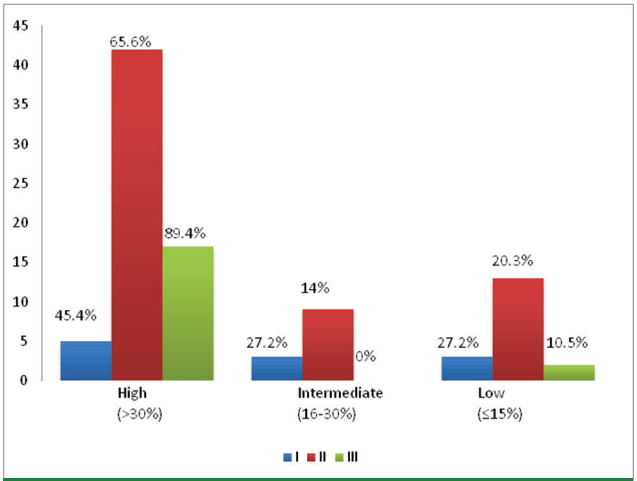


[Table/Fig-1]: Scoring of Ki67 staining in all patients (n=100).

Distribution of breast carcinoma cases based upon molecular subtypes showed that maximum patients in the study were under the category of luminal A subtype [n=30(30%)], followed by luminal B subtype [n=25(25%)] and Her-2 type category [n=23(23%)]. 22 (22%) patients were in the triple negative category.

Association of Ki67 scoring in relation to age of patients (n=100) showed that 12 patients were under 35 years of age, out of which 7(58.3%) showed strong positivity, whereas, 2(16.6%) showed low Ki67 immunostaining. The other subgroup with patients over 35 years of age (n=88). 60(68.1%) patients showed strong positivity whereas, 18(20.4%) showed low positivity for Ki67 immunostaining. There was no association of gender with Ki67, which was validated by the Independent samples 't'-test.

A total 100 patients, 96(96%) were females. Out of these, 67.7% patients had strong positivity for Ki67 immunostaining. The other subgroup comprises of male patients (4%) of which 2 (50%) patients showed strong positivity with Ki67 immunostaining.



[Table/Fig-2]: Shows that likelihood ratio is significant. Ki67 scoring in association with grade of Infiltrating Ductal Carcinoma (IDC) (n=94).

	Value	df	p-value
Likelihood Ratio	13.801	6	0.032
Pearson Chi-Square	10.989	6	0.089

[Table/Fig-3]: Goodness-of-Fit Tests[a,b].
a. Model: Poisson
b. Design: Constant + KiGrade + Mcclass

Ki67t	Sum of Squares	df	Mean Square	F	p-value
Between Groups	2890.965	3	963.655	2.915	0.038
Within Groups	31740.223	96	330.627		
Total	34631.188	99	-		

[Table/Fig-4]: Statistical analysis using ANOVA.

Variables			Mc-class	Ki Grade
Kendall's tau_b	Mc class	Correlation Coefficient	1.000	0.254**
		p- value (2-tailed)	-	0.004
		Number of observations	100	100
	Ki Grade	Correlation Coefficient	0.254**	1.000
		p- value (2-tailed)	0.004	-
		Number of observations	100	100
Spearman's Rho	Mc class	Correlation Coefficient	1.000	0.284**
		p- value (2-tailed)	-	0.004
		Number of observations	100	100
	Ki Grade	Correlation Coefficient	0.284**	1.000
		p- value (2-tailed)	0.004	-
		Number of observations	100	100

[Table/Fig-5]: Correlations.
** Correlation is significant at the 0.01 level (2-tailed).

Majority of patients were of IDC (n=94). Out of these, 64(68%) patients showed high proliferating index for Ki67 immunostaining. Out of 5 patients of ILC, 3 (60%) showed high proliferating index for Ki67 immunostaining. One case of mucinous carcinoma showed low proliferating index for Ki67 immunostaining.

[Table/Fig-2] shows that out of 64 patients of IDC Grade II, 42 (65.6%) showed high proliferating index, whereas 13 (20.3%) showed low proliferating index for Ki67 immunostaining. The remaining 9 (14%) patients showed intermediate proliferating index for Ki67 immunostaining. Out of 19 patients of IDC Grade III, 17 (89.4%) showed high proliferating index for Ki67 immunostaining. Out of 11 patients of IDC Grade I, 5 (45.4%) showed high proliferating index for Ki67 immunostaining. This table shows that the mean Ki67 levels are significantly different in different molecular classification.

