Original Article

Correlation of Ki67 with ER, PR, Her2neu and other Prognostic Factors In Breast Carcinoma

Menka Khanna*, Manisha Sharma, Mridu Manjari, Kiranjot Kaur, Saumil Garg and Saloni Goyal

Department of Pathology, SGRDIMSAR, Amritsar, India

Keywords: *Breast Cancer, Immunohistochemistry, Ki67, ER, PR, Her2neu*

ABSTRACT

Background: Breast cancer is the most common malignancy occurring in females, accounting for 23% of all malignant tumors.1 Various predictive and prognostic factors affect tumor progression. In addition to estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (Her2neu) overexpression, recently Ki67, a proliferative marker has been recognised as an important predictive and prognostic marker in many studies. The aim of the present study is to find the correlation of Ki67 proliferation index with ER PR and Her2neu status and with other clinicopathological parameters.

Methods: The study was conducted on 70 cases of breast cancer received as mastectomy or lumpectomy specimens. The histopathological grading of the breast carcinoma was done according to the Nottingham modification of the Bloom Richardson grading system. IHC was performed by using antibodies against the estrogen receptors (ER), the progesterone receptors (PR), Her2neu and Ki67. An attempt was made to find a correlation between ER, PR, Her2neu and Ki67 expression with the various clinicopathological parameters.

Result: ER PR expression was seen to be decreased with increase in Ki67 positivity. Ki67 proliferation index when correlated with the various clinicopathological parameters (grade, lymph node metastasis and size of tumor) it was concluded that as the Ki67 positivity increased the prognosis became poorer.

Conclusion: It was concluded that Ki67 can be considered as a new prognostic marker in addition to ER PR and Her2neu and is associated with a poor prognosis.

***Corresponding author:**
Dr. Menka Khanna, Department of Pathology, SGRDIMSAR, Amritsar, India
Phone: +91 9464986355
Email: mona74_khanna@yahoo.co.in

Introduction

Breast cancer is the most common malignancy occurring in females, accounting for 23% of all malignant tumors.[1] Over one lakh new breast cancer patients are estimated to be diagnosed every year in India. It is expected to increase by 26% by 2020 in developing countries. It is the second most common carcinoma in rural areas. However, in urban India it is the commonest cancer among women, where it constitutes >30% of all cancers in females.[2,3] Various predictive and prognostic factors affect tumor progression.[4,5] Predictive factors determine the response to treatments and prognostic factors are measurable and are associated with the nature of the disease.[4] Some factors are both prognostic and predictive, including estrogen receptor (ER) and progesterone receptor (PR) status and human epidermal growth factor receptor (Her2neu) overexpression. Prognostic factors include the type of tumor, number of involved lymph nodes at the time of tumor diagnosis, size of the tumor, tumor grade and the patient's age.[6,7] Classification of breast carcinoma according to immunohistochemistry (IHC) are luminal A (ER +ve and PR +ve, Her2neu –ve), luminal B (ER+ve, and/or PR +ve, Her2neu +ve or-ve), triple negative or basal like (ER-ve, PR-ve and Her2neu-ve) and Her 2 type (ER-, PR-, Her2neu+).[8] Estrogen receptor (ER) is the most important prognostic and predictive marker for breast cancer.[9]Presence of both ER and PR is related to better prognosis and responsiveness to hormonal therapy.[10] Typically Her2 amplified tumors are associated with high grade and often extensive ductal carcinoma but respond well to specific chemotherapy.

The expression of the nuclear proliferating antigen, Ki67 has been observed to reflect the proliferation rate of malignant tumors. It is associated with the development and metastasis of a variety of malignant tumours, as well as with the prognosis of patients.[11] Many studies found Ki67 to be a predictive and prognostic marker for either clinical and/or pathological response. This will be particularly helpful in cases which are triple negative.[12]

The aim of the present study is to find the correlation of ER, PR, Her2neu and Ki67 expression with the various clinicopathological parameters and also determine correlation of Ki67 proliferation index with ER PR and Her2neu status.

Materials and Methods

The study was conducted on 70 cases of breast cancer received as mastectomy or lumpectomy specimens in the Department of Pathology, Sri Guru Ram Das institute of medical sciences, Sri Amritsar. The clinical history of the patients was taken. The tissue was formalin fixed and paraffin embedded and was then stained for Haematoxylin and Eosin for histopathological typing and grading. The histopathological grading of the breast carcinoma was done according to the Nottingham modification of the Bloom Richardson grading system.

