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Original Research Article

Clinicopathological relevance of Ki-67 expression and its association with immunoprofile in breast cancer

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ABSTRACT

Background: Ki-67 is a widely used proliferation marker in breast carcinoma; however, its prognostic relevance remains inconsistent due to variability in assessment and lack of standardized cut-offs. Its correlation with established clinicopathological parameters and hormone receptor status continues to be an area of active investigation. Objectives were to evaluate Ki-67 expression and determine its association with clinicopathological features and immunohistochemical markers (ER, PR, HER2/neu) in invasive breast carcinoma.
Methods: This hospital-based cross-sectional study included 88 cases of primary invasive breast carcinoma. Ki-67 expression was assessed by immunohistochemistry and categorized as low (<20%) or high (≥20%). Associations with tumor size, grade, lymph node status, lymphovascular invasion, perineural invasion, and hormone receptor status were analyzed using Chi-square/Fisher's exact test. A $p<0.05$ was considered statistically significant.
Results: High Ki-67 expression (≥20%) was observed in 44.3% of cases. Significant associations were noted between high Ki-67 expression and larger tumor size ($p=0.018$), higher histological grade ($p=0.013$), lymphovascular invasion ($p=0.001$), perineural invasion ($p=0.002$), and HER2/neu positivity ($p=0.001$). No significant association was found with age, lymph node metastasis, ER status, PR status, combined ER/PR status, or triple marker status.
Conclusions: Ki-67 expression demonstrates significant association with adverse pathological features and HER2/neu positivity, supporting its role as an adjunct prognostic marker in invasive breast carcinoma. Incorporation of Ki-67 into routine evaluation may enhance risk stratification, particularly in resource-limited settings; however, standardization of assessment methods remains essential for its optimal clinical utility.

Keywords: Breast carcinoma, Ki-67, Proliferation index, ER, PR, HER2/neu, Prognostic markers

INTRODUCTION

Breast cancer is the most common malignancy among women worldwide and a leading cause of cancer-related mortality, accounting for approximately 2.3 million new cases and 685,000 deaths annually GLOBOCAN 2020 global cancer statistics.[1] In India, it constitutes a significant proportion of female cancers, with rising incidence attributed to urbanization, lifestyle changes, and improved detection rates.[2]

Breast carcinoma is a biologically heterogeneous disease, and its clinical behavior is influenced by multiple prognostic and predictive factors, including tumor size, histological grade, lymph node status, and molecular subtype.[3] Immunohistochemical markers such as estrogen receptor (ER), progesterone receptor (PR), and human

epidermal growth factor receptor 2 (HER2/neu) have become integral to therapeutic decision-making and prognostication.[4]

Ki-67, a nuclear non-histone protein expressed during all active phases of the cell cycle except G0, serves as a marker of cellular proliferation. Its expression reflects tumor growth fraction and has been proposed as a useful prognostic and predictive biomarker in breast carcinoma.[5] Several studies have demonstrated that high Ki-67 expression is associated with aggressive tumor characteristics, poor differentiation, and unfavorable outcomes.[6] However, its clinical utility remains controversial due to inter-observer variability, differences in scoring methods, and lack of universally accepted cut-off values.

The International Ki-67 in Breast Cancer Working Group has emphasized the need for standardization in pre-analytical, analytical, and interpretative aspects of Ki-67 assessment to improve reproducibility and clinical applicability.[7] Despite these limitations, Ki-67 continues to be widely used, particularly in resource-limited settings, where access to advanced molecular testing may be restricted.

Given these considerations, the present study was undertaken to evaluate Ki-67 expression in invasive breast carcinoma and to correlate it with clinicopathological parameters and established immunohistochemical markers (ER, PR, HER2/neu), thereby assessing its potential role as an adjunct prognostic marker.

METHODS

This hospital-based cross-sectional observational study was conducted over a period of one year (March 2020 to February 2021) in the Department of Pathology, Himachal Pradesh, after obtaining approval from the Institutional Scientific and Ethics Committees.

A total of 88 cases of primary invasive breast carcinoma undergoing radical or modified radical mastectomy were included. Cases with benign lesions, carcinoma in situ, recurrent or metastatic tumors, and patients who had received neoadjuvant chemo-radiotherapy were excluded.

