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Original Article

Association of Ki-67 Proliferation Index with Axillary Lymph Node Metastasis in Early Breast Cancer: A Retrospective Study

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ABSTRACT

Objective: To determine frequency of axillary lymph node involvement and its association with Ki67 in early-stage breast cancer patients.

Study design: Retrospective Chart Review.

Place and Duration of Study: Breast Surgery Department at Shaukat Khanum Memorial Cancer Hospital & Research Centre, Lahore, Pakistan, from Mar to Aug 2025.

Methodology: Data were retrospectively reviewed for 165 patients diagnosed with early-stage breast cancer between January 2020 and June 2022. Records were reviewed for cases with histologically confirmed early breast cancer, patients undergoing axillary staging, and documented Ki-67 index. The retrieved data included patients' age at diagnosis, clinical and pathological staging, Ki-67 expression, Estrogen (ER), Progesterone (PR) and HER2 status.

Results: A total of 165 cases were reviewed. Mean age was 43.2±7.4 years. Axillary lymph node involvement was seen in 38.8% of patients. Median value of Ki67 was 25 (IQR=15-50). The Ki67 range was 2-90. Nearly three-quarters of patients had Ki67 ≥14% (75.8%). Frequency of high Ki67 expression was higher among patients with axillary metastasis as compared to those not having axillary metastasis, but it failed to achieve statistical significance (79.7% versus 73.3%, p=0.348).

Conclusion: The high expression of Ki67 (≥14%) was common in early-stage breast cancer patients. Frequency of axillary lymph node involvement was lower than higher Ki67% expression. Moreover, Ki67% was not found to be associated with axillary lymph node involvement in early-stage breast cancer patients.

Keywords: Axillary Lymph Node Metastasis, Breast Carcinoma, Early Stage Disease, Tumor Proliferation Marker, Ki-67.

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INRODUCTION

Breast cancer (BC) is the most often diagnosed malignancy in females, accounting for greater than one in ten newly diagnosed cancers each year. BC is the second predominant cause of cancer-associated fatalities among females at the global level.[1] Despite modern detection techniques, BC BC continues to be a significant challenge because of the complex etiologies and heterogeneous presentations, which hinder standardized management and prevention.[2] Additionally, the incidence of BC continues to rise around the world and deserves to be an area of focus for developmental oncological research and public health interventions.[3]

Prognostic and predictive factors are essential for the care of early breast cancer and comprise a large number of parameters. Clinical parameters include tumor size and the involvement of axillary lymph nodes (ALN), which provide information about the stage of disease and the possibility of spread.[4] Pathological parameters include histological grade and lymphovascular invasion; molecular parameters include estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), along with proliferation indices, namely the Ki-67 labeling index, which assesses tumor aggressiveness.[5,6] Of all the prognostic and predictive factors described above, ALN status is the most predictive of disease-free and overall survival, as it directly reflects the spread of disease, dictates staging, and is pertinent to the prescribing of adjuvant therapy. The Ki-67 antigen is a nonhistone, labile nuclear protein that was first defined in 1983. The Ki-67 has been closely correlated with the cell cycle and is expressed in proliferating cells during G1, S, G2, and M phases but is lacking in resting G0 and early G1 phase cells (12). Ki67 has been utilized by BC researchers as a metric of cell proliferation for many decades. The role of Ki67 in differentiating response to endocrine therapy or chemotherapy and in estimating prolonged outcomes among early-stage invasive

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disease is of specific interest. Ki67 score data are available before surgery, making it relevant to identifying patients who are appropriate for neoadjuvant therapy or for determining the best neoadjuvant regimen. Higher Ki67 expression has frequently been tied to undesirable outcomes.[3]

