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# Association Between Grades and Ki-67% in Breast Cancer

## Shumaila Nawaz Khan\*, Ghulam Haider, Kaneez Zainab Rabail, Saima Zahoor, Abdul rehman, Aakash Ramchand, Munazza Anwer, Mehwish Jabeen

*Innah post graduate medical center (JPMC), Pakistan.*
*\*Corresponding author: Shumaila Nawaz Khan.*

## Abstract

**Background:** Ki-67 is a key proliferative marker in breast cancer, often associated with tumor aggressiveness and grade. Despite its established role, the correlation between Ki-67% and histological grade remains inconsistent across studies. This study aims to explore the association between Ki-67% expression and tumor grades in breast cancer patients.

**Methods:** A cross-sectional study was conducted at the Department of Medical Oncology, Jinnah Postgraduate Medical Centre, Karachi, over six months. A sample of 78 breast cancer patients was included, divided into groups based on Ki-67% expression (<20% and >21%). Histological grading was assessed using the Scarff-Bloom-Richardson grading system, while demographic and clinical data, including age, marital status, and BMI, were collected and analyzed using SPSS version 24.0.

**Results:** High Ki-67 expression (>21%) was observed predominantly in Grade III tumors, with 33 cases (91.7%) compared to Grade II with 52 cases (66.7%) and Grade I with 3 cases (50%) (p=0.008). No significant associations were found between Ki-67% and other variables, including tumor size, axillary lymph node involvement, and TNM stage.

**Conclusion:** This study demonstrates a strong association between higher Ki-67% and advanced histological grades in breast cancer, suggesting that Ki-67 may serve as a valuable prognostic indicator. However, further research is needed to clarify its role in predicting clinical outcomes across diverse patient populations.

**Keywords:** Ki-67; breast cancer; tumor grade; histological grade; prognosis; proliferation marker

## Introduction

Breast cancer is the most common cancer in females, it accounts for one-third of all malignancies affecting women, this cancer has high metastatic potential leading to high mortality. Early detection of this disease leads to improved outcomes and increased survival rate [1]. In Asian countries, breast cancer is the most common with a peak age between 50 and 64 years [2]. Urban females are more likely to develop breast carcinoma than rural women [3]. There are vast clinical presentations and behaviors in different patients and racial populations due to genetic heterogeneity [4]. Various clinicopathological factors help evaluate the prognosis and determine the appropriate management strategy in breast cancer patients [3-5]. Factors include patient age, tumor size, lymph node status, histological type, grade, lymphovascular invasion, hormonal receptor status, human epidermal growth factor receptor 2 (Her2neu) expression, and Ki-67 labeling index [3-5]. Various molecular techniques are used to ascertain the molecular classification of breast carcinoma. The different molecular classification helps determine suitable, specific, and personalized targeted breast cancer treatments [6].

The Ki-67 antigen is a labile, non-histone nuclear protein identified in the early 1980's. Ki-67 regulates the cell cycle, is associated with cellular proliferation, and is the most widely used proliferation marker [7]. Previous studies have demonstrated that Ki-67 is expressed in all the active cell cycle phases, and not the resting $G_0$ phase. Furthermore, Ki-67 has been used as a biomarker to assess the growth fraction of a given cell population [8,9]. Biology of breast cancer with high ki67 % and grade looks similar but whether there is a definite and clear correlation between high grade and high ki 67% and vice versa is not yet clear. Therefore, we wanted to clear this dilemma in this study. Immunostaining techniques that use monoclonal Ki-67 antibodies can assess the growth fraction of neoplastic cell populations [10]. Although Ki-67 is an accepted prognostic marker, the role of the protein in the management of BC is unclear. At present, a standard operating procedure, or generally accepted cut-off definition, is not defined for Ki-67 [11].

Despite the observation that high Ki-67 levels are associated with worse prognosis and survival rates in patients with early BC [12], the marker has not yet been implemented for routine clinical use. Due to insufficient quality assurance and existing data, The

College of American Pathologists (CAP) has not advised the routine use of Ki-67 screening for the prognosis of patients with BC [13]. However, at the 2011 and 2013 St. Gallen Consensus Conferences, the use of Ki-67 screening was recommended for the analysis of cellular proliferation, and for identifying the differentiation status of luminal A and B tumors [14,15]. A study by Nigam JS et al [16] reported the positive vs negative expression rate of Ki-67 in 39.13% vs 10.87% among patients for grade 2 breast cancer, respectively. Although there is still no consensus over an optimal cutoff value used to decide chemotherapy, several studies found that a high ki67 index is associated with a higher rate of relapse and worse breast cancer survival [17].