[Table/Fig-3-6] shows the association of ER, PR and Her-2/neu with Ki67 immunostaining. Maximum patients (n=30) were of Luminal A subtype, out of which 15(50%) showed high proliferating index. In the Luminal B subtype, 16(64%) patients showed high proliferating index and in the Her-2 subtype, 17(73.9%) patients showed high proliferating index. Of TNBC subtype 19(86.3%) patients showed high proliferating index for Ki67 immunostaining [Table/Fig-7-13].

DISCUSSION

Breast cancer is a common type of malignancy in women and its incidences in India have steadily increased over the years [8].

Assessment of overall prognosis and treatment decision is attributed to the role of prognostic factors, including growth factors and oncogenes [9].

Tumour proliferation marker Ki67 is widely used as a prognostic marker in cancer patients [7].

Ki Grade	Mc class	Observed		Expected		Residual	Standardized Residual	Adjusted Residual	Deviance
		Count	%	Count	%				
Low	Luminal A	10	10.0%	6.000	6.0%	4.000	1.633	2.182	1.489
	Luminal B	5	5.0%	5.000	5.0%	.000	.000	.000	.000
	Her 2 neu	2	2.0%	4.600	4.6%	-2.600	-1.212	-1.544	-1.367
	TNBC	3	3.0%	4.400	4.4%	-1.400	-.667	-.845	-.709
Medium	Luminal A	5	5.0%	3.900	3.9%	1.100	.557	.713	.533
	Luminal B	4	4.0%	3.250	3.3%	.750	.416	.515	.401
	Her 2 neu	4	4.0%	2.990	3.0%	1.010	.584	.713	.555
	TNBC	0	0%	2.860	2.9%	-2.860	-1.691	-2.052	-1.691
High	Luminal A	15	15.0%	20.100	20.1%	-5.100	-1.138	-2.363	-1.192
	Luminal B	16	16.0%	16.750	16.8%	-.750	-.183	-.368	-.185
	Her 2 neu	17	17.0%	15.410	15.4%	1.590	.405	.802	.398
	TNBC	19	19.0%	14.740	14.7%	4.260	1.110	2.184	1.062

[Table/Fig-6]: Cell Counts and Residuals[a,b].
a. Model: Poisson b. Design: Constant + KiGrade + Mcclass

Hormone treatment of patients with breast cancer widely depends on the ER and PR stats of primary tumours. ER and PR negative tumours show virtually no response, whereas patients with ER and PR positive tumours exhibit a significantly higher response. Heterogeneity of hormone receptors within the same tumour mass has also been reported in some studies [10].

In our study, maximum number of patients were (49%) between 31-50 years of age and the mean age of diagnosis was 24.5 years.

In a study done on a total of 11,780 breast cancer patients in India, revealed that more than 60% of patients were in the age group of 31-50 years [11]. In another study, Mudduwa LK et al., found the mean age of occurrence of breast cancer to be 52.5 years [12]. In our study, maximum patients were in the fourth decade that is in agreement with the general data as breast cancer occurs a decade earlier in Indian women as compared with the women of developed countries [13].

A few reviews have archived that breast carcinoma is somewhat more successive in the left breast than in the right [14]. In our study 52% had right breast involvement as compared to 48% with left breast involvement, which is discord according to the other reported studies.

According to the American Cancer Society, the most common symptom of breast cancer is a lump or mass. Conceivable indications of breast cancer include redness, swelling of the entire or part of a breast, breast or nipple pain, nipple discharge, nipple retraction, thickening of the nipple or breast skin, dimpling, and skin irritation. However, breast cancers can be tender, soft, or with regular edges or also painful. But, a mass that is painless, hard, and has irregular edges is more likely to be cancerous [15]. The most common presentation in our study was appearance of a breast lump in 57% (57/100) patients which was also associated with pain and nipple discharge in 30% and 12% patients respectively.