Literature documents a positive correlation of tumor cell proliferation activity with tumor progression, and it is recognized as a crucial prognostic indicator. A higher rate of proliferation activity indicates rapid tumor growth, which is linked to higher histological grade, likelihood of invasion, metastasis, and aggressive biological behavior.[7] Thus, tumors with raised Ki67 expression are more likely to spread beyond the primary site, causing a higher likelihood of regional spread of ALN and local recurrence. Consequently, high expression of Ki67 may be a potential predictor of nodal metastasis, helping in preoperative risk assessment and management planning. In spite of the biological plausibility of an association between Ki67 expression and axillary lymph nodes presence, the literature is inconclusive and controversial on this topic. Some authors indicate a correlation between these two factors, whereas other authors have not revealed any connection.[8] Heterogeneity in results can be explained by various features of populations, tumor biology, and clinical and histopathological examination methods.[9] Due to the inconsistent results, more studies are needed to clarify the association of Ki67 and axillary lymph node involvement. Moreover, the literature on this subject is also lacking in Pakistan. Therefore, we designed this study with the purpose of revealing this association in our population, as the establishment of this association would improve preoperative risk stratification and help improve surgical and neoadjuvant treatment planning strategies.

METHODOLOGY

This retrospective chart review was conducted by the Department of Breast Surgery at Shaukat Khanum Memorial Cancer Hospital & Research Centre, Lahore, Pakistan. The study was carried out after obtaining ethical permission from the hospital ethics committee (EX-04-02-25-01). The overall study duration was from Mar to Aug 2025. Data of 165 patients diagnosed with early-stage breast cancer were reviewed for the period from Jan 2020 to June 2022. A sample size of 145 patients was estimated, taking a 24.7%[10] frequency of axillary lymph involvement, 95% confidence interval and 7% margin of error. Sample size computation was performed using the single proportion approach on the online calculator OpenEpi.

Inclusion Criteria: Records were consecutively reviewed for cases with histologically confirmed early breast cancer, patients undergoing axillary staging, and documented Ki-67 index.

Exclusion Criteria: Cases with metastatic or recurrent diseases, contraindications to axillary staging, or incomplete records were excluded. The retrieved data included patients' age at diagnosis, clinical and pathological staging, and expression of estrogen (ER), progesterone (PR), HER2 and Ki67.

The American Joint Committee on Cancer (AJCC) TNM classification system, revised in 2017, was employed to stage all breast cancer cases.[11] Hormone receptor status for estrogen (ER) and progesterone (PR) was evaluated using immunohistochemistry (IHC), and tumors were categorized as positive when at least 1% e of the tumor cells exhibited nuclear staining.[12] HER2 expression was also assessed via IHC, with fluorescence in situ hybridization (FISH) used to confirm HER2 gene amplification in uncertain cases.[13] Based on hospital protocol, ER, PR, and HER2 results, patients were stratified into four molecular subtypes: Luminal A (ER+ and/or PR+, HER2−), Luminal B (ER+ and/or PR+, HER2+), HER2-enriched (ER−, PR−, HER2+), and Triple-negative (ER−, PR−, HER2−).

Two pathologists reviewed each slide, and a third expert was consulted to settle any discrepancies in the findings. The International Ki-67 in Breast Cancer Working Group's recommendations were followed when evaluating Ki-67.[14] Using the MIB-1 (M7240, DAKO) antibody and immunohistochemistry, the expression of Ki-67 was identified. At least 500 tumor cells were counted to determine the fraction of Ki67-positive cells. Regardless of staining intensity, the Ki67 labeling index (LI) was defined as the proportion of positive tumor cells among all cells in the scored area. Low expression was defined as Ki-67<14% and high expression as Ki-67≥14%.

Frequencies and percentages were computed for categorical variables like age groups, site, morphology, clinical stage, pathological stage, axillary lymph node involvement, estrogen status, progesterone status, Her2neu status, molecular classification, and Ki-67 groups. Numerical variables such as age were expressed as Mean±SD. Non-normal variable 'Ki67 values' was expressed as median with interquartile range (IQR). The assumption of

normality was tested with the Shapiro-Wilk test. Chi-square or Fisher's exact test was applied to compare patients' features among those having Ki67 values <14% and ≥14%. The *p*-value less than or equal to 0.05 was taken as statistically significant.