We anticipated that a high Ki-67% would correspond to a higher grade of breast cancer, and vice versa; however, some studies did not demonstrate a clear association between these parameters. Therefore, we conducted this study to examine the correlation between Ki-67% and breast cancer grade and to determine if any significant association exists between them.

## Methods and Materials

The study was designed as a cross-sectional analysis and conducted in the Department of Medical Oncology at Jinnah Postgraduate Medical Centre (JPMC) Hospital, Karachi, over a six-month period following approval. A sample size of 39 participants per group was estimated using the W.H.O. sample size calculator, based on a frequency of grade 2 breast cancer among women with positive versus negative Ki-67% (39.13% vs. 10.87%) with a test power (1-β) of 90% and a significance level (α) of 5%. Non-probability consecutive sampling was employed to select participants.

Inclusion criteria consisted of female patients aged 18-75 with histologically confirmed breast cancer samples that included both grade and Ki-67% assessment. The study encompassed all stages of breast cancer. Exclusion criteria included patients unwilling to participate, those under 18 years of age, and male patients.

After obtaining Institutional Review Board (IRB) approval, eligible women with breast cancer were enrolled from the outpatient department. Study details were thoroughly explained to participants, and written informed consent was obtained. Baseline demographic and clinical data, including age, residence, education, ethnicity, marital status, height

(measured using a wall-mounted scale in cm), weight (measured with a digital scale in light clothing), and BMI (calculated as weight in kg divided by height in m²), were recorded in a predesigned proforma. Each participant underwent breast cancer screening, immunohistochemical staining, and histological grading based on their histopathology report.

For immunohistochemical analysis, breast tissue samples were fixed in 10% formaldehyde for 24 hours, dehydrated, cleared, embedded in paraffin, and sectioned into 5 µm slices. The slices were baked at 65°C, dewaxed with xylene, hydrated with graded ethanol, treated with 3% hydrogen peroxide, and incubated at 37°C for 10 minutes to inactivate endogenous peroxidase. After antigen retrieval through microwave heating and blocking with normal goat serum, the slices were incubated at 4°C overnight with a primary Ki-67 antibody. The following day, biotin-labeled secondary antibody incubation was performed at room temperature for 30 minutes, followed by development with diaminobenzidine, counterstaining with hematoxylin, differentiation with hydrochloric acid ethanol, dehydration with graded ethanol, clearing with xylene, and mounting with neutral gum for microscopic examination. Phosphate-buffered saline was used in place of the primary antibody as a negative control. The Ki-67 marker index was considered positive if 20% or more of the tumor cells exhibited nuclear staining, categorizing participants into Group A (Ki-67% positive) and Group B (Ki-67% negative). Histological grading of breast cancer was performed according to the Scarff-Bloom-Richardson grading system.

Data were entered and analyzed using SPSS version 24.0. Continuous variables such as age, age at menarche, tumor size, weight, height, BMI, and parity were summarized as mean ± SD or median (IQR), while categorical variables, including residential status, marital status, educational status, ethnicity, tumor sidedness, histological type and grade, multifocal/multicentric status, axillary lymph node involvement, TNM stages, nuclear grade, hormone replacement therapy, use of oral contraceptives, family history of cancers, family history of breast cancer, menopausal status, and lactation, were presented as frequencies and percentages. The association between breast cancer grades and Ki-67% was assessed using the Chi-square or Fisher's Exact test, with an odds ratio greater than 1 considered significant. Potential confounders (age groups, BMI, age at menarche, tumor size, residential status, marital

status, educational status, ethnicity, tumor sidedness, histological type, multifocal/multicentric status, axillary lymph node involvement, TNM stages, nuclear grade, hormone replacement therapy, use of oral contraceptives, family history of cancers, family history of breast cancer, and menopausal status) were controlled through stratification, and post-stratification analyses were conducted using the Chi-square or Fisher's Exact test. A p-value ≤ 0.05 and an OR >1 were considered statistically significant. Data visualization was performed using bar graphs and pie charts where appropriate.

## Results

The mean age at presentation for the participants was 47.3 years with a standard deviation of 12.63. The mean age at menarche was 12.72 years with a standard deviation of 1.64 (Table 1).

**Table 1:** The age distribution of participants

| Age | Standard deviation |
|---|---|
| <30 years | 9 (7.5%) |
| 30-50 years | 75 (62.5%) |
| 51-70 years | 34 (28.3%) |
| >70 years | 2 (1.7%) |

Ethnic distribution included Urdu, 54 (45%); Sindhi, 32 (26.7%); Punjabi, 22 (18.3%); Pashto, 5 (4.2%); Balochi, 3 (2.5%); and Other, 4 (3.3%) (Table 2).