All specimens were fixed in 10% neutral buffered formalin for 24–48 hours. Gross examination was performed, and representative sections were processed for histopathological evaluation using hematoxylin and eosin staining. Tumors were graded according to the Nottingham modification of the Bloom–Richardson grading system.

Immunohistochemistry (IHC) for Ki-67, ER, PR, and HER2/neu was performed using the BioGenex XMatrix Fully Automated Processing System (i6000™ Infinity).

Interpretation of IHC

Ki-67

Ki-67 labeling index (LI) was calculated as the percentage of positively stained tumor nuclei among at least 500 malignant cells. Both hotspot and random field assessment methods were used. Low<20% and high-≥20% (as recommended by international consensus guidelines).[7]

ER and PR

Evaluated using the Allred scoring system, combining proportion and intensity scores (0–8). A score ≥3 was considered positive.[8]

HER2/neu

Assessed based on membranous staining using ASCO/CAP HER2 testing guidelines.[9] Only 3+ staining was considered positive.

Statistical analysis

Data were entered into Microsoft excel and analyzed using Epi Info version 7.2.2.0. Associations between Ki-67 expression and clinicopathological parameters were evaluated using Chi-square or Fisher's exact test. A $p<0.05$ was considered statistically significant.

RESULTS

A total of 88 mastectomy specimens were analyzed. The age of patients ranged from 31 to 90 years, with a mean age of 54.10±12.51 years, and the majority (60.2%) belonged to the 41-60-year age group. Most tumors were located in the upper inner quadrant (29.5%), followed by the lower inner quadrant (26.1%). The majority of cases (69.3%) had tumor size ranging from 2-5 cm (pT2). Histopathologically, grade II carcinoma was the most common (66%), while grade I and III each accounted for 17% of cases. Axillary lymph node metastasis was observed in 52 out of 88 cases (59.1%), with additional involvement of Rotter's lymph nodes in 4 cases. Lymphovascular invasion was present in 69.3% of cases, and perineural invasion was seen in 45.5%.

High Ki-67 expression (≥20%) was observed in 39 cases (44.3%), whereas 49 cases (55.7%) showed low expression. On correlation analysis, no statistically significant association was found between Ki-67 expression and age ($p=0.151$) or lymph node metastasis ($p=0.126$). Although higher Ki-67 expression was noted in tumors >5 cm and in higher-grade tumors, the associations were statistically significant (tumor size: $p=0.018$; tumor grade: $p=0.013$). Significant association was observed between high Ki-67 expression and lymphovascular invasion ($p=0.001$) as well as perineural invasion ($p=0.002$).

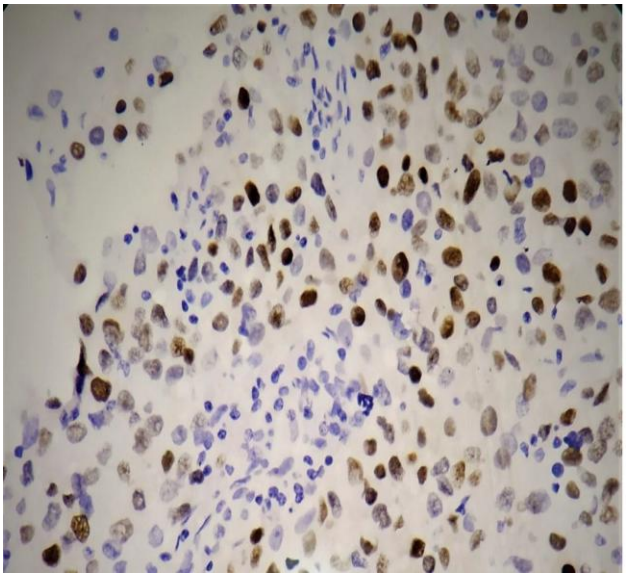

Figure 1: ER positive tumor with Allred score 4+3=7 (10×).

