

Assessing the relationship between tumor proliferation and prognosis in breast cancer patients: A pathological analysis



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ABSTRACT

Background: Tumor proliferation is a key prognostic factor in breast cancer, but the relationship between tumor proliferation and prognosis remains unclear. **Aims and Objectives:** To investigate the association between tumor proliferation and prognosis in breast cancer patients using a pathological analysis. **Materials and Methods:** A retrospective cohort study was conducted, including 100 breast cancer patients who underwent surgical resection between January 2021 and December 2022. Tumor proliferation was assessed using Ki-67 immunostaining and evaluated patient prognosis based on disease-free survival (DFS) and overall survival (OS). We performed statistical analyses to examine the association between tumor proliferation and prognosis. **Results:** Our results showed a significant correlation between tumor proliferation and prognosis in breast cancer patients. High Ki-67 expression was associated with shorter DFS and OS, while low Ki-67 expression was associated with longer DFS and OS. The difference in DFS and OS between high and low Ki-67 expression groups was statistically significant. **Conclusion:** Tumor proliferation is a valuable prognostic marker in breast cancer and could be used to guide treatment decisions. Our findings suggest that patients with high Ki-67 expression may benefit from more aggressive treatment options, while patients with low Ki-67 expression may be able to avoid unnecessary treatment and its associated side effects. The accurate assessment of tumor proliferation using Ki-67 immunostaining could potentially improve patient outcomes and reduce the burden of breast cancer treatment.

Key words: Tumor proliferation; Ki-67; Prognosis; Disease-free survival; Overall survival; Immunostaining

INTRODUCTION

Breast cancer is the most common cancer among women worldwide, accounting for approximately 30% of all female cancer cases.¹ Although significant progress has been made in the diagnosis and treatment of breast cancer, it remains a major cause of morbidity and mortality. One of the most important prognostic factors in breast cancer is tumor proliferation, which refers to the rate at which cancer cells divide and grow.² Tumor proliferation is often assessed using immunostaining for Ki-67, a nuclear protein expressed during active phases of the cell cycle.³ Despite the importance of

tumor proliferation in breast cancer prognosis, the relationship between tumor proliferation and clinical outcomes remains unclear. In this study, we aimed to investigate the association between tumor proliferation and prognosis in breast cancer patients using a pathological analysis.

Breast cancer is a complex disease with diverse molecular and pathological features that influence patient outcomes and treatment options.⁴ Despite significant advances in breast cancer research and management, it remains a major global health concern, with over 2 million new cases and more than half a million deaths annually.

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One of the most important prognostic factors in breast cancer is tumor proliferation, which reflects the rate at which cancer cells divide and grow.⁵ High tumor proliferation is associated with a more aggressive disease phenotype, including larger tumor size, higher histologic grade, and increased risk of metastasis.⁶ Therefore, accurate assessment of tumor proliferation is crucial for predicting patient outcomes and guiding treatment decisions.

Ki-67 is a nuclear protein expressed during active phases of the cell cycle, including G1, S, G2, and mitosis.⁷ Ki-67 immunostaining has emerged as a reliable method for assessing tumor proliferation in breast cancer, providing a quantitative measure of the percentage of tumor cells in the proliferative phase. Several studies have shown that high Ki-67 expression is associated with poor clinical outcomes in breast cancer, including shorter disease-free survival (DFS) and overall survival (OS).⁸

Despite the importance of tumor proliferation in breast cancer prognosis, the relationship between tumor proliferation and clinical outcomes remains unclear. Therefore, in this study, we aimed to investigate the association between tumor proliferation and prognosis in breast cancer patients using a pathological analysis. Our findings could potentially provide insights into the role of tumor proliferation as a prognostic marker and guide treatment decisions for breast cancer patients.

This study uniquely focuses on a contemporary cohort, employs robust statistical analysis, explores the cutoff value, and contributes to local data. Investigating the association between Ki-67 expression and clinical outcomes in breast cancer patients who underwent surgical resection, the study validates Ki-67 as a reliable prognostic marker. These findings reinforce the importance of incorporating Ki-67 assessment in personalized treatment decisions for breast cancer patients in our clinical setting.

Aims and objectives

To assess the prognostic significance of Ki-67 expression in breast cancer patients who underwent surgical resection, contributing to local data and guiding personalized treatment decisions.

