

To Study Clinicopathological Correlation of Ki67 in Invasive Breast Cancer

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Abstract:

Introduction- Ki67 expression is a biomarker for proliferation. Expression of ki 67 and its Association with other prognostic factors in breast cancer is recognised significantly.

Aim-(I) To classify breast cancer on the basis of Ki 67 and hormone status(molecular subtypes).(3)To correlate ki67 with other clinicopathological parameters.

Method- 77 breast cancer women with invasive ductal carcinoma(MRM specimen) were included in this study. Hormone receptor status, HER2 status and ki67 index were determined for breast cancer subtype. Age, tumor size, lymph node involvement, histological grade, nuclear grade, and lymphovascular invasion were evaluated and correlated with Ki67 index.

Statistics used -Chi square test

Results- In our study with 77 breast cancer patients, patients with age>50 years , 26 cases show more than 20% ki67 index in comparison 9 cases show <20% positivity. After analysing the histological grade with ki67 index , 54 cases having histological grade 2 & 3 showing > 20% Ki67 index. Hormonal receptor her2/ neu expression was directly correlated with ki67 index. In her2 neu study , out of 26 positive cases, 22 cases showed >20%. While there is significant inverse correlation between Ki67 with ER and PR status of patients.

Conclusion- Patients with low ER and PR expression have higher value of ki67. Other factors eg. Older age, tumor size , higher histological and nuclear grade, Her2/neu positivity are also correlated with increased Ki67 index. Ki67 can be used as predictive and prognostic marker in breast cancer patients when integrated with subtypes of breast cancer, specially in triple negative and ER positive cases.

Key words: Ki67 index, breast cancer

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I. Introduction

Breast cancer is the most common type of cancer and the leading cause of cancer-associated mortalities¹. An understanding of the clinicopathological parameters associated with breast cancer is essential to personalized treatment². Different Parameters eg. age, tumor size, histological grade, mitotic counts, lymphovascular invasion, lymph node involvement, Hormonal receptor expression , Her2/ neu expression and Ki67 index are used as predictive or prognostic factors.

Ki-67 is a nuclear protein being associated with cellular proliferation and was originally identified by Gerdes et al.³ A number of studies have demonstrated, Ki67 is an important biomarker used in routine clinical and pathological practice, with potential applications in prognosis, used to predict responses or resistance to chemotherapy and endocrine therapy, even for molecular subtyping of breast cancer.⁴In the 2017 St Gallen / Vienna consensus, distinction between Luminal A like and Luminal B like by IHC describes important categories in luminal breast cancer which is prognostically different.⁵ According to ESMO clinical practice guidelines, breast cancer is classified into five molecular subtypes based on the expression of four markers ; ER, PR , Her2/neu and Ki67.⁶In contrast to St Gallen consensus , The American Society of Clinical Oncology (ASCO) Tumor Marker Guidelines Committee did not advise the use of Ki-67 for prognosis in patients with newly identified breast cancer because of insufficient quality assurance.⁶

Therefore, understanding the association of Ki67 with pathological characteristics (including histological grade, tumor size and lymph node metastasis) and immunohistochemical indexes [including the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2/neu) status] is important for subtyping breast cancers, clinical evaluations and guiding treatment strategies.

II. Material And Methods

In our study, 77 breast cancer women with invasive ductal carcinoma (MRM specimen) were included for this study. The histopathological findings were analyzed. Hormone receptor status, Her2/neu status and ki67 index (% of Ki67 positive cancer nuclei) were determined. IHC was done on the thin cut sections on polylysine coated slides. ER and PR immunohistochemical staining was performed using antibodies of Biocare diagnostic. IHC staining was done using concentrated and prediluted rabbit monoclonal antibody clone SP1 for ER (1:50-1:100) and clone SP2 for PR (dilution 1:100-1:200). The cut-off value for a positive result was positive staining for ER and PR in $\geq 1\%$ of tumor cells in 10 selected tumor sub-regions⁷. Intensity were graded using Allred scoring for ER and PR expression. Her2/neu immunohistochemical staining was performed using concentrated and prediluted monoclonal antibody of Biocare diagnostic (dilution 1:50-1:100). Her2/neu expression were analysed by using modified 2013 ASCO/CAP guidelines.

Ki67 immunohistochemical staining was performed using antibodies of Biocare diagnostics. At least three fields in particular staining 'hot-spots' were selected in order to represent the spectrum of staining observed upon the initial overview of the entire section. The cancer cells in the three micrographs were manually counted (500–1,000 cells were counted), and the percentage of positively-stained cancer cells were considered to be the Ki67 score.⁸

External and Internal controls were set and normal epithelial elements also served as our internal controls. Age, tumor size, lymph node involvement, histological grade, nuclear grade, and vascular invasion were other parameters those determined and correlated with Ki67 index.