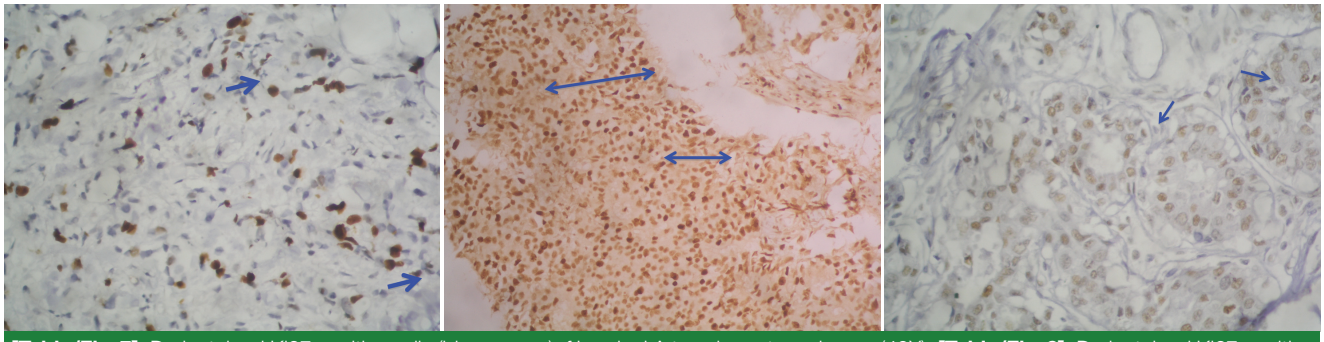
Osbrone C et al., deduced in a review that unmarried ladies will probably be determined to have breast cancer Stage II-IV versus Stage I and insitu malignancy and they were at an expanded danger of death from Breast Cancer [16]. In our study, there were 5 unmarried patients, of which all had infiltrating ductal carcinoma, Stage II-III, which was concordant with the above study.

The risk of breast cancer increases with the number of affected first-degree relatives, especially if the cancer occurred at a young age. Most family risk is considered to be probably due to interaction of low-risk susceptibility genes and some non genetic factors [17]. In our study, 7% patients had positive family history of breast cancer. All these patients had history of breast cancer in their first degree relatives.

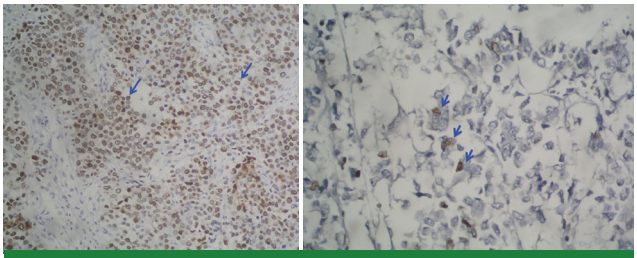
The location of breast carcinoma is usually indicated in relation to breast quadrants. Approximately 50% occur in the upper outer quadrant, 15% in the upper inner quadrant, 10% in the lower outer quadrant, 5% in the lower inner quadrant, 17% in the central region (within 1cm if the areola), and 3% are diffuse, either massive or multifocal [16]. Our study had 52% cases in the upper outer quadrant followed by 26% cases in the upper inner quadrant, and 11%, 6% and 3% in lower outer, sub-areolar region and lower inner quadrant respectively.

According to Kumar et al., five main histological types were IDC, which comprised of maximum number of cases, followed by lobular carcinoma, tubular carcinoma, mucinous carcinoma and medullary carcinoma [18]. In our study, it was observed that 94% cases were of IDC, 5% were infiltrating lobular carcinoma and there was 1 case (1%) of mucinous carcinoma.

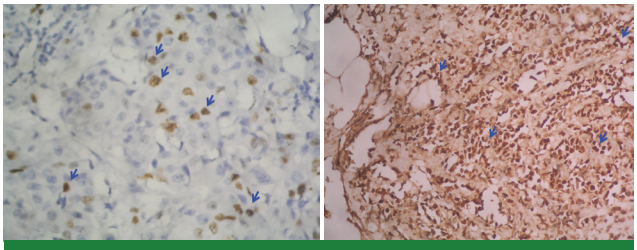
The two most broadly utilized frameworks throughout the years for the microscopic grading of invasive carcinoma have been those of Bloom and Richardson, constructed chiefly with respect to architectural features (degree of tubule arrangement) and Black, in view of the level of nuclear atypia. These are determined by visual microscopic examination of routinely stained sections. Elston proposed the Nottingham modifications of the Bloom-Richardson system which also subsumes the evaluation of mitotic activity. By adding up the scores for tubule formation, nuclear pleomorphism, and mitotic count, each of which is given 1,2, or 3 focuses, a grade is acquired [19]. In our study 94 (94%) cases of breast cancer were graded according to Nottingham Modification



[Table/Fig-7]: Dark stained Ki67 positive cells (blue arrows)of Luminal A type breast carcinoma (40X). **[Table/Fig-8]:** Dark stained Ki67 positive cells (blue arrows) of Luminal B type breast carcinoma (40X). **[Table/Fig-9]:** Dark stained Ki67 positive cells (blue arrows) of Her-2/neu type breast carcinoma (40X).