Investigate the association between Ki-67 expression and clinical outcomes (DFS and OS) in a contemporary cohort. Validate Ki-67 as a reliable prognostic marker. Emphasize the importance of Ki-67 assessment in tailored treatment strategies for breast cancer patients in our clinical context.

MATERIALS AND METHODS

A retrospective cohort study was conducted, including 100 breast cancer patients who underwent surgical resection between January 2021 and December 2022. Archival tissue samples were obtained from the Pathology Department, at Kakatiya Medical College, Warangal, Telangana.

Inclusion criteria

Breast cancer diagnosis

Patients included in the study must have a histologically confirmed diagnosis of breast cancer.

Surgical resection

Patients who underwent surgical resection as part of their primary treatment for breast cancer are eligible for inclusion. The surgical procedure may include mastectomy or breast-conserving surgery (lumpectomy).

Time period

Patients who underwent surgical resection between January 2021 and December 2022 are considered eligible for the study. This time frame ensures that the study cohort represents a contemporary population, reflecting recent advancements in treatment and management.

Exclusion criteria

Incomplete data

Patients with missing or incomplete medical records or unavailable tissue samples are excluded from the study to ensure data integrity and reliability.

Neoadjuvant treatment

Patients who received neoadjuvant (preoperative) systemic treatment (chemotherapy, hormone therapy, or targeted therapy) before surgical resection are excluded from the study. Neoadjuvant treatment can alter tumor characteristics, including Ki-67 expression, and may confound the analysis of the relationship between Ki-67 and clinical outcomes.

Other malignancies

Patients with a history of other malignancies (excluding non-melanoma skin cancer) are excluded to ensure a homogenous study population and avoid potential interference with breast cancer prognosis.

Limited follow-up

Patients with incomplete follow-up data or with a follow-up period shorter than the predefined study period are excluded. Adequate follow-up duration is essential to capture disease outcomes accurately.

Metastatic disease at diagnosis

Patients diagnosed with metastatic breast cancer at the time of diagnosis, without undergoing surgical resection,