Study Design: Retrospective observational study

Study Location: This was a tertiary care teaching hospital based study done in department of Pathology SMS Medical college & Attached Hospital, Rajasthan University of Health Sciences, Jaipur, Rajasthan

Study Duration: September 2016 to August 2017

Sample size: 77 patients

Statistical Analysis

The correlation of Ki67 as a categorical variable was determined using the χ^2 -test. Chi square test was used to analyse the significance of correlation between the expression of Ki67 and other parameters. Two-tailed $P < 0.05$ was considered to indicate a statistically significant difference.

III. Result

In study with 77 female patients, mean age of diagnosis is 50.3 years. Out of 53 patients, 39 cases had ≥ 2 cm (73.58%) showed $>20\%$ Ki67. As per RB grading score- 5 patients had score of grade I (6.49%), 52 patients (67.53%) had grade II score and 20 patients had grade III (25.97%). Approximately 75% of patients in both categories of age variables had $>20\%$ of Ki67 index. The findings are given in table 1.

After analysing the histological grade, cases with grade II and III histology; 75% showed $>20\%$ Ki67 whereas 25% of these cases showed $<20\%$ index.

Hormonal receptor her2/neu expression was directly correlated with ki67 indexing. 84.62% her2/neu positive and equivocal cases showed ki67 $>20\%$. But this was not statistically significant. This could be because of small sample size.

86.36% ER negative cases had Ki67 index $>20\%$. The same was seen in PR negative cases, where Ki67 index was $>20\%$. There was significant inverse correlation between Ki67 index and ER/PR status of patients. (p value < 0.05).

In this study, Ki67 positivity could not be correlated with histological variables like lymphovascular invasion and lymph node status. We noticed that patients with Ki67 index $> 20\%$ were more in age < 50 years, tumor size > 2 cm and high histological grade comparatively.

Cases were divided into five subtypes based on the expression levels of ER, PR and Her2/neu and the Ki67 proliferation index as per Emad et al⁶. The findings are given in table 2.

Table1: The correlation between Ki67 with other factors in breast cancer female patients

Variables	Ki67<20%	Ki67>20%	total	P value
Age(years)<50	10	32	42	0.846925337
>50	9	26	35	
Total	19	58	77	
Tumor size(cm)<2	5	19	24	0.598733669
>2	14	39	53	
total	19	58	77	
Histological grade -I	1	4	5	0.181302639
II	10	42	52	
III	8	12	20	
Total	19	58	77	
Nuclear grade-I	0	3	3	0.284828895
II	11	40	51	
III	8	15	23	
Total	19	58	77	
Lymph node involvement-Yes	8	27	35	0.735494956
No	11	31	42	
Total	19	58	77	
Vascular invasion- Yes	10	27	37	0.645257134
No	9	31	40	
total	19	58	77	
ER- Positive	13	20	33	0.009475
Negative	6	38	44	
Total	19	58	77	
PR-Positive	14	17	31	0.000619399
Negative	5	41	46	
Total	19	58	77	
Her2/neu- Score 3, positive	1	11	12	0.298319577
Score 2, equivocal	3	11	14	
Negative	15	36	51	
Total	19	58	77	

Statistical analysis: The correlation of Ki67 as a categorical variable was determined using the χ^2 -test. Chi square test was used to analyse the significance of correlation between the expression of Ki67 and other parameters. Two-tailed $P < 0.05$ was considered to indicate a statistically significant difference.

Table2 : Numbers of patients based on Ki67 and types of tumor

	Luminal A (ER+, PR+, Her2-)	Luminal B (ER+, PR+/-)		Her2 positive (ER-,PR-)	Basal type (triple negative)
		Her2 +ve	Her2 -ve with High Ki67		
High ki67	0	9	10	11	24
Low ki67	9	5		0	5
Total	9(11.6%)	14(18.18%)	10(12.98%)	11(14.28%)	29(37.66%)

*4 cases showed only PR positivity out of which 2 had high ki67 and 2 had low ki67.

- Luminal A subtype:** ER- and/or PR-positive, Her2/neu-negative and a low Ki67 proliferation index of $\leq 20\%$;
- Luminal B (high Ki67) subtype:** ER- and/or PR-positive, Her2/neu-negative and a high Ki67 index ($>20\%$);
- Luminal B (HER2-positive) subtype:** ER- and/or PR-positive, Her2/neu-positive and any Ki67 index;
- HER2-positive (non-luminal) subtype:** ER- and PR-negative, Her2/neu-positive and any Ki67 index;
- Triple-negative subtype:** ER-, PR- and Her2/neu-negative, and any Ki67 index .