[Table/Fig-10]: Dark stained Ki67 positive cells (blue arrows) of TNBC type breast carcinoma (40X). **[Table/Fig-11]:** Low proliferating Ki67: Only very few dark stained Ki67 positive cells (blue arrows) (40X).



[Table/Fig-12]: Intermediate proliferating Ki67: Few dark stained Ki67 positive cells (blue arrows) (40X). **[Table/Fig-13]:** High proliferating Ki67: Many groups of dark stained Ki67 positive cells (blue arrows) (40X).

of Bloom Richardson system, out of which 68% cases were in Grade II, followed by 20% cases in Grade III and 12% cases in Grade I. However, in a large study from India, Shet et al., found that 70% cases belonged to Grade III while 28% cases were seen in Grade II and only 2% cases were seen in Grade I. This was possibly due to the fact that this study was done in a tertiary cancer hospital wherein large numbers of referral cases were in very advanced stage [11].

The Ki67 antigen is a labile, non histone nuclear protein that is firmly connected to the cell cycle. It is communicated in mid-G1, S, G2, and M phases of multiplying cells, however, not in quiet or resting cells of the G0 and early G1 phases [5]. A study evaluated the predictive and prognostic significance of Ki67 in patients with invasive breast cancer receiving neoadjuvant treatment and concluded that Ki67 had predictive and prognostic value and was a feasible marker for clinical practice [20]. In our study on Ki67 immunostaining, 69% (n=69) patients showed highly proliferating index (Ki67 >30%), 20% (n=20) patients showed low proliferating index (Ki67 ≤15%) and 11% (n=11) patients showed intermediate proliferating index (Ki67= 16%-30%).

IHC was created over 30 years back and it is utilized for arrangement of breast malignancy into ER positive and ER negative tumours. Breast cancer can be classified into three sub-groups (i) ER/PR positive (ii) ER negative or HER-2 positive and triple negative (ER, PR and HER-2 negative) on the basis of receptor status [21].

In Stage I breast cancer, the absence of ER is by all accounts the most imperative component for foreseeing prior repeat and poorer survival. In Stage II breast cancer, PR content has all the earmarks of being superior to ER content in foreseeing illness free survival and it is as critical in anticipating general survival. The advantages of adjuvant endocrine treatment are better anticipated by the nearness or non attendance of PR than by the nearness or non appearance of ER [22]. Her-2 is a trans-membrane tyrosine kinase receptor belonging to a family of epidermal growth factor receptors. It is amplified in 20 to 30% of breast cancers and is considered a marker of poor prognosis [5].

In our study, maximum patients were Luminal A type (n=30, 30%), followed by Luminal B type (n=25, 25%), the Her2 type category (n=23, 23%) and TNBC (n=22, 22%). A cross-sectional review led at GMC Medical College, Bhopal, on 70 invasive breast carcinoma, found the most widely recognized molecular subtype to be Luminal A (27.1%), trailed by Luminal B, Her-2/neu positive (25.7% each) and basal-like molecular subtype (15.7%). The slightest regular subtype was normal-like including 5.7% of aggregate cases [23]. Our study comprised of maximum number of Luminal. A subtype which was concordant as proved by other studies.

In our study, out of 100 patients, 88 (88%) were above 35 years of age and 12 were less than 35 years. In patients above 35 years age group, out of 88, 60 (68.1%) showed high proliferating index and 18 (18%) showed low proliferating index with Ki67 immunostaining. In patients less than 35 years of age, out of 12, 7 (58.3%) showed high proliferating index and 2 (28.5%) showed low proliferating index with Ki67 immunostaining. The proportion of positive and negative Ki67 immunostaining was statistically not significant at 0.05 level of significance (p=0.49). In a study done in Milan, Italy, 13% were aged <35 years ('very young') and 87% were aged 35–50 years ('less young'). Compared with less young patients, the very young patient group had a higher expression of Ki67≥20% of cells stained. They deduced, that contrasted with less youthful premenopausal patients, extremely young ladies have a more prominent shot of having an endocrine-inert tumour, and will probably give a higher grade tumour with more extensively proliferating and vessel invading disease [24]. In our study we did not find any relation of Ki67 expression in relation to age group.

