Original article:

Evaluation of KI-67 Index as a Prognostic Marker in Different Molecular Subtype in Patients of Carcinoma Breast

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Abstract

Objective: Ki-67 is an important marker of cell proliferation. As a prognostic marker it has been studied in many cancers like breast, neuroendocrine tumors etc. The aim of this study was to know the prevalence of Ki-67 and try to establish its role as a prognostic marker in different molecular subtypes in patients of carcinoma breast.

Methods: Total 56 patients of early breast carcinoma were included in the study. These patients were evaluated by histology and molecular classification by IHC for various markers like ER, PR, Her2 Neu, Ki-67 and EGFR. Patients were divided by <10% (low) and >15% (high) of Ki-67 level in different subgroups of molecular classification and tried to correlate them clinically and histologically.

Results: We analysed the data using molecular classification and divided them into Estrogen positive (luminal A and luminal B/luminal Her2 hybrids) and Estrogen negative (Triple negative or basal cell type, HER-2 Neu type and normal breast like phenotype) subtypes. ANOVA-F test was used to categorise variables and measure the test of significance. All luminal A, 88.9% of Her-2 Neu type and 85.7% of normal basal like subtypes had low (<10%) Ki-67 levels (non-significant) and 50% of each luminal B and triple negative had high (>15%) Ki-67 levels with p value-0.001. Average of Ki-67 was higher in triple negative (25.62) than luminal B (18.12 %).

Conclusion: Ki-67 is an important prognostic marker and helpful in molecular classification of breast carcinoma. It may be used in treatment planning and follow up, although further studies are required to establish a relation between Ki-67 and overall and disease-free survival.

Key words: Ki-67, Carcinoma Breast, Molecular Classification.

INTRODUCTION

Worldwide breast cancer is one of the most common cancer among women and foremost cause of cancer death.[1] In India, for the year 2012, 144,937 women were newly detected with breast cancer and 70,218 women died of it and for every 2 women newly diagnosed with breast cancer, one woman is dying of it. In comparison, in USA in the year 2012, incidence was 232,714 with 43,909 deaths and one death for 5-6 breast carcinoma patients. In China in year 2012, incidence was 187,213, with 47,984 deaths and one death for 4 breast carcinoma patients.[2] Since more patients (in India) turn up in later stages, they do not survive long even with the best treatment they may get, and hence the mortality is fairly high.

Carcinoma breast is a heterogeneous disease with several subtypes. Prognostic factors of breast carcinoma include age of patient, tumor size,

axillary lymph node involvement, Nottingham Prognostic Index (histological grade), lympho-vascular invasion and extra capsular spread. A number of predictive factors has evolved and established a role in treatment response like ER. PR, HER2 Neu. Some other factors also play some role in the molecular classification of breast carcinoma and predicts survival like Ki-67 (proliferation marker) and EGFR. Other markers like Mitosin, Cyclin-E and Cyclin-D1 are currently under investigation and not used as a routine.[3]

Ki-67 is a nuclear protein and during cell cycle its expression varies. It is present in all active phases of cell cycle, except the G0 phase. Ki67 levels are low during the G1 and early S phase peak in mitosis and decreases during anaphase and telophase.[4]

In molecular classification it is the estrogen receptor which divides it into two either positive or negative subtypes. Differentiation of Luminal A and B/Her2 Neu depends on Ki-67 proliferation index.[5] As it has an important therapeutic and prognostic implication. Ki-67 is also used among the 21 selected genes in Oncotype DX assay, used to predict the benefits of chemotherapy and risk of recurrence in patients with early breast cancer like size <2cm, node-negative, ER positive and HER2 Neu negative.[6]

Main aim of this study was to categorise breast carcinoma patients into different subtypes of molecular classification and to analyse the correlation of Ki-67 index among these different subtypes. We also included clinico-pathological factors into our cohort.

MATERIALS & METHODS

Study was carried out in Department of Surgical Oncology in collaboration with Department of Pathology at Dharamshila Hospital & Research Centre, Delhi. This retrospective study was carried out in patients of breast carcinoma attending Surgical Oncology OPD. Total 56 patients of carcinoma breast (stage-I and stage-II) undergoing upfront surgery (either MRM or breast conservative surgery) were included in the study. An informed consent was taken from all the recruits.

The tissue obtained was subjected to histopathological and immuno-histochemistry (IHC) analysis (Figure-1 & 2). As we used manual method for IHC, reagents like PAP and antibodies by Thomas Boenisch, editor director immunohistochemistry laboratory DAKO corporation, Santa Borbora, California (Bio genex laboratories) were utilised. This method allows the specific demonstration of cells and tissue antigens in variety of fixed tissues.