Among 77 breast cancer patients, there were 9 (11.6%) with luminal A , 14 (18.18%) with luminal B subtype, 10 (12.98%) with luminal B subtype- Her2neu negative with high Ki67 index, 11 (14.28%) with Her2 neu positive and 29 (37.66%) were triple negative types. In triple negative cases, 82.75% cases showed high Ki67 index.

IV. Discussion

Large number of women are diagnosed with breast cancer annually worldwide. Treatment protocol for breast cancer doesn't end up in having clinical examination with radiological and histological diagnosis per se. Introduction of efficient biomarkers for the timely prognosis of breast cancer is great concern. This study was focused on prognostic and predictive value of hormone receptors, Epithelial growth factor receptor, and proliferation index with clinicopathological parameters for treatment plan. ER, PR and Her2/neu routinely used immunohistochemically for subtyping breast cancer. Now many consensus concern regarding new additional markers to know proliferation rate and their validation. The proliferation rate of the breast cancer cell is routinely measured by histological grade, mitotic count, lymphovascular invasion and tumour size. It can also be measured by IHC and gene expression profiling of ki67. Ki67 has potential to emerge as prognostic and predictive tool for breast cancer patients, and has become an important biomarker in routine clinical practice. Number of studies done in past established correlation of ki67 with other clinicopathological parameters though the findings are controversial.⁹

Pinder SE et al demonstrated a significant association between the Ki67 index and the histological grade, size and type of the tumors.¹⁰ The study by Haroons also showed a significant association between Ki67 expression and tumor grade, PR, Her2/neu and lymph node status. However, no correlation was identified between the ER status and tumor size.¹¹

A meta-analysis of 12,155 patients demonstrated that the Ki-67 positivity show a higher risk of recurrence in patients with early breast cancer. The study concluded that the high levels of Ki-67 are associated with worse prognosis.¹² According M E Perez et al, Ki-67 has been demonstrated as an independent and predictor of survival.¹³ In a meta-analysis investigating the proliferation markers and survival in early breast cancer included data from 32,825 patients and concluded that higher expression of Ki-67 was associated with bad survival rates.¹⁴

Jinzhong et al found that Higher levels of ER and PR were correlated with declining Ki67 scores¹⁵, while higher levels of Her2/neu were associated with increasing Ki67 scores, results indicated that there was an increased proliferative activity in the breast cancer cells with lower levels of ER and PR, or higher levels of Her2, and that Ki67 is an accurate biomarker that denote tumor cellular proliferate activity. They also found Ki67 score increased with increasing tumor size in the early stages of breast cancer which suggest proliferative activity increases with the progression of a tumor.

In our study we also found the same thing with regard to ER, PR as to higher level of ER and PR associated with low ki67 and lower ER and PR associated with higher ki67 and the results are statistically significant. We get same results with regard to Her2 but we didn't find statistically significant correlation between the two this can be assumed because of our smaller sample size.

St Gallen consensus is continuously working on usefulness of Ki67 in breast cancer patients for subtyping.^{16,17} In our study we found triple negative breast cancer are exclusively associated with higher ki67. Also majority of patients in HER2 type cohort have higher ki67(11 of 11). These results are similar to a previous study which also identified higher Ki67 expression levels in triple-negative and Her2/neu-positive subtypes compared with the luminal subtypes.¹⁸

We also found higher ki67 in age group <50yr, tumor size >2cm but they didn't turn out to be statistically significant. Higher ki67 index was also found in histological and nuclear grade 2 patients they seems to be because of higher number of patients among these grades.

V. Conclusion

Instead of limitation of resources and small sample size in our study, there is significant association between Ki67 and other clinicopathological parameters. Analysing the data of MRM specimen, it is concluded that patients with low ER and PR expression have higher the value of ki67. Ki 67 can also be used to identify breast cancer subtyping which are differ in Ki67 expression., Other factors eg. Older age, tumor size, higher histological grade, higher nuclear grade, Her2/neu positivity are also correlated with increased Ki67 index. So it can be a predictive and prognostic marker in breast cancer patients by integration of subtyping of breast cancer, specially in triple negative and ER positive cases. We can also able to distinguish Luminal B subgrouping of breast cancers from luminal A subgroups, prognosis is different in both groups.

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