Expression of Ki67 in Triple Negative Breast Carcinoma and Its Correlation with Clinicopathological Variables – A Study from a Tertiary Care Center in Thrissur, Kerala

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ABSTRACT

BACKGROUND

Breast cancer is the most common malignancy in females. Triple negative breast carcinomas (TNBC), one of the molecular subtypes of breast carcinoma, are not sensitive to hormonal therapy and are reported to have aggressive behaviour. The present study was done to evaluate the expression of Ki67 in triple negative breast cancer using immunohistochemistry (IHC) and correlate the Ki67 expression with other clinicopathological variables.

METHODS

Based on the IHC status (ER, PR, HER neu), 50 triple negative breast carcinoma cases were selected from January 2019 to June 2020 for a cross-sectional study in Department of Pathology, Government medical college Thrissur. Ki67 immunohistochemical staining was done on the tissue sections and the Ki67 score was correlated with clinicopathological variables.

RESULTS

Of the 50 cases, majority (74 % cases) had high Ki67 expression (score of more than 50 %). High Ki67 score was strongly associated with presence of lymphovascular emboli (LVE) (P value < 0.05). Even though most of the patients were above 50 years (72 %), no significant correlation was seen between age and Ki67 score. The association with tumour size, histopathological type, tumour grade and lymph node status were not statistically significant.

CONCLUSIONS

Ki67 expression was high in triple negative breast cancer with mean score of 62 %. High Ki67 score correlated with presence of LVE. High Ki67 would predict increased proliferation of breast cancer cells and could be considered as a prognostic marker.

KEYWORDS

TNBC- Triple Negative Breast Cancer, Ki 67 Score, Clinicopathological Variables

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BACKGROUND

Invasive breast carcinoma (IBC) refers to the large and heterogeneous group of malignant epithelial neoplasms of the glandular elements of the breast. Carcinoma of breast is the most common cancer in women accounting for about 23 % of all carcinomas in women globally and 27 % in affluent countries. It is the second most common cancer overall.

The aetiology of breast cancer is multifactorial, involves diet, reproductive factors and hormones. From descriptive epidemiological data it has emerged that, the breast cancer is a disease of affluent societies characterized by a high-calorie diet rich in animal fat and proteins, combined with a lack of physical exercise. Breast cancer has several biological subtypes.[1] It is a disease with unpredictable aggressiveness. The molecular biomarkers like ER, PR, HER2/neu are used in the classification and routine clinical management of patients with invasive breast cancer. Of all the other markers, Ki 67 is the one marker which reflects the proliferation potential of breast cancer.[2] This can be used as an independent biomarker to know the response of tumour cells to chemotherapy

The advancement of molecular biology has led to new modified classification of breast cancer into four subtypes according to the various molecular characteristics.

The St. Gallen international expert consensus 2011 defined the breast cancer subtypes on immunohistochemistry (IHC) as[3]:

Luminal A (ER + and/or PR +, Her2 − and low Ki67 - < 14 %)
Luminal B (ER + and/or PR +, Her2 +; ER + and/or PR +, Her2 −, high Ki67 - > 14 %)
Her2 - positive (ER −, PR − and Her2 +)
Triple-negative type (ER −, PR − and Her2 −).

Among the various subtypes, triple negative breast carcinoma (basal like subtype) is a highly diverse group of cancer, accounts for 15 % and is associated with the worst prognosis.[4,5,6] These are the most common tumour type observed in patients with germline BRCA1 mutations. These tumours have a "basal-like" pattern of mRNA expression resembling many genes that are expressed in normal myoepithelial cells.

TNBCs are highly invasive and exhibit certain organ oriented metastasis. The majority of these breast cancers are high-grade with high nuclear cytoplasmic ratio, a solid growth pattern and geographical necrosis. They typically have very high mitotic counts, while some tumours in this group are having favourable prognosis, such as secretory carcinoma and adenoid cystic carcinoma, necessitating the creation of a "low-grade" category of basal-like carcinoma. Triple-negative breast cancer subtyping is necessary to identify better molecular-based therapies.