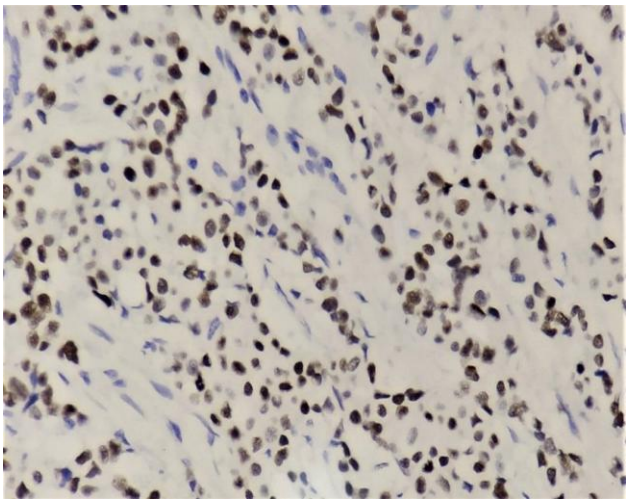

Figure 2: PR positive tumor with Allred score 4+3=7 (10×).

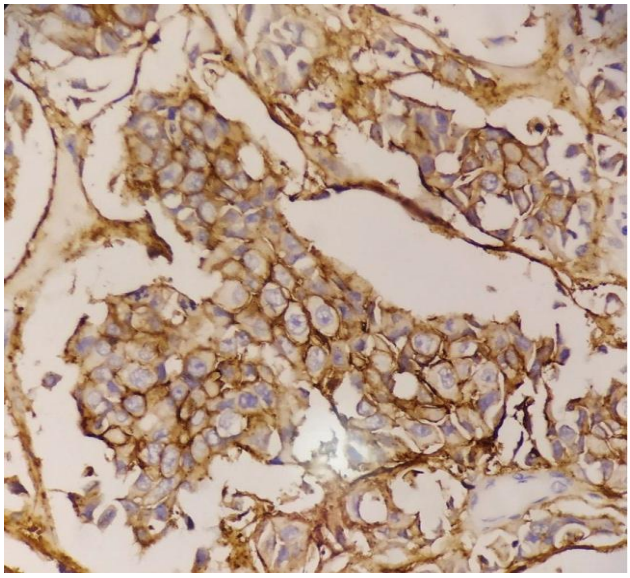

Figure 3: HER 2 neu 3+ (10×).

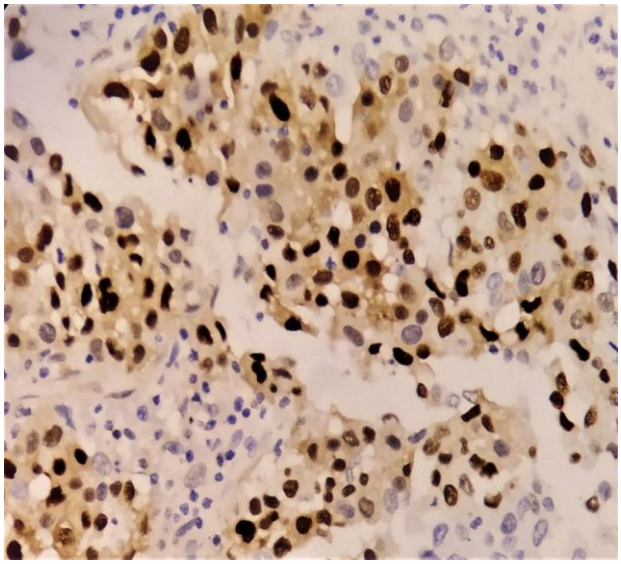

Figure 4: Ki 67 approx. 60% (30×).

Figure 1: Clinicopathological parameters and Ki-67 correlation, (n=88).

Parameters	Category	Ki67 <20%, N (%)	Ki67 >20%, N (%)	Total, N (%)
Histologic grade	Grade I	12 (24.5)	3 (7.7)	15 (17.0)
	Grade II	33 (67.3)	25 (64.1)	58 (65.9)
	Grade III	4 (8.2)	11 (28.2)	15 (17.0)
ER/PR status	ER+ PR+	24 (49.0)	15 (38.5)	39 (44.3)
	ER+ PR- / ER- PR+	5 (10.2)	6 (15.4)	11 (12.5)
	ER- PR-	20 (40.8)	18 (46.2)	38 (43.2)
HER2/neu status	Positive	5 (10.2)	17 (43.6)	22 (25.0)
	Negative	44 (89.8)	22 (56.4)	66 (75.0)
Triple marker status	Triple positive	1 (2.0)	6 (15.4)	7 (8.0)
	Triple negative	16 (32.7)	10 (25.6)	26 (29.5)
	Others	32 (65.3)	23 (59.0)	55 (62.5)
Overall Ki67 distribution	<20%	49 (100.0)	-	49 (55.7)
	>20%	-	39 (100.0)	39 (44.3)