In our study, it was observed that 96 patients (96%) were females, out of which 65 (67.7%) showed high proliferating index with Ki67 immunostaining and 20 (20.8%) patients showed low proliferating index with Ki67 immunostaining. Out of 4 male patients, 2 (50%) patients showed high proliferating index with Ki67 immunostaining and 2 (50%) patients showed intermediate proliferating index with Ki67 immunostaining. The proportion of Ki67 immunostaining in males and females was not statistically significant at 0.05 level of significance (p=0.067). In a study on male breast carcinoma, the monoclonal antibody MiB1 (Ki-67), was identified as a potential prognostic marker in breast carcinoma in which a higher percentage correlated with increased tumour mitotic index and tumour grade. Previous studies showed that 20–40% of male breast carcinomas

were positive for MiB1.These carcinomas had frail to solid relationship between high MiB1 scores and androgen receptor antagonism and worse prognosis. However, higher MiB1 did not correlate with lymph node metastasis, tumour Grade, Stage, or disease free survival [25].

Lymph node metastasis is one of the most important prognostic factors in carcinoma breast [26]. Feng-yan Li et al., expressed that the prognostic estimation of Ki67 is perceived in breast disease patients with negative axillary nodes, yet is less certain in breast tumour patients with positive axillary lymph nodes [27]. In our study, it was proved that out of 45 (45%) patients in the N0 category, 28 (62.2%) showed high proliferating index and 12 (26.6%) showed low proliferating index with Ki67 immunostaining. Out of 55 (55%) patients in node positive category, 39 (70.9%) showed high proliferating index, 8 (14.5%) showed intermediate proliferating and low proliferating index each.

In our study, maximum patients were of IDC (n=94). Out of which, 64% showed high proliferating index for Ki67 immunostaining. Out of 5 patients of infiltrating lobular carcinoma (ILC), 60% (n=3) showed high proliferating index and the only case of mucinous carcinoma (n=1) showed low proliferating index for Ki67 immunostaining. A review observed Ki67 expression to be essentially higher in IDC cases (64%) as opposed to IDC/ductal carcinoma in situ (IDC/DCIS) (49.7%) [5].

In our study, we observed that high proliferation index was more commonly seen in Grade III IDC (89.4%), followed by Grade II IDC (65.6%) and least number of patients of Grade I IDC (45.4%) showed high proliferating index for Ki67 immunostaining. Bouzubar N et al., stated that an excellent correlation exists between the histological grade of malignancy of breast tumours and their Ki67 status. They found that 31% of well differentiated Grade I tumours, 45% Grade II tumours and 72% of Grade III tumours were Ki67 positive (≥5% tumour nuclei stained) [28].

In our study, maximum number of patients of TNBC (86.3%) showed high proliferating index for Ki67 immunostaining, followed by 73.9% patients of Her2/neu type breast cancer. 64% patients of Luminal B subtype showed high proliferating index followed by 50% patients of Luminal A subtype. In a study, out of total 202 cases of breast cancer, 150 (74.3%) were Ki67 positive. They observed that out of 24 cases of TNBC, 20 (83.3%) cases over expressed Ki67. By contrast, out of 178 cases of non-TNBC, 130 (73%) were Ki67-positive [29]. Thus, our result was in concordance with this published study.

CONCLUSION

Breast cancer in women is a major health burden worldwide. It is also the primary cause of global cancer death among women. The study revealed that breast cancer occurs more commonly in young patients in the age group of 31-50 years and involves the right breast more frequently.

Tumours with high proliferating index (Ki67 >30%) were more commonly of IDC grade III and axillary lymph nodes were also more commonly involved in tumours with a high proliferating index.

It was also observed that high proliferating index tumours were mostly large in size. However, we could not find any correlation with various molecular subtypes of breast carcinoma.Though not statistically significant, it was observed that TNBC were most aggressive and showed highest rate of proliferation and Ki67 expression.

We conclude that Ki67 immunostaining can be used as an important biomarker for proliferation and can improve prediction of the prognosis of breast cancer patients and the sensitivity of chemotherapy. It has proved to be a clinically useful marker. Ki-67 expression along with clinical factors can improve prediction of the prognosis of breast cancer patients. The use of Ki67 may be suitable for inclusion in everyday clinical practice.

We strongly feel that in future, larger studies should be done to know the biological behaviour of these tumours and to determine the relationship of Ki67 high proliferating index tumours with various molecular subtypes which will help in better understanding of these tumours to ascertain prognosis and future treatment planning.

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