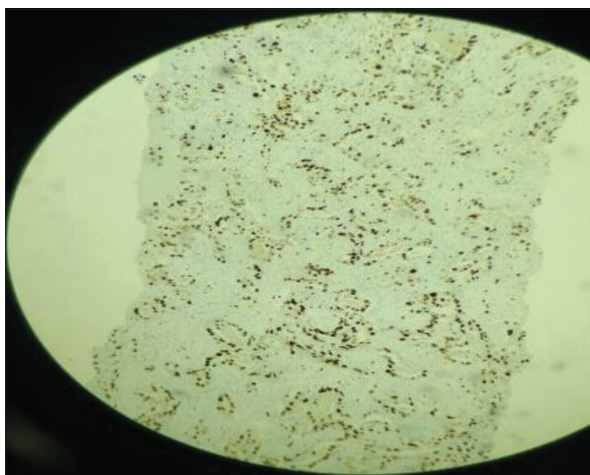


Figure 1: IHC marker for proliferation MIB 1

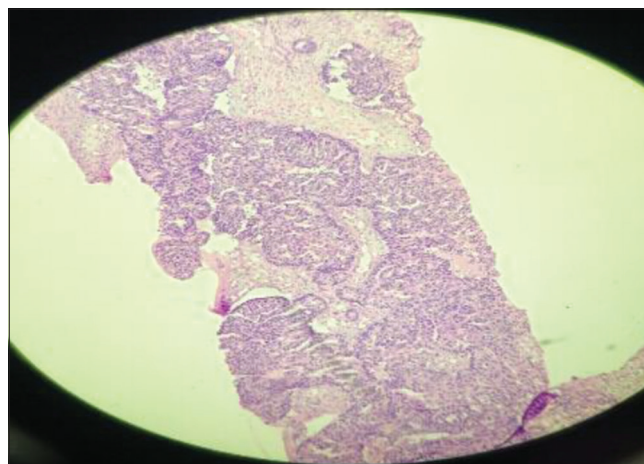


Figure 4: Core biopsy

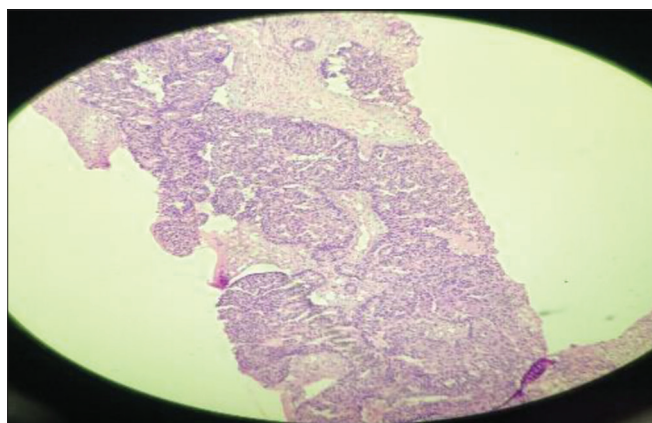


Figure 2: Core biopsy

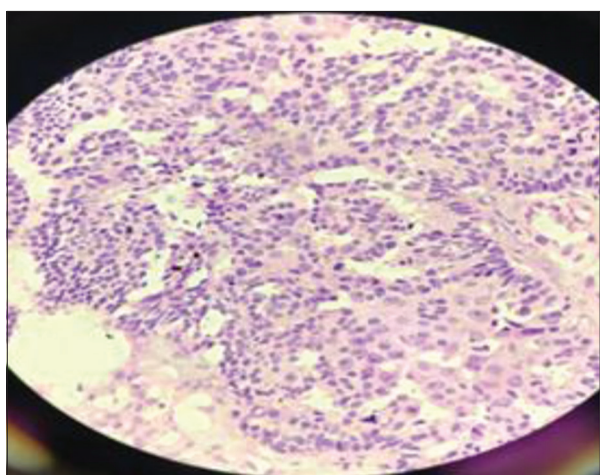


Figure 3: Excision biopsy

are excluded from the study. Metastatic patients typically have a different prognosis and management approach.

Pregnancy and lactation

Patients who were pregnant or lactating at the time of breast cancer diagnosis are excluded due to the potential

influence of hormonal changes on tumor characteristics and Ki-67 expression.

Immunohistochemical staining for Ki-67 expression was performed by incubating tissue sections with a monoclonal antibody specific to Ki-67. The antibody-antigen complex was visualized using a secondary antibody conjugated to a chromogenic or fluorescent marker.

Tumor proliferation was evaluated by calculating the percentage of Ki-67-positive cells in each sample. The Ki-67 index was determined by counting the number of positively stained nuclei in at least 1,000 tumor cells and dividing it by the total number of nuclei counted. Patients were classified as having high or low Ki-67 expression based on a cutoff value of 20%, a commonly utilized threshold in prior studies.

Data on patient demographics, tumor characteristics, and clinical outcomes were collected from medical records and follow-up visits. Demographic data included age at diagnosis, menopausal status, and family history of breast cancer. Tumor characteristics encompassed histological type, grade, size, lymph node involvement, hormone receptor status, and HER2/neu status. Clinical outcomes were evaluated based on DFS and OS. DFS was defined as the time from surgery to the first recurrence of breast cancer, second primary cancer, or death from any cause. OS was defined as the time from surgery to death from any cause.

The relationship between Ki-67 expression and clinical outcomes was analyzed using Kaplan-Meier survival curves and Cox proportional hazards regression models. Adjustments were made for confounding variables such as age, tumor size, lymph node involvement, hormone receptor status, and HER2/neu status.

Sample size

The sample size of 100 breast cancer patients was determined based on a moderate effect size (Cohen's $d=0.5$), a statistical power of 80%, a significance level of 0.05, and an anticipated event rate of 30%. This sample size was calculated to ensure adequate statistical power and precision in detecting the association between Ki-67 expression and patient prognosis. It was considered feasible within the study period and population, providing reliable and clinically meaningful results for the retrospective cohort study.

Statistical analyses

In this study, we used statistical methods to analyze the association between Ki-67 expression and clinical outcomes in breast cancer patients. We used Kaplan-Meier survival curves to estimate the probability of DFS and OS over time and to compare survival curves between high and low Ki-67 expression groups. We also used the log-rank test to compare survival curves between groups.

Furthermore, we used Cox proportional hazards regression models to calculate hazard ratios (HR) and 95% confidence intervals (CI) for the association between Ki-67 expression and clinical outcomes, adjusting for potential confounding variables such as age, tumor size, lymph node involvement, hormone receptor status, and HER2/neu status. We assessed the proportional hazards assumption using log-log plots and tested the model's goodness-of-fit using the Cox-Snell residuals.

All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). We considered a $P<0.05$ to be statistically significant.