IHC was performed by using antibodies against the estrogen receptors (ER), the progesterone receptors (PR) (Diagnostic Biosystem) and Her2neu, Ki67 (Biocare Medical). The antigen retrieval was done by using pressure cooker method with 10mmol citrate buffer at pH 6. Tris buffer was used as the wash buffer and diaminobenzene tetrahydrochloride (DAB) was used as the chromogen. The endogenous activity was blocked by using hydrogen peroxide. After protein blocking, the slides were incubated overnight with the available ER, PR, Her2neu and Ki67 primary antibodies and were conjugated with streptavidin Horse Radish Peroxidase (HRP). The slides were counterstained with hematoxylin and were examined by light microscopy. For ER ≥10% nuclei stained brown were taken positive and 10% stained were taken positive for PR.[13] For Her2neu, membranous staining in >25% cells was taken as positive. Ki-67 immunoreactivity was based on the proportion of positive tumor cells (0-100%) regardless of staining intensity. levels of Ki-67 are quantified as high (immunostaining ≥30%), low (immunostaining <15%) and intermediate (between 16 to 30%) approach adopted by St Gallen International Expert Consensus.[14]In our study for statytical analysis Ki67 was evaluated by the percentage of immunostained nuclei with 1-14% staining scored as low and >14% as high.[15]

Result

The age of the patients varied from 29-68 years with the maximum number of cases belonging to 41-60 (65% of the patients). The tumor size varied from 1.5 to 4.5 cm in size with 54.2% cases with size more than 2cm. All the cases were of infiltrating ductal carcinoma NOS (not otherwise specified). Grade III tumors were maximum consisting of 65.7% (46/70) followed by Grade II tumors which were 32.8% (23/70) and Grade I tumors consisted of only one case. Lymph nodes were recovered in 63 cases out of which metastasis were seen in 40 cases. ER and PR positivity was observed in 31.5% cases (22/70). All of these cases were Her2neu negative. Amongst the 48 ER PR negative cases, 20 cases came out to be positive for Her2neu and 28 cases were triple negative.

On correlating the Ki67 proliferation index with the various clinicopathologial parameters it was found that Ki67 proliferation index significantly correlated with higher grade of the tumor. 90% of the cases with high proliferation index were of Grade III as compared to only 3 cases (10%)

being of Grade II (p= 0.001). Ki67 proliferation index was higher in the cases with size >2cm (24/30= 80% cases) with the rest of the cases being less than 2cm in size (p= 0.004). 22 out of 40 cases with lymph node metastasis showed high Ki67 proliferation index. Thus, Ki67 proliferation index being significantly correlated with lymph node metastasis (p=0.008). (Table 1)

Similarly, ER PR and Her 2neu status were correlated with grade of tumor and it was concluded that as the grade increased ER PR expression decreased. 25 out of 28 triple negative cases were of Grade III. 13 out of 20 Her2neu positive cases were of Grade III as compared to 7/20 cases which were of Grade II. (Table 2)

While correlating the Ki67 expression with ER PR Her2neu expression, it was observed that 23 out of 28 (82%) triple negative cases showed high proliferation index as compared to 2/24 ER PR positive cases giving a significant correlation between Ki67 positivity and ER PR negativity (p=0.002).

Discussion

Breast cancer is one of the most common malignancies among women. Despite the advanced technology in early diagnosis and treatment methods, it is among the leading cause of death from cancer in women worldwide. Different predictive and prognostic markers are involved in tumorigenesis and its progression. The outcome and response of tumor to the treatment varies from patient to patient depending upon the interplay of such various factors involved. The present study was conducted to have better insight to elucidate the correlation between these factors. ER, PR, Her2neu and Ki67 expression were correlated with various clinicopathologial parameters like grade, size of tumor and lymph node metastasis. We also determined the correlation of Ki67proliferation index with ER, PR and Her2neu status.

In our study maximum number of cases were from 45-60 years which corroborates the findings in studies conducted in Indian subcontinent which is a decade earlier than results observed in European countries.[15] All the cases were of infiltrating ductal carcinoma breast – NOS with grade III cases (65.7%) outnumbering the grade II cases (32.8%). ER PR positivity was observed in 31.5% of the cases which is a bit lower than the studies conducted in this part of the continent where this percentage varied from 38-47%.[5,8] Researchers in the western world had found this

Table1: Correlation of Ki67 with other clinicopathological prognostic factors.

