Section: Pathology

Original Research Article

Ki-67 EXPRESSION IN TRIPLE NEGATIVE BREAST CANCER-APROGNOSTIC MARKER

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Abstract

Background: Breast cancer is a multifactorial disease, consisting of distinct biological subtypes, with different natural evolution and a broad spectrum of clinical, pathologic, and molecular characteristics, with different prognoses and therapeutic implications. Due to the disease heterogeneity, there is a relentless pursuit in identifying certain predictive markers concerning disease prognosis and treatment response. Specific markers are used to predict the treatment response and guide the therapeutic plan. **Objective:** The present study was undertaken to analyse the prognostic parameter Ki-67 expression in histopathological and immunohistochemical evaluation of specimens of breast cancer, in relation to triple-negative breast cancer (TNBC). **Methods:** The study was based on the histopathologicaland immunohistochemistry findings in specimens of breast cancer, obtained from MRM or BCT in 155 patients in the department of Pathology, at a multi-speciality hospital.IHC slides were evaluated for review of the ER, PR, HER-2neu, and Ki67 status in each case.M **Results**: Majority of patients (60%) were in 4th& 5th decade of their life with common morphological feature of grade II infiltrating duct carcinoma (74.2%). Out of 155 cases, 63.8%, 55.48% and 22.58% cases were ER, PR and Her2/neu receptor positive respectively. 20.64% cases were triple negative. 76.2% of grade III and 41.7% of grade II tumor had Ki-67 index more than 20%. Eleven out of 21 grade III tumours (52.38%) were triple negative breast cancer. 59.37% of triple negative cases had Ki-67 index more than 20%. **Conclusion:** A significant association was found between high Ki-67 index and triple negative cancers of the breast indicative of a poor prognosis. Triple negative breast cancer prevalence has increased in Indian scenario (20%) which is positively correlated with poor prognostic parameters and with increased expression of Ki-67 (59.37%). High-Ki 67 expression detected by IHC has been reported as the strongest individual prognostic factor of breast cancer death or recurrence in patients.

INTRODUCTION

Breast cancer is a multifactorial disease, consisting of distinct biological subtypes, with different natural evolution and a broad spectrum of clinical, pathologic and molecular characteristics, with different prognosis and therapeutic implications. Worldwide breast cancer is the most common invasive malignancy in women. It comprises 22.9% of invasive cancers in women and 16% of all female cancers.

Breast cancer survival rates vary greatly worldwide, ranging from 80% in North America, Sweden and Japan to around 60% in middle-income countries and below 40% in low-income countries.[1] The low survival rates in less developed countries can be explained mainly by a lack of early detection programme, resulting in a high proportion of women presenting in late stages of the disease. Since last few years, there have been outstanding advances in breast cancer management leading to early detection of disease and the development of more effective treatments with significant decline in breast cancer deaths and improved outcome for the patients. Breast cancer is no longer seen as a single disease but rather a multifaceted disease comprising of distinct biological subtypes with diverse natural history, presenting a varied spectrum of clinical, pathological and molecular features with different

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prognostic and therapeutic implications. Current breast cancer treatment strategies rely on the characterization of estrogen and progesterone hormone receptor protein expression status and more recently on HER2/neu protein expression or gene amplification.

Triple-negative breast cancers (TNBC), are defined as tumors that are negative for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2/neu). Nowadays TNBC represent the focus of increasing interest at the clinical, biological and epidemiological level,[2,3,4] due to the aggressive behaviour of the tumor, poor prognosis and present lack of targeted therapies.[5,6]Most triple negative cancers display distinct clinical and pathological characteristics with a high proportion of these tumours occurring at a younger age of onset and in African–American women.[7,8] Triple negative tumours typically demonstrate high histological grade and are the most common breast cancer subtype in *BRCA1* carriers.

The proliferation marker Ki-67 has repeatedly been confirmedas an independent predictive and prognostic factor in early breast cancer.[9] Breast cancer with high Ki-67 expression responds better to chemotherapy,[10] but is associated with poor prognosis. In addition, TNBC is associated with a higher expression of Ki-67 than non-TNBC.[11] Its application as a pharmacodynamic intermediate marker of the effectiveness of medical therapy also holds great promise for rapid evaluation of new drugs.

OBJECTIVES

The present study was undertaken to analyse the prognostic parameter Ki-67 expression in histopathological and immunohistochemical study of breast cancer with relation to triple-negative breast cancer (TNBC). A lot of studies have been done in the past regarding expression of Ki-67 in breast cancer but few studies have assessed the relationship between triple-negative breast cancer and Ki-67 expression.