In 2011, Lehmann et al. have introduced six molecular subtypes of TNBC from their analyses of 587 TNBCs.[7]

1. Basal-like 1 (BL1)
2. Basal-like 2 (BL2)
3. Immunomodulatory (IM)
4. Mesenchymal (M)
5. Mesenchymal stem-like (MSL)
6. Luminal androgen receptor (LAR) subtypes

These subtypes were reported to display distinct gene expressions, therapeutic target and different clinical outcomes. The Basal-like 1 (BL1) subtype showed increased expression of cell cycle–related gene. Ki67 level will be very high (average 70 %). The potential therapeutic target includes antimitotic agent-Taxanes, DNA-PK inhibitors, TORC inhibitors.

Basal-like 2 (BL2) subtype showed elevated expression of genes involved in growth factor signalling. The BL2 subtype was suggestive of basal/myoepithelial differentiation, with high levels of p63 and CD10 expression. This also had high Ki67 proliferation rate (average 70 %. The potential therapeutic target includes antimitotic agent-Taxanes, growth factor receptor inhibitors.

Mesenchymal (M) and Mesenchymal stem-like (MSL) subtypes had increased expression of genes involved in cell motility and cell differentiation pathways. Metaplastic carcinomas have shared molecular features with M and MSL subtypes. Compared with the M subtype, the MSL subtype showed lower levels of proliferation genes (low Ki67 %) and shared molecular features of claudin-low subtype. The potential therapeutic target includes PI3K/mTOR inhibitors.

The immunomodulatory (IM) subtype had enrichment of genes involved in immune cell responses and showed the same genetic signature for medullary breast carcinoma-a TNBC with a good prognosis.

The luminal androgen receptor (LAR) subtype expresses luminal CK18 and has increased expression of genes involved in hormonal pathways, including androgen receptor. The LAR subtype corresponds to the molecular apocrine subtype described previously by Farmer et al.[8]

Thus TNBC should be viewed as a group of different diseases that have similar phenotypes but different genotypes with distinct molecular characteristics. Lehmann et al. study pointed out that each of the molecular subtypes in TNBC has distinct "druggable" targets for potential targeted therapy.[7] This finding can change the current chemotherapy regimen usually employed for TNBC to individually targeted therapy based on specific molecular subtypes.

Various treatment options are available for management of patients with invasive breast carcinoma including surgical management, hormonal therapy, chemotherapy and radiation therapy. TNBC cases are not sensitive to hormonal therapy and till date no specific targeted medication has been found for TNBC.[9]

Uncontrolled proliferation is a distinct characteristic of malignancy and is assessed through various methods, including counting mitotic figures in stained tissue sections, incorporation of labelled nucleotides into deoxyribonucleic acid (DNA), and flow cytometric evaluation of cell fraction in S phase. Dowsett et al. reviewed that the most common measurement involves immunohistochemical assessment of Ki-67 antigen.[10]

Ki-67 is a nuclear nonhistone protein present in all active phases of cell cycle, except the G_0 phase. Its role as a proliferation marker attracts considerable interest.[2] Despite the controversies regarding the standardization of Ki67,

there is a strong data in the literature to show that Ki67 is an excellent prognostic and predictive biomarker.

Objectives

The aim of current study is to evaluate the expression of Ki67 in TNBC tissues using immunohistochemistry and to correlate Ki 67 expression with other clinicopathological parameters like tumour size, tumour grade, histopathological type, lymph node metastasis and lymphovascular emboli.

METHODS

This is a cross sectional study conducted in the Pathology Department, Government Medical College, Thrissur from January 2019 to June 2020. After institutional ethical committee clearance, we selected 50 TNBC cases based on immunohistochemical staining status (ER, PR, HER2neu), among the cases of invasive carcinoma received. Only those cases with modified radical mastectomy were included.