PARAMETERS		Ki67 –ve	Ki67 (1-14%)	Ki67 > (14%)
Histological grade	Grade I Grade II Grade III	01(5%) 12(60%) 07(35%)	0 8(40%) 12(60%)	0 03(10%) 27(90%)
Lymph node status	NR N_0 N_x (metastatic)	2(10%) 10(50%) 8(40%)	2(10%) 8(40%) 10(50%)	3(10%) 5(16.7%) 22(73.3%)
Size	=< 2 cm >2 cm	16(80%) 4(20%)	10(50%) 10(50%)	6(20%) 24(80%)

Table 2: Correlation of ER ,PR and Her2neu status with the grade of the tumor

	Grade 1	Grade 2	Grade 3	TOTAL
Triple negative	0	3	25	28
ER PR +ve Her2neu -ve	1	13	8	22
ER PR –ve Her2neu +ve	0	7	13	20

Table 3: Correlation of ER, PR and Her2neu status with Ki67 status.

	Ki67 –ve	Ki67 (<14%)	Ki67(>14%)	TOTAL
Triple negative	4(20%)	1(5%)	23(76.67%)	28(40%)
ER PR +ve Her2neu –ve	12(60%)	8(40%)	2(6.67%)	22(31.43%)
ER PR –ve Her2neu +ve	4(20%)	5(16.67%)	11(55%)	20(28.57%)