**Table 2:** Sociodemographic variables of participants

| Variables | N | % |
|---|---|---|
| **Age Group** | | |
| <30 years | 9 | 7.5 |
| 30-50 years | 75 | 62.5 |
| 51-70 | 34 | 28.3 |
| >70 years | 2 | 1.7 |
| **Marital status** | | |
| Married | 102 | 85 |
| Unmarried | 18 | 15 |
| **Parity** | | |
| Yes | 92 | 76.7 |
| No | 28 | 23.3 |
| **Ethnicity** | | |
| Sindhi | 32 | 26.7 |
| Urdu | 54 | 45 |
| Punjabi | 22 | 18.3 |
| Pashto | 5 | 4.2 |
| Balochi | 3 | 2.5 |
| Other | 4 | 3.3 |
| **Family history of cancer** | | |
| Yes | 49 | 40.8 |
| No | 71 | 59.2 |
| **Family history of breast cancer** | | |
| Yes | 42 | 35 |
| No | 78 | 65 |
| **Menopausal Status** | | |
| Premenopausal | 63 | 52.5 |
| Postmenopausal | 57 | 47.5 |
| **Body Mass Index** | | |
| <18 | 4 | 3.3 |
| >18-23 | 4 | 3.3 |
| 23-25 | 62 | 51.7 |
| 25-30 | 45 | 37.5 |

| | | |
|---|---|---|
| >30 | 5 | 4.2 |
| **Lactation** | | |
| Yes | 92 | 76.7 |
| No | 28 | 23.3 |

The clinical characteristics of patients were as follows: cancer was located on the left side in 43 (35.8%), right side in 71 (59.2%), and bilateral in 6 (5%) patients. Histologically, 101 (84.2%) had infiltrating ductal carcinoma, 17 (14.2%) had lobular carcinoma, and 2 (1.7%) had other types. Grading revealed that 6 (5%) had grade I, 78 (65%) had grade II, and 36 (30%) had grade III cancer. The Ki67 index was <15% in 14 (11.7%) and 15-60% in 85 (70.8%) patients, and 21 (17.5%). Tumor size was <2 cm in 6 (5%), 2-5 cm in 58 (48.3%), and >5 cm in 55 (45.8%), with 1 (0.8%) unknown. Multifocal/multicentric tumors were present in 29 (24.2%) patients. Axillary lymph node involvement was observed in 90 (75%) patients. TNM staging showed 7 (5.8%) in stage I, 46 (38.3%) in stage II, 54 (45%) in stage III, and 13 (10.8%) in stage IV. Nuclear grading was grade I in 9 (7.5%), grade II in 75 (62.5%), and grade III in 36 (30%). HER2 status was positive in 45 (37.5%), negative in 67 (55.8%), borderline in 5 (4.2%), and unknown in 3 (2.5%). Estrogen receptor status was positive in 75 (62.5%) and negative in 45 (37.5%), while progesterone receptor status was positive in 57 (47.5%) and negative in 63 (52.5%) (Table 3).

**Table 3:** Clinical characteristics of patients

| Characteristics | N | % |
|---|---|---|
| **Side** | | |
| Left | 43 | 35.8 |
| Right | 71 | 59.2 |
| Bilateral | 6 | 5 |
| **Histological type** | | |
| Infiltrating duct | 101 | 84.2 |
| Lobular | 17 | 14.2 |
| Others | 2 | 1.7 |
| **Histological grade** | | |
| I | 6 | 5 |
| II | 78 | 65 |
| III | 36 | 30 |
| **Ki67 Categories** | | |
| <15% | 14 | 11.7 |
| 15-30% | 85 | 70.8 |
| >60% | 21 | 17.5 |
| **Tumor size** | | |
| <2 cm | 6 | 5 |
| 2-5 cm | 58 | 48.3 |
| >5 cm | 55 | 45.8 |
| 5 | 1 | 0.8 |
| **Multifocal /Multicentric** | | |
| Yes | 29 | 24.2 |
| No | 91 | 75.8 |
| **Axillary lymphnode** | | |
| Yes | 90 | 75 |
| No | 30 | 25 |
| **TNM Stage** | | |
| I | 7 | 5.8 |
| II | 46 | 38.3 |
| III | 54 | 45 |
| IV | 13 | 10.8 |
| **Nuclear stage** | | |
| I | 9 | 7.5 |

| | | |
|---|---|---|
| II | 75 | 62.5 |
| III | 36 | 30 |
| **HER2 status** | | |
| Positive | 45 | 37.5 |
| Negative | 67 | 55.8 |
| Borderline | 5 | 4.2 |
| Unknown | 3 | 2.5 |
| **Estrogen Receptor** | | |
| Positive | 75 | 62.5 |
| Negative | 45 | 37.5 |
| **Progesterone Receptor** | | |
| Positive | 57 | 47.5 |
| Negative | 63 | 52.5 |