We analysed the data after getting all the information and by using Molecular Classification given by Perou et al.[7] divided them into Estrogen positive (luminal HER-2, luminal A and luminal B/HER2 Neu) and Estrogen negative (Triple negative or basal cell type, HER-2Neu type and normal breast like phenotype) subtypes.

Luminal A Tumors: These are ER positive, PR positive or negative, HER2 negative, and EGFR negative.

Luminal B Tumors: They are ER positive and either HER-2 Neu positive or having high Ki-67 index (≥ 15%).[5]

HER-2 Neu Type: ER, PR negative and HER-2 Neu positive.[8]

Triple Negative: ER, PR and HER-2 Neu negative and EGFR positive.[7]

Normal Breast Like (NBL): All markers are negative.[9]

Patients were divided by low (<10%) or high (>15%) level of Ki-67 levels in different subgroups of molecular classification and values between these two levels considered as equivocal, these cases were not included in the study. We also compare them clinically and histologically and tried to establish some correlation among them.

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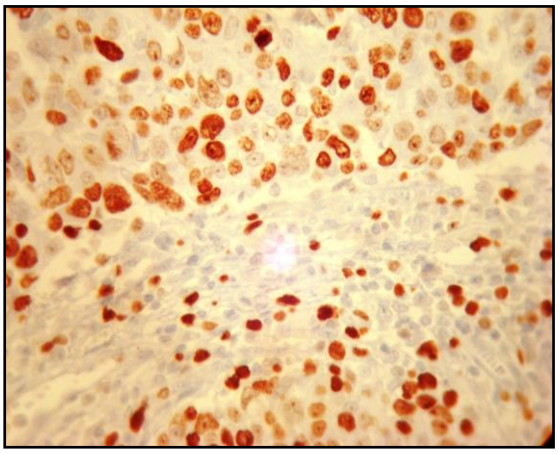

Figure 1: High Ki-67 Index (40X View)

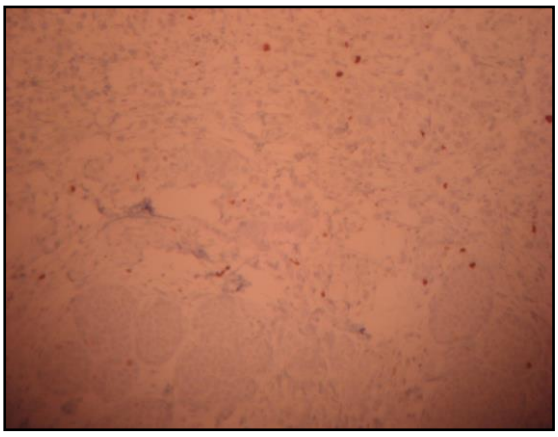

Figure 2: Low Ki-67 Index (10X View)

RESULTS

We included 56 patients in our study. ANOVA-F test was used to categorise the variables and measure the test of significance. For the detection of Her-2 Neu receptor on specimen by IHC in equivocal cases (patients who had two "++" positive points), we performed FISH test to confirm it negative or positive. Demographic data and histological findings of patients were placed separately for each group of Ki-67 (Table-1). All luminal A, 88.9% of Her-2 Neu type and 85.7% of normal basal like subtypes had low (<10%) Ki-67 levels and 50 % of each luminal B and triple negative had high (>15%) Ki-67 levels with 'P' value 0.001 (significant). Nottingham Prognostic Index in histological prognostic criteria also had low 'P' value (0.149), but it did not reach up to the level of significance.

Overall Ki-67 index was highest in triple negative cases average 25.62 %, range (5%- 60%) and lowest in Luminal-A average 6.45% with range (<5%-10%). (Table-2)

Table 1: Association between Clinico-Pathological Variables and Expression of Ki-67 in Early Carcinoma Breast Patients

Variables	Ki-67 Index (N=56)		P-Value
	<10%, N=46	≥15%, N=10	
1. Age			
< 50 Years	17	4	NS
>50 Years	29	6	
2. Tumor Stage			
T1	14		NS
T2	26	10	
T3	6		
3. Histological Subtype			
IDC	32	9	NS
ILC	8		
Others	6	1	
4. Histological Grade			
I	3		NS
II	20	1	
III	23	9	
5. Lymph Node Status			
Present	19	5	NS
Absent	27	5	

Table 2: Correlation of Ki-67 Index with different subtypes of Molecular classification of Breast Carcinoma