Regarding hormone receptor status, 52.3% of cases were ER-positive and 48.9% were PR-positive, while HER2/neu positivity was observed in 25% of cases. Combined ER+/PR+ status was seen in 44.3% of cases, whereas 43.2% were ER-/PR-. Triple-negative and triple-positive cases constituted 29.5% and 8.0%, respectively. Ki-67 expression did not show a statistically significant association with ER status (p=0.198), PR status (p=0.981), combined ER/PR status, or triple marker status (p=0.056). However, a significant association was found between high Ki-67 expression and HER2/neu positivity (p=0.001).

DISCUSSION

Ki-67 is a well-recognized marker of cellular proliferation and has been extensively studied as a prognostic and predictive biomarker in breast carcinoma. However, despite its widespread use, variability in assessment methods and lack of standardized cut-off values continue to limit its uniform clinical applicability.[6,7]

In the present study, the mean age of patients was 54.10±12.51 years, with the majority (60.2%) in the 41-60-year age group, which is consistent with previously reported Indian and global data.[1,2] Histologically, grade II tumors constituted the majority (66%) of cases, in concordance with studies by Hungund et al and Nigam et al suggesting that intermediate-grade tumors are most commonly encountered.[10,12]

A key finding of the present study was that high Ki-67 expression (≥20%) was observed in 44.3% of cases. Importantly, high Ki-67 expression showed a statistically significant association with larger tumor size (p=0.018) and higher histological grade (p=0.013). These findings are consistent with previous studies, including Liang et al which demonstrated that increased proliferative activity correlates with aggressive tumor characteristics and early relapse.[8]

Furthermore, in the present study, high Ki-67 expression was significantly associated with lymphovascular invasion (69.3% of cases; p=0.001) and perineural invasion (45.5% of cases; p=0.002), indicating a strong relationship between tumor proliferation and invasive potential. Similar observations have been reported by Elkablawy et al who found that elevated Ki-67 expression is linked to adverse pathological features.[13]

With respect to lymph node status, no statistically significant association was observed between Ki-67 expression and axillary lymph node metastasis (p=0.126) in the present study, although metastasis was present in 59.1% of cases. This finding is in agreement with studies by Ahmed et al and Neelakanth et al suggesting that tumor proliferation and nodal spread may be influenced by independent biological mechanisms.[14,15]

Regarding hormone receptor status, 52.3% of cases were ER-positive and 48.9% were PR-positive in the present study. However, no statistically significant association was observed between Ki-67 expression and ER (p=0.198), PR (p=0.981), or combined ER/PR status. These findings are comparable to those reported by Reddy et al and Nigam et al although some studies have demonstrated an inverse relationship between hormone receptor positivity and proliferation index.[11,12,16] Such discrepancies may be attributed to methodological differences, including variability in Ki-67 cut-off values.

A significant association was observed between Ki-67 expression and HER2/neu positivity in the present study (p=0.001), with a higher proportion of HER2-positive tumors showing elevated Ki-67 levels. This finding is consistent with previous reports indicating that HER2-positive tumors exhibit higher proliferative activity and aggressive clinical behavior.[13,16]

In the present study, triple-negative breast cancers constituted 29.5% of cases, while triple-positive tumors accounted for 8.0%. Although higher Ki-67 expression was observed in triple-positive cases, the association between Ki-67 and triple marker status was not statistically significant (p=0.056). This is in contrast to some studies reporting higher Ki-67 expression in triple-negative tumors, and the difference may be due to the relatively small sample size in the present study.

One of the major challenges in the clinical application of Ki-67 is the lack of standardized scoring protocols and universally accepted cut-off values. The international Ki-67 working group has emphasized the need for standardization to improve reproducibility and clinical utility.[6] Variability in these factors likely contributes to differences observed across studies.