RESULTS

A total of 165 cases were studied with a mean age of 43.2±7.4 years. The age range was 22-59 years. The cancer site in the majority of cases was an overlapping lesion of the breast (43%). More than three-fourths of patients had infiltrating ductal carcinoma (89.1%). More than half of patients had stage IIA cancer (66.1%). ER and PR were positive in 75.2% and 64.8% of patients, respectively, whereas HER2 was negative in most of the patients (69.7%). ALN involvement was seen in some patients (38.8%). The majority of cases were classified as the Luminal A molecular subtype (60.6%). Table-I displays a summary of patients' features.

Table-I: Demographic Data of Study Participants (n=165)

Variables	Groups	n(%)
Age	<40 years	49(29.7)
	40-49 years	85(51.5)
	50-59 years	31(18.8)
Site	Overlapping lesion	71(43.0)
	Upper outer-quadrant	51(30.9)
	Upper inner quadrant	18(10.9)
	Lower outer quadrant	12(7.3)
	Central portion	7(4.2)
	Lower inner-quadrant	2(1.2)
	Axillary tail	2(1.2)
	Breast, NOS	1(0.6)
	Nipple	1(0.6)
Morphology	Infiltrating duct carcinoma, NOS	149(90.3)
	Lobular carcinoma, NOS	11(6.7)
	Carcinoma, NOS	2(1.2)
	Cribriform	1(0.6)
	Paget disease	1(0.6)
	Encapsulated papillary carcinoma	1(0.6)
Clinical stage	Stage 0	11(6.7)
	Stage 1a	36(21.8)
	Stage IIa	90(54.5)
	Stage IIb	28(17)
Pathological stage	Stage 0	11(6.7)
	Stage 1a	78(47.3)
	Stage IIa	54(32.7)
	Stage IIb	22(13.3)
Axillary lymph node involvement	Yes	64(38.8)
	No	101(61.2)
Estrogen status	Positive	124(75.2)
	negative	41(24.8)
Progesterone status	Positive	107(64.8)
	Negative	58(35.2)
Her2neu status	Positive	50(30.3)
	Negative	115(69.7)
Molecular classification	Luminal A	100(60.6)
	Luminal B	26(15.8)
	Her2neu enriched	24(14.5)
	Triple negative	15(9.1)

Median value of Ki67 was 25 (IQR=15-50). The Ki67 range was 2-90. Nearly three-quarters of patients had Ki67 ≥14% (75.8%). Table-II compares patients' features with Ki67 <14% and ≥14%. Age (*p*=0.358), site (*p*=0.390), morphology (*p*=0.060), clinical stage (*p*=0.519), and pathological stage (*p*=0.872) were not considerably different among patients with low and high Ki67 values. Patients receiving chemotherapy also had higher Ki67 values than those who were not receiving chemotherapy (*p*<0.001). Frequency of negative ER (*p*=0.013) and PR (*p*<0.001) was higher among patients with high Ki67 expression than those who had positive ER and PR, respectively. The frequency of higher Ki67 expression was higher in HER2-positive patients than HER2-negative patients (*p*=0.043). All of the triple-negative patients had high Ki-67 expression (*p*<0.001).