Variable	All cases N=56	Luminal A N=24	Luminal B/Luminal-HER2 hybrids N=8	Her2/Neu type N=9	Triple negative N=8	Normal Breast Like (NBL) N=7	P value
NPI	**4.85**	4.32	5.47	5.02	5.13	4.66	**0.149**
Average Ki-67 Index (%)	**11.16%**	6.45%	18.12%	6.67%	25.62%	10.7%	**0.001**
Ki-67 Index, Range	**<5-60%**	<5-10%	<5-40%	<5-15%	<5-60%	<5-30%	**NA**

DISCUSSION

Breast cancer is characterized by heterogeneity, exhibiting a wide variety of clinical presentations and disease aggressiveness in different patients and ethnic populations and poses a major challenge in diagnosis and treatment. Invasive breast cancers were classified according to histologic type for many decades. More recently, gene expression profiling analysis by IHC or microarray analysis indicates that breast cancers can be classified into different molecular subtypes, thereby enabling the prediction of different prognosis, clinical outcome and response to therapy. Although IHC-based assays do not provide as much tumor biology as gene-based ones do, (as 5 % tumors are negative on IHC even they do express the concern gene) they are increasingly used as a surrogate for molecular gene profiling since they allow classification of tumors at affordable costs and in the absence of fresh tissue specimens. Currently used histological classification system for breast cancer is not very accurate to predict the prognosis or to select the treatment protocol for an individual patient. Some breast cancer patients having higher histological score and poor differentiation have complete treatment response and long-term survival without metastasis and recurrence. Contrary to this, patients with low grade and well differentiated tumors may have metastatic disease even after best treatment. Thus, there may be a need for a different classification system as molecular classification. This would result in less frequent use of chemotherapy with considerable advantages like reducing in toxicity and costs.[5]

Perou et al. were the first to provide a classification system based on gene expression analysis, which consisted of four major molecular classes of breast cancer: luminal-like, basal-like, normal-like, and HER-2 positive.[7] Subsequent studies suggested the existence of more molecular classes and this led to addition of a fifth category, with the molecular spectrum now expanding to luminal A, luminal B, HER2 over expressing, basal-like, and normal-like.[10]

Initially Ki67 monoclonal antibody was used to stain proliferating cells only in unfixed tissues, not in formalin-fixed paraffin-embedded samples. The development of new antibodies MIB-1 and MIB-3[11] in 1992 caused successful staining in paraffin-embedded samples. MIB-1 was superior to other antibodies because of the simplicity of the technique and a good association with Ki67 expression on tissue during frozen section.[12] Manually scoring systems are based on percentage of tumor cells stained by the antibody. We used manual method to detect scoring system of Ki-67, but it is tedious to perform and is poorly reproducible. Currently, automated readers are used to score large series of samples and are also applicable on FNAC specimens

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but this method may count non-malignant nuclei also and cause a potential error.[13] In pathology, newer technique tissue microarray has been used and if the sample size is only 0.6-mm tissue, results are valid and reproducible also.[14]

Normally, in healthy breast tissue and in epithelium adjacent to the fibroadenoma, Ki-67 expression is of low levels (<3%).[15] Ki67 is expressed exclusively in estrogen receptor (ER)-negative cells, meaning that under normal circumstances ER-positive cells are less likely to proliferate. But in malignancy this division does not occur, as expression of Ki-67 increases gradually from benign breast disease to ductal carcinoma in situ to invasive breast cancer.[16] Finally, Ki-67 expression can be used to differentiate some epithelial subtypes of breast carcinoma as its high levels are found in sebaceous and lipid-rich breast carcinomas.[17]

Relation of Ki-67 with other biologic markers of breast carcinoma also has been studied. The ER, PR have an inverse relation with Ki-67, as these receptors are found in least proliferating tumors.[18] Its relationship with expression of HER2 Neu and EGFR is conflicting.[19] Tumors with higher rates of proliferation as measured by Ki-67, it is found that oncogene p53 is often mutated.[20] Ki-67 has an inverse relation with Bcl-2 gene and Ki-67/Bcl2 index has been used as an independent predictive factor for survival in patients of ER positive breast carcinoma.[21,22]