Immunohistochemical staining of formalin-fixed paraffin-embedded tissue was done. 4 micrometre thick sections were obtained. Sections were deparaffinized and dehydrated. Antigen retrieval was performed by pressure cooking and stained with primary antibody Ki67. Ki67 proliferative index is expressed as the percentage of positively stained cells among a total number of invasive cells, counting 1000 cells. Tissues were considered to be positive for Ki67 when the proportion of positively stained cells was > 5 %.[8]

≤ 5 %	Negative
6 – 25 %	+
26 – 50 %	++
≥ 50 %	+++

Table 1. Ki 67 Grading

Statistical Analysis

Data thus obtained was entered in Microsoft office excel sheet. This was then analysed using software statistical package for social sciences (SPSS) version 20. Qualitative data expressed as proportions and quantitative data in mean and standard deviation. Fishers exact test was used and the P value < 0.05 was considered statistically significant.

RESULTS

The age of the patients ranged from 30 to 72 years and mean age was 55 years. The maximum number of patients was in the age group of 60 - 70 years. The major histopathological type was invasive carcinoma breast – no special type (NST), accounting for 82 %.

Ki 67 Score	Frequency	Percentage
5 % - 25 %(+)	2	4 %
25 % - 50 % (++)	11	22 %
≥ 50 % (+++)	37	74 %

Table 2. Ki67 Score

Ki67 Score	Age Group 30 - 40 Years	40 - 50 Years	50 - 60 Years	60 - 70 Years	➢ 70 Years
5 - 25 %	0	0	2	0	0
25 - 50 %	0	3	2	5	1
> 50 %	3	8	9	14	3

Table 3. Distribution of Ki67 Score among Different Age Groups. Association between Ki67 and Age was not Found to be Statistically Significant (P Value > 0.01)

The association between Ki67 and age was not found to be statistically significant (P value > 0.01)

Characteristics		Ki67Score < 50 % (n = 13)	≥ 50 % (n = 37)	Total (N = 50)	P Value
Histopathological type	Invasive carcinoma NST	10 (76.9 %)	31 (83.8 %)	41 (82 %)	0.400
	Metaplastic carcinoma	3 (23.1 %)	4 (10.8 %)	7 (14 %)	
	Carcinoma with medullary features	0 (0 %)	2 (5.4 %)	2 (4 %)	
MBR tumour grade	I	1 (7.7 %)	0 (0 %)	1 (2 %)	0.199
	II	3 (23.1 %)	15 (40.5 %)	18 (36 %)	
	III	9 (69.2 %)	22 (59.5 %)	31 (62 %)	
Tumour size	<2 cm	2 (15.4 %)	1 (2.7 %)	3 (6 %)	0.162
	>2cm	11 (84.6 %)	36 (97.3 %)	47 (94 %)	
Lymphovascular emboli	Present	0 (0 %)	19 (51.4 %)	19 (38 %)	*0.001
	absent	13 (100 %)	18 (48.6 %)	31 (62 %)	
Lymph node metastasis	Present	7 (53.8 %)	12 (32.4 %)	19 (38 %)	0.171
	absent	6 (46.2 %)	25 (67.6 %)	31 (62 %)	

Table 4. Correlation of Expression of Ki67 with the Clinicopathological Factors

*Fisher's exact test has been done to check the association of Ki67 with the various clinico-pathological factors and it showed that there was a statistically significant relation between Ki 67 score and lymphovascular emboli (P = 0.001)