MATERIALS AND METHODS

The present study was a retrospective as well as prospective study in the Department of Pathology conducted at a multi-speciality hospital, from January 2013 to November 2015. It was based on the histopathological and immunohistochemistry findings of breast specimens received as Modified Radical Mastectomy (MRM) and Breast Conservation Therapy (BCT)in 155 patients with a diagnosis of breast cancer. The available bio-clinical data like age, menopausal status, parity, and other relevant parameters were obtained from available clinical records.

H&E stained slides of selected cases were re-evaluated for histological type of the tumor and grade of the tumor using the modified Bloom-Richardson method. Mitotic count was performed on Nikon ECLIPSE 50i microscope at 40x magnification and in 10 high-power fields (HPFs). Other parameters such as vascular and lymphatic invasion, lymph node status, size of the tumor and presence or absence of carcinoma in situ were also studied.

All the selected cases were then evaluated for hormone receptor, ER, PR, Her2/neu and Ki-67 expression by immunohistochemistry using specific antibodies. Immunohistochemical assessment of Ki-67antigen was done using Mouse anti-human monoclonal antibody, diluted in PBS, pH7.6, containing 1% BSA and 0.09% sodium azide.

Statistical Analysis was performed for Mean, SD and percentage. Comparison between groups was performed by using chi-square test for categorical variables and t-test for continuous variables. A p-value less than 0.05 was considered as significant. Data analysis was performed by using statistical software SPSS v20.0

Inclusion Criteria

The study included retrospective and prospectiveprimary operable cases of Modified Radical Mastectomy and breast conservative surgery with axillary node dissection.

Exclusion Criteria

Tru-cut biopsies, outside slides and blocks referred for opinion, neo-adjuvant cases were excluded.

RESULTS

In the present study of 155 cases of invasive breast carcinomas, mean age of the patients was 55.8 years (with an age range of 29 to 95 years). A total of 93 patients (60%) were in 4th and 5th decade of their life. (Table1)

Table 1: Age Group distribution

Age group	Number of patients	Percentage (%)
≤ 40	9	5.8
41 – 50	42	27.1
51 – 60	51	32.9
61 – 70	39	25.2
71 – 80	11	7.1
> 80	3	1.9
Total	155	100.0

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Table 2: Correlation of Receptor status with proliferation index

		Ki 67		Total	P-value
		≤ 20%	> 20%		
ER	Positive	68	31	99	< 0.001
	Negative	18	38	56	
PR	Positive	60	26	86	< 0.001
	Negative	26	43	69	
Her 2/neu	Positive	11	24	35	< 0.001
	Negative	75	45	120	

By using Chi-square test p-value <0.05, therefore there is significant association between Ki 67 with ER, PR, and Her2/neu findings.
Receptor status assessment was done in all 155 cases. Out of 155patients, 99 had ER + receptor status (63.8%), 86 were PR + (55.48%) & Her2/neu receptor was positive in 22.58% patients.
32 out of 155 patients were triple negative (20.64%).

- Nuclear staining by IHC for Ki-67 was assessed & calculated in the form of percentage of tumour cells taking up the stain. 44.5% of the cases (69 out of 155) had a Ki-67 index >20%.
- There is significant association between Ki-67 with ER, PR, and Her2/neu (By using Chi-square test p-value < 0.001).
- 52.38%(11 out of 21 patients) of higher histological grade (grade 3) were triple negative as compared to 47% of non-TNBC tumors suggesting that TNBC phenotype is associated with higher histological grade of the tumors and a worse prognosis (p<0.05).

Table 3: Correlation of TNBC with proliferation index

Ki-67	Triple Negative		Total
	Yes	No	
< 20%	13	73	86
>20%	19	50	69
Total	32	123	155

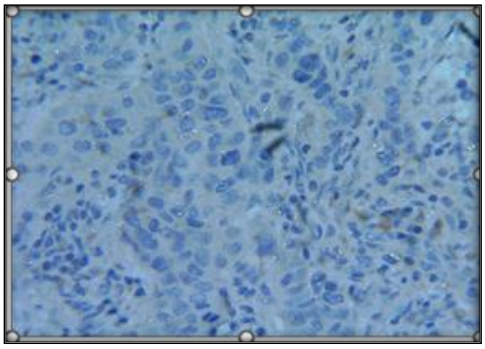
P value 0.073

Among TNBC group, 59.37% of the cases (19 out of 32) had Ki-67 > 20%. (Table3)