Wiesner et al. reviewed the role of Ki-67 as a marker in routine clinical use in breast cancer patients and concluded that its expression is statistically significant for overall survival and a trend towards disease free survival.[23] Grading of tumor does not correlate with Ki-67 levels so they conclude that Ki-67 has an independent prognostic value. In our study we had some correlation with NPI as it contains low "P" value but it could not be reached to the level of significance. In a meta-analysis de Azambuja et al.[24] produced the data from various studies which included node negative and node-positive patients and analyse the data for disease-free survival. In addition, they analysis of data from 9 studies about the overall survival and its relation with Ki67. They concluded that Ki67 positivity had an adverse effect on disease free as well as overall survival in a given population and partly in the subgroups of patient with node-positive disease. Molino et al. in a study of 126 patients concluded that Ki-67 had an adverse effect on overall and partially with disease free survival and stated that Ki-67 may identify a different phenotype of breast carcinoma.[25]

Klintman et al. showed the Ki-67 is a prognostic factor in node negative breast carcinoma which is restricted to ER positive and histological grade 2 patients.[26] Railo et al. concluded that Ki-67 was the only prognostic marker for long term survival in a study of patients with T1 and node negative breast carcinoma.[27]

Billgren et al. analysed the significance of Ki67/MIB-1 antibody as a proliferation in FNAC specimens. They concluded that Ki67 had significant prognostic value, which is independent of tumor size, ER status and lymph node positivity.[28]

The appropriate cut-off value of Ki-67 is still a matter of debate among different onco-pathologists as this hamper the comparison between studies. The St. Gallen consensus[29] had classified tissues according to the Ki-67 index of <15%, 16-30% and >30% as low, intermediate and highly proliferating respectively but different studies use different cut-off value of Ki-67. We used <10% as low and >15 % as high index and between these were equivocal and we did not include in the study. In our study Ki-67 was low in Her2 Neu and basal type variety probably due to facts as we used manual method for evaluation of Ki-67, heterogeneity in its expression into the tumor and may be low sample size as 8 and 7 patients respectively.

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Although Ki-67 has been investigated extensively as a prognostic and predictive marker, it is still not using widely as a marker in clinical practice. Interpretation of the data of different studies is difficult to correlate since standardization/ regulation of pathological assessment is not yet accomplished.

There is a wide variation in handling of tissue, timing of fixation, dilution of formalin and its pH, testing methods (manual or by automated reader) and procedure of antigen retrieval. These manoeuvres are not validated worldwide.[30] Furthermore, inter-observer variability is high in assessment and factors such as selection of the tumor area for test, type of antibody has used and human error play a significant role. Computer assisted image[31] analysis can enhance the accuracy and inter-observer reproducibility of assessment. Although computer assisted form can measure much larger number of cells that may be more representative of whole tumor section and ameliorate the problem of tumor heterogeneity, but it has a limited capacity to exclude inflammatory and normal stromal cells.

To summarise, as large number of studies regarding the prognostic value of Ki67 have been published, it might be helpful in future in identifying patients with a favourable diagnosis and in selecting appropriate treatment.

References

1. World Health Organization International Agency for Research on Cancer (June 2003). "World Cancer Report".
2. International Agency for Research on Cancer, World Health Organization. (Taken from- http://globocan.iarc.fr)
3. Esteva FJ, Hortobagyi GN. Prognostic molecular markers in early breast cancer. Breast Cancer Res 2004; 6: 109–18.
4. Key G, Becker MH, Baron B et al. Modalities of synthesis of Ki67 antigen during the stimulation of lymphocytes. Cytometry 1991; 12: 42–9.
5. Zepeda-Castilla EJ, Recinos-Money E, Cuellar-Hubbe M et al. Molecular classification of breast cancer. Cir Cir. 2008; 76:87-93.
6. Paik S, Tang G, Shak S, Kim C et al. Gene expression and benefit of chemotherapy in women with node negative, estrogen receptor-positive breast cancer. J Clin Oncol. 2006; 24: 3726–34.
7. Perou CM, Sorlie T, Eisen MB et al. Molecular portraits of human breast tumours. Nature. 2000; 406:747-52.
8. Carey LA, Perou CM, Dressler LG et al. Race and the poor prognosis basal-like breast cancer (BBC) phenotype in the population-based Carolina Breast Cancer Study. J Clin Oncol. 2004(Suppl): Abstr 9510.
9. Yu KD, Shen ZZ, Shao ZM. The immunohistochemically “ER-negative, PR-negative, HER2-negative, CK5/6-negative, and HER1-negative” subgroup is not a surrogate for the normal-like subtype in breast cancer. Breast Cancer Res Treat. 2009; 118:661-3.
10. Kaufmann O, Fietze E, Mengs J et al. Value of p63 and Cytokeratin 5/6 as Immunohistochemical Markers for the Differential Diagnosis of Poorly Differentiated and Undifferentiated Carcinomas. Am J Clin Pathol. 2001; 116:823-30.
11. Key G, Becker MH, Baron B et al. New Ki-67-equivalent murine monoclonal antibodies (MIB 1–3) generated against bacterially expressed parts of the Ki-67 cDNA containing three 62 base pair repetitive elements encoding for the Ki-67 epitope. Lab Invest 1993; 68: 629–36.