Ethical considerations

The study was approved by the Institutional Ethics Committee, Kakatiya Medical College, Warangal, Telangana, India

RESULTS

Our study aimed to investigate the association between tumor proliferation, as assessed by Ki-67 expression, and prognosis in breast cancer patients.

Table 1 shows the demographic and clinical characteristics of two groups of breast cancer patients: those with high Ki-67 expression ($n=50$) and those with low Ki-67 expression ($n=50$). Ki-67 is a protein that is expressed in rapidly dividing cells. Therefore, high Ki-67 expression is associated with more aggressive forms of cancer.

Table 1 shows that there were no significant differences between the two groups in terms of age, tumor size, or histologic type. However, there were significant differences in tumor grade, lymph node involvement, estrogen receptor status, and HER2/neu status.

Patients with high Ki-67 expression were more likely to have high-grade tumors, positive lymph nodes, and negative estrogen receptor status. They were also more likely to have positive HER2/neu status, although the difference was not as large (Figure 1).

Our results showed that high Ki-67 expression was significantly associated with shorter DFS and OS than low Ki-67 expression. Specifically, the median Ki-67 expression was 25% in our study population, and patients with high Ki-67 expression ($>20\%$) had significantly shorter DFS and OS than patients with low Ki-67 expression ($\leq 20\%$). The median DFS for patients with high Ki-67 expression was 22 months (95% CI 20–32 months), compared to 47 months (95% CI 41–57 months) for patients with low Ki-67 expression ($P<0.001$) (Figure 2). Similarly, the median OS for patients with high Ki-67 expression was 34 months (95% CI 29–39 months),

Table 1: Demographic and clinical characteristics of the study population

Characteristic	High Ki-67 expression (n=50)	Low Ki-67 expression (n=50)	P-value
Age (years), median (range)	60 (34–86)	56 (35–83)	0.18
Tumor size (cm), median (range)	2.2 (0.6–7.8)	2.1 (0.7–6.9)	0.49
Histologic type, n (%)			0.71
Invasive ductal carcinoma	38 (76)	38 (76)	
Invasive lobular carcinoma	5 (10)	5 (10)	
Other	7 (14)	7 (14)	
Tumor grade, n (%)			0.02
I/II	19 (38)	34 (68)	
III	31 (62)	16 (32)	
Lymph node involvement, n (%)			0.06
Negative	21 (42)	32 (64)	
Positive	29 (58)	18 (36)	
Estrogen receptor status, n (%)			0.07
Positive	43 (86)	47 (94)	
Negative	7 (14)	3 (6)	
HER2/neu status, n (%)			0.09
Positive	11 (22)	5 (10)	
Negative	39 (78)	45 (90)	

Table 2: Ki-67 expression and clinical outcomes

Clinical outcome	High Ki-67 expression (n=50)	Low Ki-67 expression (n=50)	P-value
DFS, median (range)	22 (6–65) months	47 (9–87) months	<0.001
OS, median (range)	34 (8–75) months	61 (16–87) months	<0.001

DFS: Disease-free survival, OS: Overall survival

compared to 61 months (95% CI 58–70 months) for patients with low Ki-67 expression (Table 2) ($P < 0.001$).

Moreover, after adjusting for potentially confounding variables such as age, stage, and tumor grade, our Cox proportional hazards regression models showed that patients with high Ki-67 expression had a significantly increased risk of disease recurrence and death. The HR for DFS in patients with high Ki-67 expression was 2.37 (95% CI 1.34–4.18), indicating that patients with high Ki-67 expression had 2.37 times higher risk of disease recurrence compared to patients with low Ki-67 expression (Figures 3 and 4). Similarly, the HR for OS in patients with high Ki-67 expression was 2.86 (95% CI 1.48–5.53), indicating that patients with high Ki-67 expression had 2.86 times higher risk of death compared to patients with low Ki-67 expression (Tables 3 and 4).

DISCUSSION

In this study, we conducted a comprehensive analysis of the association between tumor proliferation, as assessed by Ki-67 expression, and patient prognosis in breast cancer. The results revealed a significant correlation, with patients exhibiting high Ki-67 expression experiencing worse clinical outcomes than those with low Ki-67 expression. These findings resonate with a wealth of existing literature that consistently underscores the prognostic value of tumor proliferation markers in breast cancer. The assessment of tumor proliferation using Ki-67 immunostaining emerges as a powerful tool for predicting patient outcomes and guiding personalized treatment decisions in breast cancer management.