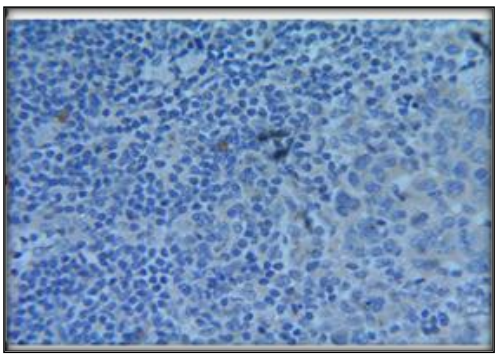
Table 4: Comparison of Ki-67 index among TNBC & non-TNBC in various studies

	Ki-67 index	TNBC	NON TNBC	p Value
Park et al	High	113(69.3%)	110(13.1%)	<0.001%
	Low	50(30.7%)	729(86.9%)	
Patil et al	High	51(37.5%)	21(4%)	<0.001%
	Low	85(62.5%)	495(96%)	
Present Study	High	22(71%)	47(38%)	0.0009%
	Low	9(29%)	77(62%)	

IDC-Grade 3-TNBC



ER –Neg



PR-Neg

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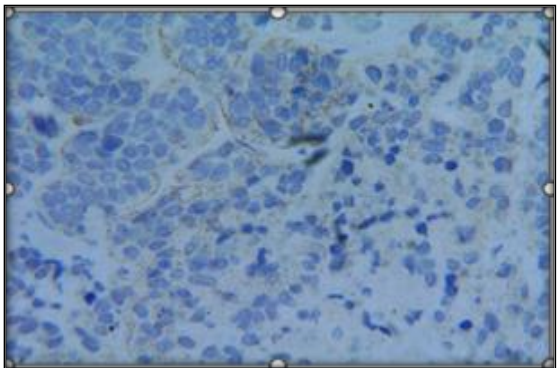
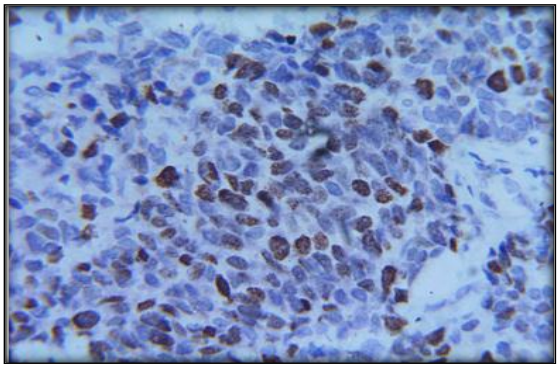


Her2/ Neu-Neg



Ki 67- >20%

DISCUSSION

Breast cancer is one of the most frequently diagnosed cancers in developed countries[12]. But now, breast cancer incidence has been on a rise in developing countries too, especially in urban areas.[13]

It is well known that breast cancer prognosis depends on various clinicopathological factors including metastatic status of lymph nodes, tumor size, tumor grade, hormone receptor status, HER2/neu status and Ki-67 labeling index.

Most of the patients in the current study were in the age group of 51 to 60 years, with a mean age of 55.8 years, whereas study by SEER cancer statistics review reported average age as 61 years and Pakseresht et alreported 47.7 years.[14] The mean age of breast cancer patients in developed countries is almostone decade higher as compared to Indian studies.[15,16] Prevalence of breast cancer in femalepatients(98.06%,152/155) is much higher as compared to male breast cancer (1.94%, 3/155) which is similar to the study doneby Sedighi et al.[17]Most common histological type of breast cancer observed in our study was infiltrating ductal carcinoma similar to that found in literature.[18,19]

We found 75.6% tumorsto be of histological grade II, which is different from the findings of Shet et al who had 70% of the tumorsof histological grade III [20]. The histological grade of invasive breast tumors showed a significant association with hormone receptor status and proliferation index, suggesting that low grade tumors show positivity with estrogen and progesterone receptors and low proliferation index (p-value <0.05).[20,21,22]

Prevalence of TN breast cancer was observed in 20%. Similar findings were reported by Carey(26.4%)[23] Bauer et al (12.4%)[24] and Sen et al (27.8%).[25]