The present study has certain limitations, including its relatively small sample size, single-center design, and lack of follow-up data. These factors limit the generalizability of the findings and preclude evaluation of the prognostic impact of Ki-67 on survival outcomes.

CONCLUSION

High Ki-67 expression is significantly associated with adverse clinicopathological features, including larger tumor size, higher histological grade, lymphovascular invasion, perineural invasion, and HER2/neu positivity in invasive breast carcinoma. These findings support the role of Ki-67 as a valuable adjunct prognostic biomarker that may aid in risk stratification and therapeutic decision-making. Although no significant association was observed with ER, PR, or lymph node status, routine assessment of Ki-67, when performed using standardized methodology, may enhance the prognostic evaluation of breast cancer, particularly in resource-limited settings. Further multicentric studies with larger sample sizes and long-term follow-up are warranted to validate its

prognostic significance and establish standardized assessment protocols.

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REFERENCES

1. Sung H, Ferlay J, Siegel RL, Mathieu L, Isabelle S, Ahmedin J, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide. CA Cancer J Clin. 2021;71(3):209-49.
2. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of breast cancer and associated risk factors. Breast Cancer (Dove Med Press). 2019;11:151-64.
3. Harbeck N, Penault-Llorca F, Cortes J, Michael G, Nehmat H, Philip P, et al. Breast cancer. Nat Rev Dis Primers. 2019;5(1):66.
4. Wolff AC, Hammond MEH, Allison KH, Brittany EH, Pamela BM, John MSB, et al. Human epidermal growth factor receptor 2 testing in breast cancer: ASCO/CAP guideline update. Arch Pathol Lab Med. 2018;142(11):1364-82.
5. Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. J Clin Oncol. 2005;23(28):7212-20.
6. Nielsen TO, Leung SCY, Rimm DL, Andrew D, Balazs A, Sunil B, et al. Assessment of Ki67 in breast cancer: updated recommendations from the International Ki67 Working Group. J Natl Cancer Inst. 2021;113(7):808-19.
7. Dowsett M, Nielsen TO, A'Hern R, John B, Charles CR, Jack C, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 Working Group. J Natl Cancer Inst. 2011;103(22):1656-64.
8. Liang Q, Ma D, Gao RF, Ke-Da Y. Effect of Ki-67 expression levels and histological grade on early relapse in breast cancer. Sci Rep. 2020;10(1):7648.
9. Ilić IR, Stojanović NM, Radulović NS, Vesna VŽ, Pavle JR, Aleksandar SP, et al. Quantitative ER immunohistochemical analysis in breast cancer using Allred scoring. Medicina (Kaunas). 2019;55(8):461.
10. Hungund BR, Malur PR, Godhi AS. Ki-67 expression and its association with conventional prognostic markers in breast carcinoma. Int J Health Sci Res. 2019;9(1):215-22.
11. Reddy P, Devi SM. Correlation of ER, PR, HER2/neu and Ki-67 with prognostic factors in breast carcinoma. Trop J Pathol Microbiol. 2020;6(5):349-61.
12. Nigam JS, Kumar T, Shreekant B, Surabhi, Ruchi S, et al. Association of Ki-67 with clinicopathological factors in breast cancer. Cureus. 2021;13(6):e15621.
13. Elkablawy MA, Albasri AM, Mohammed RA, Akbar SH, Magdy MN, Ahmed SA, et al. Ki67 expression in breast cancer and its correlation with prognostic parameters. Saudi Med J. 2016;37(2):137-41.
14. Neelakanth SS, Suchitha S, Manjunath VG. Ki-67 immunostaining in breast carcinoma and correlation with prognostic factors. Indian J Pathol Oncol. 2017;4(1):98-102.
15. Ahmed ST, Ahmed AM, Musa DH, Farhad KS, Muzahm Al-K, Intisar SP. Proliferative index (Ki-67) in breast duct carcinoma and its prognostic significance. Asian Pac J Cancer Prev. 2018;19(4):955-9.
16. Kanyılmaz G, Yavuz BB, Aktan M. Prognostic importance of Ki-67 in breast cancer and its relationship with other prognostic factors. Eur J Breast Health. 2019;15(4):256-61.

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