12. McCormick D, Yu C, Hobbs C et al. The relevance of antibody concentration to the immunohistological quantification of cell proliferation-associated antigens. Histopathology 1993; 22: 543–547. 2008; 26: 5569–75.
13. Zabaglo L, Ormerod MG, Dowsett M. Measurement of proliferation marker Ki67 in breast tumour FNAs using laser scanning cytometry in comparison to conventional immunocytochemistry. Cytometry B Clin Cytom 2003; 56: 55–61.
14. Ruiz C, Seibt S, Al Kuraya K et al. Tissue microarrays for comparing molecular features with proliferation activity in breast cancer. Int J Cancer 2006; 118: 2190–4.
15. de Lima GR, Facina G, Shida JY et al. Effects of low dose tamoxifen on normal breast tissue from premenopausal women. Eur J Cancer 2003; 39: 891–8.
16. Allred DC, Mohsin SK, Fuqua SA. Histological and biological evolution of human premalignant breast disease. Endocr Relat Cancer 2001; 8: 47–61.
17. Tavassoli FA, Devilee P (eds). World Health Organization Classification of Tumors, Tumors of the Breast and Female Genital Organs, ed 2. Lyon, IARC Press, 2003.
18. Rudas M, Gnant MF, Mittlbock M et al. Thymidine labeling index and Ki-67 growth fraction in breast cancer: comparison and correlation with prognosis. Breast Cancer Res Treat 1994; 32: 165–75.
19. Nicholson RI, McClelland RA, Finlay P et al. Relationship between EGF-R, c-erbB-2 protein expression and Ki67 immunostaining in breast cancer and hormone sensitivity. Eur J Cancer 1993; 29: 1018–23.
20. Pinder SE, Wencyk P, Sibbering DM et al. Assessment of the new proliferation marker MIB1 in breast carcinoma using image analysis: associations with other prognostic factors and survival. Br J Cancer 1995; 71: 146–9.
21. Liu S, Edgerton SM, Moore DH et al. Measures of cell turnover (proliferation and apoptosis) and their association with survival in breast cancer. Clin Cancer Res 2001; 7: 1716–23.
22. Ali HR, Dawson SJ, Blows FM, et al. A Ki67/BCL2 index based on immunohistochemistry is highly prognostic in ER-positive breast cancer. J Pathol 2012; 226: 97–107.
23. Wiesner FG, Magener A, Fasching PA et al. Ki-67 as a prognostic molecular marker in routine clinical use in breast cancer patients. Breast 2009; 18: 135–41.
24. de Azambuja E, Cardoso F, de Castro G Jr et al. Ki67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer 2007; 96: 1504–13.
25. Molino A, Pedersini R, Micciolo R et al. Relationship between the thymidine labeling and Ki67 proliferative indices in 126 breast cancer patients.Appl Immunohistochem Mol Morphol2002;10:304–9.
26. Klintman M, Bendahl PO, Grabau D et al. South Sweden Breast Cancer Group: The prognostic value of Ki67 is dependent on estrogen receptor status and histological grade in premenopausal patients with node-negative breast cancer. Mod Pathol 2010; 23: 251–9.
27. Railo M, Lundin J, Haglund C et al. Ki-67, p53, ER receptors, ploidy and S phase as long-term prognostic factors in T1 node-negative breast cancer. Tumour Biol 2007; 28: 45–51.
28. Billgren AM, TAni E, Liedberg A et al. Prognostic significance of tumor cell proliferation analyzed in fine needle aspirates from primary breast cancer. Breast Cancer Res Treat 2002; 71: 161–70.
29. Colozza M, Sidoni A, Piccart-Gebhart M. Value of Ki67 in breast cancer: the debate is still open. Lancet Oncol 2010; 11: 414–5.
30. Eichbaum MH, Kaltwasser M, Bruckner T et al. Prognostic factors for patients with liver metastases from breast cancer. Breast Cancer Res Treat 2006; 96: 53–62.
31. Demichelis F, Barbareschi M, Dalla Palma P et al. The virtual case: a new method to completely digitize cytological and histological slides. Virchows Arch 2002; 441: 159–64.