Table-II: Comparison of Demographic, Clinical, and Pathological Characteristics Between Patients with Low and High Ki-67 Expression (n=165)

Variables	Groups	Ki67 expression		*p*-value
		Low (<14)	High (≥14)	
Age	<40 years	13(26.5)	36(73.5)	ŧ0.358
	40-49 years	17(20.0)	68(80.0)	
	50-59 years	10(32.3)	21(67.7)	
Site	Upper-inner quadrant	5(27.8)	13(72.2)	†0.390
	Upper-outer quadrant	8(15.7)	43(84.3)	
	Lower-inner quadrant	1(50.0)	1(50.0)	
	Lower-outer quadrant	4(33.3)	8(66.7)	
	Central portion	2(28.6)	5(71.4)	
	Overlapping lesion	19(26.8)	52(73.2)	
	Axillary tail	0(0.0)	2(100.0)	
	Breast, NOS	1(100.0)	0(0.0)	
	Nipple	0(0.0)	1(100.0)	
Morphology	Infiltrating Duct Carcinoma, Nos	30(20.4)	117(79.6)	†0.060
	Carcinoma, Nos	1(50.0)	1(50.0)	
	Cribriform Carcinoma, Nos	1(100.0)	0(0.0)	
	Encapsulated papillary carcinoma with invasion	0(0.0)	1(100.0)	
	Lobular Carcinoma, Nos	6(54.5)	5(45.5)	
	Paget Disease	0(0.0)	1(100.0)	
Clinical stage	Stage 0	2(18.2)	9(81.8)	ŧ0.519
	Stage 1a	10(27.8)	26(72.2)	
	stage 2a	24(26.7)	66(73.3)	
	stage b	4(14.3)	24(85.7)	
Pathological stage	Stage 0	3(27.3)	8(72.7)	ŧ0.872
	Stage a	16(27.1)	43(72.9)	
	Stage a	16(23.5)	52(76.5)	
	Stage b	5(18.5)	22(81.5)	
Chemotherapy	Yes	14(14.9)	80(85.1)	ŧ*<0.001
	No	26(36.6)	45(63.4)	
Estrogen status	Positive	36(29.0)	88(71.0)	ŧ*0.013
	Negative	4(9.8)	37(90.2)	
Progesterone status	Positive	35(32.7)	72(67.3)	ŧ*<0.001
	Negative	5(8.6)	53(91.4)	
HER2 status	Positive	7(14.0)	43(86.0)	ŧ*0.043
	Negative	33(28.7)	82(71.3)	
Molecular classification	Luminal A	33(33.0)	67(67.0)	ŧ*<0.001
	Luminal B	4(15.4)	22(84.6)	
	Her2neu enriched	3(12.5)	21(87.5)	
	Triple negative	0(0.0)	15(100.0)	

*Data is expressed as n(%), ŧ Chi-square test is reported, *Significant at p<0.05*

Figure displays a comparison of the frequency distribution of axillary metastasis between patients with low and high Ki67 expression. Frequency of high Ki67 expression was higher among patients with axillary metastasis as compared to those not having axillary metastasis, with no statistical significance (79.7% versus 73.3%, *p*=0.348).

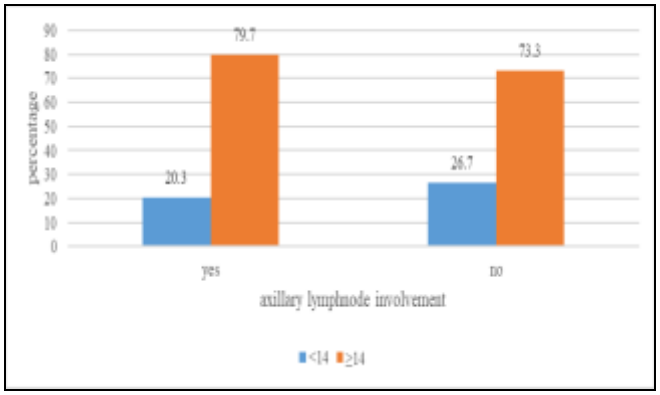

Figure: Comparison of frequency of axillary metastasis among those with Ki-67 of <14% and ≥14%(n=165)