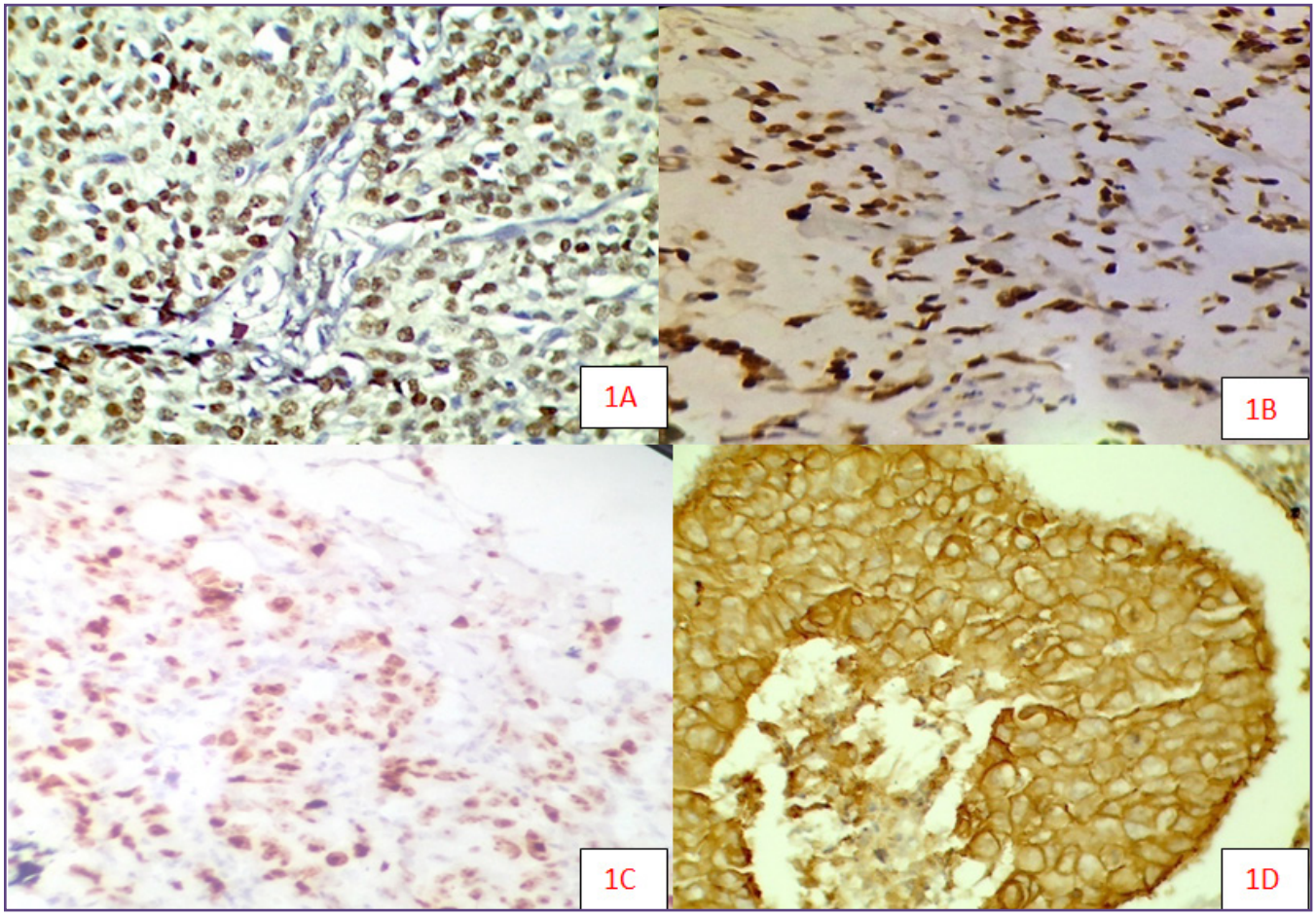

Fig 1 (A): ER positivity (Nuclear) – IHC (400X) (B): PR positivity (Nuclear) – IHC (400X) (C): Ki67 positivity (Nuclear) – IHC (400X) (D): Her2neu positivity (Cytoplasmic) – IHC (400X).

percentage to be even higher varying from 40-55%.[1,4] All ER PR positive cases showed Her2neu negativity. Among 48 ER PR negative cases, 20 cases had Her2neu positivity and 28 cases (40%) being triple negative. Similar findings had been recorded in literature with this percentage varying from 30-40% depending upon the population studied.[1,4,16]

Ki67 positivity was noted in 50 cases with 30 cases showing high proliferation index (>14%). Ki67 is a non histone nuclear protein closely linked to the proliferation of the cells. In the present study high Ki67 proliferation index was significantly correlated with higher grade of the tumor (p=0.001), increasd size (p=0.004) and lymph node metastasis (p=0.008). Previous studies had reported similar results where high Ki67 expression was associated with high histological grade, large tumor size and the presence of axillary lymph node metastasis.[17,18,19]

Among the Her2neu positive cases, 80% cases showed Ki67 positivity. In contrast to Her2neu negative cases which showed Ki67 positivity in only 68% cases. So there was direct correlation between Her2nu positivity and Ki67 positivity. Previous studies found similar correlation between Her2neu and Ki67.[19] In triple negative cases, high Ki67 proliferation index (>14%) was present in 76% patients. Many researchers also reported similar results.[21,22,23]

Ki67 has repeatedly been confirmed as an independent predictive and prognostic factor in breast cancer.[24] Breast cancer with high Ki67 expression responds better to chemotherapy but is associated with poor prognosis.[25,26] Triple negative cases also have poor survival despite a higher response to neoadjuvant chemotherapy.[22,27]

Keam et al further reported that triple negative breast cancer patients with high Ki67 expression had poor survival than triple negative breast cancer patients with low Ki67 expression.[28]

Conclusion

It was concluded that Ki67 can be considered as a new prognostic marker in addition to ER PR and Her2neu. The

breast cancers with a high Ki67 index are associated with poor prognosis and it may be helpful in triple negative cases for deciding a better treatment protocol.