The implications of our results are profound, as they underscore the critical importance of considering tumor proliferation as a key factor in the management of breast cancer patients. By identifying patients with high Ki-67 expression, health-care providers may be prompted to adopt more aggressive treatment approaches, such as chemotherapy or targeted therapies, to address the heightened risk of disease progression. Conversely, patients with low Ki-67 expression may benefit from a more conservative approach, potentially avoiding unnecessary overtreatment and the associated side effects while still ensuring optimal outcomes. The accurate assessment of tumor proliferation using Ki-67 enables clinicians to tailor treatment strategies to each patient's individual prognosis, optimizing therapeutic interventions and ultimately enhancing patient care and quality of life.

Table 3: Multivariate Cox regression analysis for DFS

Variable	HR (95% CI)	P-value
Ki-67 expression	2.37 (1.34–4.18)	0.003
Age	1.02 (0.99–1.05)	0.15
Stage	2.12 (1.34–3.36)	0.001
Tumor grade	1.49 (0.95–2.34)	0.08

DFS: Disease-free survival, HR: Hazard ratio, CI: Confidence interval

Table 4: Multivariate Cox regression analysis for OS

Variable	HR (95% CI)	P-value
Ki-67 expression	2.86 (1.48–5.53)	0.002
Age	1.02 (0.98–1.06)	0.30
Stage	1.89 (1.12–3.20)	0.02
Tumor grade	1.36 (0.72–2.55)	0.34

OS: Overall survival, HR: Hazard ratio, CI: Confidence interval

The strength of our study lies in the robustness and consistency of the findings, which align with a substantial body of existing evidence. Notably, studies by Dowsett et al., (2010) and Viale et al., (2008) reported analogous results, corroborating a significant correlation between high Ki-67 expression and adverse clinical outcomes.^{9,10} Furthermore, a comprehensive meta-analysis conducted by Liu et al., (2014) involving over 3000 breast cancer patients from eight studies reaffirmed a consistent link between elevated Ki-67 expression and worse DFS and OS.¹¹ The concordance of these findings across diverse studies underscores the reproducibility and generalizability of the association between Ki-67 expression and prognosis, enhancing the credibility of our results.

Another pivotal study by Urruticoechea et al., (2005) demonstrated that high Ki-67 expression was associated with an increased likelihood of recurrence and death in early breast cancer patients.¹² Our study's alignment with this research reinforces the clinical significance of Ki-67 as a reliable prognostic marker in breast cancer management, particularly in identifying patients at higher risk of disease relapse and mortality.

By providing additional evidence supporting the prognostic value of Ki-67 expression in breast cancer, our study contributes to the growing body of knowledge in this field. Our findings not only reinforce the existing literature but also add depth to the understanding of the relationship between tumor proliferation and

patient outcomes. Consequently, this supports the integration of Ki-67 expression assessment into the clinical decision-making process for breast cancer treatment. As suggested by Goldhirsch et al., (2013), the incorporation of Ki-67 expression data in the adjuvant chemotherapy decision-making process may assist in identifying patients who would derive the greatest benefit from such treatments.¹³

Limitations of the study

Retrospective design, which could introduce selection bias and confounding factors. In addition, our study only included a relatively small number of patients from a single institution, which limits the generalizability of our findings. Future studies with larger sample sizes and longer follow-up periods are needed to confirm our findings and explore the relationship between Ki-67 expression and other prognostic factors in breast cancer.

CONCLUSION

From the results, it can be concluded that our study provides additional evidence for the prognostic value of Ki-67 expression in breast cancer patients. Our findings support the use of Ki-67 immunostaining as a useful tool for predicting patient outcomes and guiding treatment decisions in breast cancer. Future studies should continue to explore the potential benefits of incorporating Ki-67 expression into the management of breast cancer patients.

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MAM- Concept and design of the study, results interpretation, review of literature and preparing first draft of manuscript. Statistical analysis and interpretation, revision of manuscript; **AKK**- Concept and design of the study, results interpretation, review of literature and preparing first draft of manuscript, revision of manuscript; **PS**- Review of literature and preparing first draft of manuscript. Statistical analysis and interpretation; **SRR**- Concept and design of the study, results interpretation, review of literature and preparing first draft of manuscript. Statistical analysis and interpretation, revision of manuscript.


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