DISCUSSION

In this RCR, ER and PR were positive in about three-quarters and two-thirds of patients, respectively, while HER2 was positive in about a quarter of the patients. Our results are consistent with the study conducted by Fong *et al*., regarding the positivity of hormone receptors in the greater proportion of early BC patients and HER2 status being positive in fewer cases.[15] The higher occurrence of ER/PR positivity is suggestive of the possible responsiveness to endocrine therapy, which is a central part of the management of hormone receptor-positive disease. The lower rate of HER2 positivity is consistently documented by Chidananda *et al*., but shows the role of targeted therapy in this group, as they could have aggressive activity usually associated with HER2-enriched tumors.[16]

ALN metastasis occurs when tumor cells invade surrounding tissues and then travel into lymphatic channels.4 Even for early-stage BC, microscopic spread may occur due to tumor cell turnover, lymphovascular invasion, or intrinsically aggressive tumor biology. The axilla is the primary regional drainage basin for the breast, making it the first site of metastasis.[8] Consistent with these systems, ALN metastasis was seen in 38.8% of early-stage BC. Liu *et al*., reported varying frequencies of ALN metastasis for cohorts with early-stage BC ranging from 24.5% to 48.1%.[17] Variation probably reflects tumor characteristics, staging variables, and population profiles between studies.

In this study, the median value of Ki67 was 25 out of 100, and around three-quarters of patients had expression of Ki67 (75.8%). Despite the tumors being diagnosed at an onset phase, many tumors had significant proliferative activity. The raised Ki67 expression alludes to relatively aggressive biological behavior in a significant number of instances. Lee *et al*., have reported a raised Ki67 expression in early-stage BC with values ranging from 68.6%-87.6%.[18], indicating it is clinically significant during BC classification. Variability in tumor biology, patient characteristics, laboratory techniques, and the threshold of Ki67% contributes to variance between studies regarding Ki67% expression. Despite the differences in the reported prevalence of high Ki67 expression from study to study, related to patient and methodology differences, the results included in this article show collectively that early BC has a high expression of Ki67.

Nishit *et al*., have reported associations between ER, PR, and HER2 statuses and Ki-67 expression. Usually, hormone-receptor-positive tumors, including ER and PR report with low Ki67 expression, reflecting a low degree of proliferative activity, whereas HER2-positive tumors typically are associated with higher Ki67 values, consistent with their biologically aggressive phenotype.[19] These findings suggest that HER2-positive tumors, even tumors expressing hormone receptors, are associated with an increased level of Ki67, and subsequently, this is an indicator of aggressive disease. The data support the idea that Ki67 has negative prognostic value in combination with ER, PR, and HER2, completing the characterization of the tumor and providing comprehension of the biological heterogeneity of early-stage BC.[20]

Literature has demonstrated relationships of ER, PR, and HER2 status with Ki-67; the link with ALN status is less clear in the literature.[3] There was a large proportion of patients in our cohort who had higher Ki67 expression, but no association was found between nodal metastasis and Ki67 expression.[5] It may be inferred that although Ki67 is a good marker for tumor proliferation and indicates biological aggressiveness, it is not necessarily a determinant of regional nodal metastasis. The evidence on this association is mixed in other similar studies, with some authors finding an association between nodal involvement and Ki67 expression, while others did not find this association. This variation may be due to significant differences in patient selection, tumor biology, and threshold values for defining high or low Ki67 expression. Therefore, nodal involvement is probably most closely linked to a wider range of tumor pathology than simply proliferative activity.

LIMITATIONS OF STUDY

The current investigation suffers from a few limitations. The study was performed in a single-center institution retrospectively with a limited sample size, limiting its generalizability to a diverse population. The threshold value selected in this study could be different from that of other studies. Additional investigations may be carried out to address the gaps in this study and substantiate current findings.

CONCLUSION

Although the high expression of Ki67 (≥14%) was common in early-stage breast cancer patients, axillary lymph node involvement was lower than in patients with higher Ki67% expression. Moreover, Ki67% was not found to be associated with axillary lymph node status in early-stage breast cancer patients.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SE & MSN: Data acquisition, data analysis, critical review, approval of the final version to be published.

ZT & NS: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AK: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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