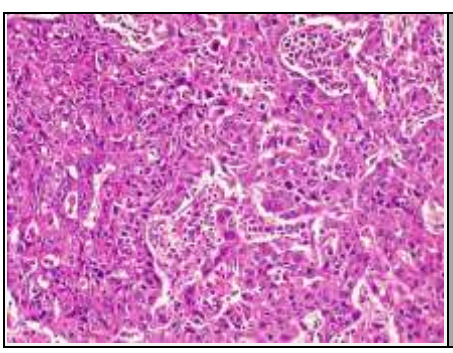

Figure 1. Invasive Carcinoma – NST Modified Bloom Richardson Grade III

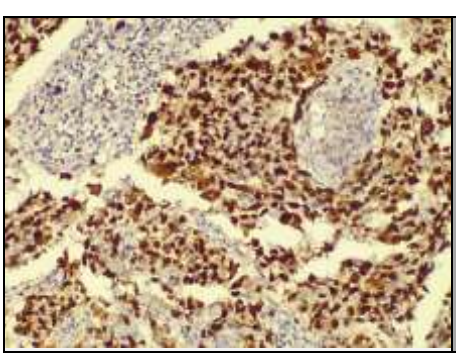

Figure 2. Invasive Carcinoma – NST, Ki67 Score 80 %

8 patients had metaplastic carcinoma of breast and one patient had carcinoma with medullary features. 94 % of triple negative carcinoma cases had tumour size of more than 2 cm. Majority of the patients (62 %) had a modified bloom Richardson (MBR) grade of III. All the 50 patients showed positive Ki67 expression (> 5 % tumour cells with positive nuclear staining). Mean Ki67 score was 62 % and median score was 82 %. Majority of patients (37 patients - 74 %) had Ki67 score of ≥ 50 %.

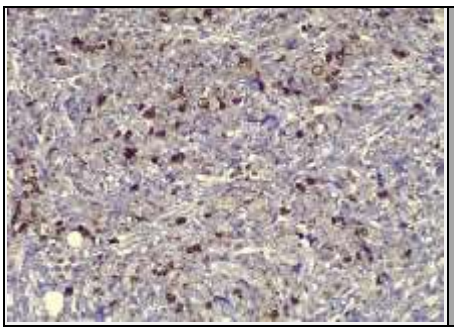

Figure 3. Invasive Carcinoma – NST with Ki67 Score 29 %

DISCUSSION

In the present study, positive expression of Ki67 was noted in all cases of TNBC with a mean score of 62 %. High Ki67 expression (Score +++/3+ > 50 %) was seen in the majority of cases (74 %) of triple negative breast carcinoma while only 26 % cases showed low Ki 67 score (Table 2). This is in consistent with the study done by Hashmi et al[11] where they noticed high Ki67 expression with mean score of 50 % in TNBC. As Ki67 is a marker of proliferation, high Ki 67 expression indicates that majority of TNBC carcinoma is associated with high proliferation.

The age of patients ranged from 30 to 72 years and the most frequent age group in the present study was between 60 and 70 years (38 %). The mean age was 55 years. A high Ki67 score of > 50 % was noted in all the patients younger than 40 years with mean Ki67 score being 76 %. 75 % of patients older than 70 years also showed high Ki67 expression of > 50 %, while 25 % of them showed expression between 25 and 50 % (Table 3). However no significant correlation was demonstrated between Ki67 and age group. But a study conducted by Mohammed et al. found that there was a significant correlation between high Ki67 score and higher age.[14] In our study, invasive carcinoma NST is the predominant subtype among the cases with high Ki 67 score (83.8 %), followed by metaplastic carcinoma (10.8 %). 3 cases of metaplastic carcinoma had Ki 67 score of less than 50 %. 2 cases of carcinoma with medullary features included in the study had Ki 67 score of > 50 %. However, no significant correlation was found between Ki67 nuclear staining and histopathological type.

Among the 37 cases with Ki 67 score of > 50 %, 22 (59.5 %) were of MBR tumour grade III and 15 (40.5 %) were of MBR grade II. Single case of TNBC with MBR grade I showed Ki 67 score of < 50 %. No statistically significant relationship is found between MBR grade and Ki 67 score, as the P value is 0.199. But previous study done by Mohammed et al. showed that a high Ki67 expression was associated with the higher grade.[12] In the present study, 97.3 % of patients (36 cases) with > 50 % Ki 67 score and 84.6 % of patients (11 cases) with < 50 % Ki 67 score had tumour size > 2 cm. It indicates lack of correlation between tumour size and Ki67 nuclear staining (P value 0.162) (Table 4). This finding is in concordance with the study conducted by Payandeh et al.[13]

Among the patients evaluated in our study, 51.4 % patients (19 cases) with > 50 % nuclear ki67 staining showed lymphovascular emboli in routine H and E evaluation while anyone in the other group with < 50 % of Ki67 score showed LVE (Table 4). Therefore, statistically significant association was noted between Ki67 score and presence of lymphovascular emboli (P value of 0.005).