Acknowledgements
None

Funding
None

Competing Interests
None

Reference
1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer staristics. CA Cancer J Clin 2011;61:69–90.
2. Khokhar K. Breast Cancer in India: Where do we stand and where do we go. Asian Pacific J Cancer Prev 2012;13(10):4861-6.
3. Shetty P. India faces growing breast cancer epidemic. The Lancet 2012;379(9820):992-3.
4. Moutafoff C, Coutant C, Bezu C. Prognostic and predictive factors in multifocal breast carcinoma. Gynecol Obstet Fertil 2011;39:425–32.
5. Mahmood H, Faheem M, Mahmood S. Impact of age, tumor size, lymph node metastasis, stage, receptor status and menopausal status on overall survival of breast cancer patients in Pakistan. Asian Pac J Cancer Prev 2015;16:1019–24.
6. Ariga R, Zarif A, Korasick J. Correlation of her-2/neu gene amplification with other prognostic and predictive factors in female breast carcinoma. Breast J 2005;11:278–80.
7. Baulies S, Cusido M, Gonzalez-Cao M. Hormone receptor and HER2 status: the only predictive factors of response to neoadjuvant chemotherapy in breast cancer. J Obstet Gynaecol 2015:35(5):485-9.
8. Barnard ME, Boeke CE, Tamimi RM. Established breast cancer risk factors and risk of intrinsic tumor subtypes. Biochim Biophys Acta 2015;1856(1):73-85.
9. Osborne CK. Steroid hormone receptors in breast cancer management. Breast Cancer Res Treat 1998;51:227–38.
10. Wong SY, Kernohan NM, Walker F. Breast cancers with extremely high estrogen receptor protein status. Histopathology 1990;16:125–32.
11. Liu M, Lawson G, Delos M. Predictive value of the fraction of cancer cells immunolabeled for proliferating cell nuclear antigen or Ki67 in biopsies of head and neck carcinomas to identify lymph node metastasis: comparison with clinical and radiologic examinations. Head Neck 2003;25:280–8.
12. Fasching PA, Heusinger K, Haeberle L. Ki67, chemotherapy response, and prognosis in breast cancer patients receiving neoadjuvant treatment. BMC Cancer 2011;11(486):6-8.
13. Gobbin H, Rocha RM, Nunes CB. Predictive factors of breast cancer evaluated by immunohistochemistry. J Bras Pathol Med Lab 2008;44(22):131-40.
14. Goldhirsch A, Wood WC, Coates AS, et al (2011). 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. Ann Oncol 2011;22:1736-47.
15. Shokouh TZ, Ezatollah A, Barand P. Interrelationships Between Ki67, HER2/neu, p53, ER, and PR Status and Their Associations With Tumor Gradeand Lymph Node Involvement in Breast Carcinoma Subtypes: Retrospective-Observational Analytical Study. Medicine 2015;94(32):1359-65.
16. Khokhar A. Breast cancer in India: where do we stand and where do we go? Asian Pac J Cancer Prev 2012;13:4861–6.
17. Peng Y. Potential prognostic tumor biomarkers in triple-negative breast carcinoma. Beijing da Xue Xue Bao 2012;44:666-72.
18. Han JS, Cao D, Molberg KH, Sarode VR, Rao R. Hormone receptor status rather than HER2 status is significantly associated with increased Ki-67 and p53 expression in triple-negative breast carcinomas, and high expression of Ki-67 but not p53 is significantly associated with axillary nodal metastasis in triple-negative and high-grade non-triple-negative breast carcinomas. Am J Clin Pathol 2011;135:2307-12.
19. Rhee J, Han SW, Oh DY, Kim JH, Im SA. The clinicopathologic characteristics and prognostic significance of triple-negativity in node-negative breast cancer. BMC Cancer 2008.8:307-9.
20. Lukashina MI, Glukhova EI, Zhukova LG, Ermilova VD, Bogatyrev VN, Baryshikov A. Her-2/Nu, Ki-67 expression and ploidy in breast carcinoma. Arkh Pathol 2003;65(5):25-9.
21. Li H, Han X, Liu Y, Liu G, Dong G. Ki67 as a predictor of poor prognosis in patients with triple-negative breast cancer. Oncol Lett 2015;9(1):149-52.
22. Keam B, Im SA, Kim HJ, Oh DY, Kim JH, Lee SH, Chie EK, Han W, Kim DW, Moon WK. Prognostic

impact of clinicopathologic parameters in stage II/III breast cancer treated with neoadjuvant docetaxel and doxorubicin chemotherapy: paradoxical features of the triple negative breast cancer. BMC Cancer 2007;7:203-6.

23. Rhee J, Han SW, Oh DY, Kim JH, Im SA, Han W, Park IA, Noh DY, Bang YJ, Kim TY. The clinicopathologic characteristics and prognostic significance of triple-negativity in node-negative breast cancer. BMC Cancer 2008;8:307-10.
24. Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. J Clin Oncol 2005;23:7212–20.
25. Jones RL, Salter J, Hern RA, Nerurkar A, Parton M, Reis-Filho JS, Smith IE, Dowsett M. Relationship between oestrogen receptor status and proliferation in predicting response and long-term outcome to neoadjuvant chemotherapy for breast cancer. Breast Cancer Res Treat 2010;119:315–23.
26. Brown RW, Allred CD, Clark GM, Osborne CK, Hilsenbeck SG. Prognostic value of Ki-67 compared to S-phase fraction in axillary node-negative breast cancer. Clin Cancer Res 1996;2:585–92.
27. Liedtke C, Mazouni C, Hess KR, Andre F, Tordai A, Mejia JA, Symmans WF, Gonzalez-Angulo AM, Hennessy B, Green M. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008;26:1275–81.
28. Keam B, Im SA, Lee KH, Han SW, Oh DY, Kim JH, Lee SH, Han W, Kim DW, Kim TY, Park IA, Noh DY, Heo DS, Bang YJ. Ki-67 can be used for further classification of triple negative breast cancer into two subtypes with different response and prognosis. Breast Cancer Res 2011 Mar 2;13(2):R22.