In the present study, majority of the tumours (74.2%) were Grade 2 on histology while 13.5% were grade 3 and only 3.2% were grade 1. Among the grade III tumor, 52.38% tumorswere triple negative as opposed to 47.6% in the non-TNBC group. This observation was statistically significant with p value 0.012. Thus, it is clear from the above observation that TNB tumours are significantly associated with higher histological grade than non-TNB tumours. This finding has been consistent with several other studies.[25,26]

High Ki-67 expression has been shown to be associated with a higher histologic grade, larger tumor size, the presence of axillary lymph node metastasis and shorter disease-free and overall survival in breast cancer patients.[27]Furthermore its higher expression in triple negative breast cancers is indicative of increased aggressiveness and poorer prognosis inthese subset of tumors.[28]

In the present study, the cut-off value for Ki-67 index was selected as 20%.[29] However there is no recommended consensus about cut-off points at this time.[30] Some studies used 10% as arbitrary cut-off value for Ki-67, whereas others have selected optimal cut-off values according to the mean or median of Ki-67.[31] In the present study, we used this cut-off value as it was close to the mean Ki-67 fraction and was reproducible with other studies done on the subject. An Indian study by Patil et al, has also demonstrated similar results, where the cut-off value usedwas 20%.[32] Park et al (2012) also reported similar results using cut-off value of 14 %.[33]

In this study, a significant correlation was found between immunohistochemical determination of Ki-67 index in tumour cells and the TN phenotype. 71% of TNBC cases had a Ki-67 index > 20% as opposed to 38% of non-TNBC cases. Most of the studies have confirmed this finding. Han et al demonstrated that Ki-67 expression is significantly higher in TN tumours as compared to high grade non-TN tumours.[28]

A significant association was found betweenhighKi-67 index and higher histological grade of the tumor with 76.2% of grade 3 tumors having a Ki-67 index >20% as compared to 42.7% of grade 2 tumors. This observation was extremely significantstatistically with p value <0.0001. Viale et al [34]in2008 also demonstrated similar association between high Ki-67 index and higher histological grade of tumors. They found 78.5% of grade 3 tumors showing high Ki-67 index (>=11%) as compared to 49.1% of grade 2 tumors (p value <0.0001).

A further powerful correlation was noted in hormone receptor status and Ki-67 corresponding with previous studies. ER status has been largely

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identified as being inversely correlated with Ki-67, with the higher rates of ER positivity shown in the lowest proliferating tumors.[28] Moreover, it could be demonstrated that high levels of Ki-67 are associated with HER2/neu positivity according to former studies.[29] A univariate analysis by Viale et al[34] concluded that higher values of Ki-67-beling index were associated with adverse prognostic factors. High (>11 %) Ki-67-labeling index was associated with larger tumors, higher tumor grade, peritumoral vascular invasion and HER2/neu positivity (each $P < 0.01$).

Our study also stated a significant association (p-value <0.001) between lower Ki-67 score (<20%) and high index of positivity of both ER (68.7%) and PR (69.7%) and high proliferation index Ki-67 (>20%) with (68.57%) positive HER2-neu score (p-value <0.001).

CONCLUSION

A significant association was found between high Ki-67 index and higher histological grade of the tumor, which is a poor prognostic indicator.

A significant association (p-value <0.001) was found between lower Ki-67 score (<20%) and high index of positivity of both ER (68.7%) and PR (69.7%).

Triple negative breast cancer prevalence has increased in Indian scenario (20%) which correlates well with poor prognostic parameters and with increased expression of Ki-67 (59.37%).

Significant association (p-value <0.001) of ER, PR and HER2neu with Ki-67 scores suggest that it can be used as a possible prognostic and predictive marker of therapy.

High Ki-67 index is positively associated with the TNBC phenotype indicating increased proliferation potential and consequently poorer prognosis.

The Oncotype Dx gene test (Genomic Health, Redwood City, CA, USA) is a commercially available reverse transcriptase PCR assay (RT–PCR) of 21 gene. It provides valuable prognostic and predictive information in patients with early-stage breast cancers. Unfortunately, the high price limits the accessibility of this test to all patients. Hence, there has been increasing interest to find simple pathology tests, which can help predict the recurrence of disease. High-Ki-67 expression detected by IHC has been reported as the strongest individual prognostic factor of breast cancer death or recurrence in patients.

One of the limitations of the use of Ki 67 value is the lack of a standardised scoring system, which should be addressed. However, there are a lot of new molecular and genetic techniques by which targeted therapy and outcome in individual cases can be further studied.

We conclude that Ki-67 can been valued as a prognostic factor associated with invasive breast cancer outcomes.

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