32.4 % of patients (12 cases) with > 50 % of Ki67 nuclear staining showed lymph node metastasis. 53.8 % of patients (7 cases) with Ki67 score < 50 % also showed lymph node metastasis (P value 0.171) (Table 4). No association was hence found between Ki67 expression and presence of lymph node metastasis. This may be explained from the previous studies,[14,15] that, TNBC often do not involve lymph node but frequently spread to distant site. Tumour with high proliferation often metastasis faster than tumour with low proliferation.

A study done by Keam et al. identified the predictive or prognostic value of Ki-67 among patients with TNBC treated with neoadjuvant chemotherapy, and the role of Ki-67 in further classification of TNBC.[16] In their study, they found that TNBC with high Ki-67 expression had poorer survival than TNBC with low Ki-67 expression, despite a higher pathological complete remission rate (triple negative paradox). Furthermore, TNBC with high Ki-67 expression showed rapid recurrence within three years, whereas TNBC with low Ki-67 expression showed a near-constant recurrence rate. Therefore TNBC with high Ki-67 should be followed-up more frequently within three years to guard for any recurrence, indicating the prognostic significance of Ki67 in TNBC.

CONCLUSIONS

In our study, all 50 cases of triple negative breast carcinoma showed positive Ki67 expression with mean Ki67 score of 62 %. Significant correlation was observed between high Ki67 score and presence of LVE, indicating the strong association between high Ki67 score and tumor aggressiveness. Despite the controversies regarding the standardization of Ki67 score, there is strong data in the literature to show that Ki67 is an excellent prognostic and predictive biomarker. So along with other prognostic indicators Ki67 may be useful for predicting the prognosis and management of triple negative breast carcinoma cases.

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REFERENCES

[1] Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes and survival in the Carolina Breast Cancer Study. JAMA 2006;295(21):2492-2502.

[2] Nishimura R, Osako T, Okumura Y, et al. Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer. Experimental and Therapeutic Medicine 2010;1(5):747-754.

[3] Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes—dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Annals of Oncology 2011;22(8):1736-1747.

[4] Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proceedings of the National Academy of Sciences 2001;98(19):10869-10874.

[5] Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406(6797):747-752.

[6] Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proceedings of the National Academy of Sciences of the United States of America 2003;100(14):8418-8423.

[7] Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121(7):2750-2767.

[8] Farmer P, Bonnefoi H, Becette V, et al. Identification of molecular apocrine breast tumours by microarray analysis. Oncogene 2005;24(29):4660-4671.

[9] Banerjee S, Reis-Filho JS, Ashley S. Basal-like breast carcinomas: clinical outcome and response to chemotherapy. J Clin Pathol 2006;59(7):729-735.

[10] Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 2011;103(22):1656-1664.

[11] Hashmi AA, Hashmi KA, Irfan M, et al. Ki67 index in intrinsic breast cancer subtypes and its association with prognostic parameters. BMC Research Notes 2019;12(1):1-5.

[12] Mohammed EA, Albasri AM, Mohammed RA, et al. Ki67 expression in breast cancer: correlation with prognostic markers and clinicopathological parameters in Saudi patients. Saudi Medical Journal 2016;37(2):137.

[13] Payandeh M, Malayeri R, Sadeghi M, et al. Expression of p53 and Ki67 in the patients with triple negative breast cancer and invasive ductal carcinoma. Am J Cancer Prev 2015;3(3):58-61.

[14] Fulford LG, Easton DF, Reis-Filho JS, et al. Specific morphological features predictive for the basal phenotype in grade 3 invasive ductal carcinoma of breast. Histopathology 2006;49(1):22-34.

[15] Wiechmann L, Sampson M, Stempel M, et al. Presenting features of breast cancer differ by molecular subtype. Annals of Surgical Oncology 2009;16(10):2705-2710.

[16] Keam B, Im SA, Lee KH, et al. Ki-67 can be used for further classification of triple negative breast cancer into two subtypes with different response and prognosis. Breast Cancer Research 2011;13(2):R22.