The association between Ki-67% and clinical variables in breast cancer patients shows significant differences in certain categories. For histological grade, higher Ki-67 levels (>60%) are more frequent in Grade III tumors (36.1%) compared to Grade I (0%) and Grade II (10.3%), with a significant p-value of 0.005. Regarding histological type, the infiltrating duct tape shows the highest proportion of patients with Ki-67 >60% (21.0%), though the association is not statistically significant (p=0.144). Tumor size does not exhibit a significant trend (p=0.223), but larger tumors (>5 cm) tend to have a higher proportion of Ki-67 >60% (25.0%). Multifocal or multicentric lesions show no significant difference in Ki-67 levels compared to unifocal lesions (p=0.437). Similarly, axillary lymph node status (p=0.337) and TNM stage (p=0.386) do not demonstrate a statistically significant relationship with Ki-67%. However, the nuclear stage shows a significant association (p=0.005), with Grade III nuclear tumors exhibiting the highest proportion of Ki-67 >60% (36.1%). HER2-positive tumors show a relatively higher percentage of Ki-67 >60% (24.4%) compared to HER2-negative tumors (11.9%), but this is not statistically significant (p=0.217). These findings highlight the relationship between higher Ki-67 levels and more aggressive tumor characteristics such as higher histological and nuclear grades (Table 4).

**Table 4:** Association Between Ki-67% and Clinical Variables in Breast Cancer Patients

| Variables | Ki67% | | | Total | p-value |
|---|---|---|---|---|---|
| | <15% | 15-60% | >60% | | |
| **Histological grade** | | | | | |
| Grade I | 2 (33.3%) | 4 (66.7%) | - | 6 (100%) | 0.005 |
| Grade II | 9 (11.5%) | 61 (78.2%) | 8 (10.3%) | 78 (100.0%) | |
| Grade III | 3 (8.3%) | 20 (55.6%) | 13 (36.1%) | 36 (100.0%) | |
| **Histological Type** | | | | | |
| Infiltrating duct | 10 (10.0%) | 69 (69.0%) | 21 (21.0%) | 101 (100.0%) | 0.144 |
| Lobular | 4 (23.5%) | 13 (76.5%) | - | 17 (100.0%) | |
| Others | - | 2 (100.0%) | - | 2 (100.0%) | |
| **Tumor size** | | | | | |
| <2 cm | - | 5 (83.3%) | 1 (16.7%) | 6 (100.0%) | 0.223 |
| 2-5 cm | 9 (15.5%) | 43 (74.1%) | 6 (10.3%) | 58 (100.0%) | |
| >5 cm | 5 (8.9%) | 37 (66.1%) | 14 (25.0%) | 56 (100.0%) | |
| **Multifocal/Multicentric lesion** | | | | | |
| Yes | 5 (17.2%) | 18 (62.1%) | 6 (20.7%) | 29 (100.0%) | 0.437 |
| No | 9 (9.9%) | 67 (73.6%) | 15 (16.5%) | 91 (100%) | |
| **Axillary lymph node** | | | | | |
| Yes | 9 (10%) | 63 (70.0%) | 18 (20.0%) | 90 (100.0%) | 0.337 |
| No | 5 (16.7%) | 22 (73.3%) | 3 (10.0%) | 30 (100.0%) | |
| **TNM Stage** | | | | | |
| I | 1 (14.3%) | 4 (57.1%) | 2 (28.6%) | 7 (100.0%) | 0.386 |
| II | 7 (15.2%) | 33 (71.7%) | 6 (13.0%) | 46 (100.0%) | |

| | | | | | |
|---|---|---|---|---|---|
| III | 3 (5.6%) | 39 (72.2%) | 12 (22.2%) | 54 (100.0%) | |
| IV | 3 (23.1%) | 9 (69.2%) | 1 (7.7%) | 13 (100.0%) | |
| **Nuclear Stage** | | | | | |
| I | 2 (33.3%) | 4 (66.7%) | - | 9 (100.0%) | 0.005 |
| II | 8 (10.7%) | 59 (78.7%) | 8 (10.7%) | 75 (100.0%) | |
| III | 3 (8.3%) | 20 (55.6%) | 13 (36.1%) | 36 (100.0%) | |
| **HER2 status** | | | | | |
| Positive | 7 (15.6%) | 27 (60.0%) | 11 (24.4%) | 45 (100.0%) | 0.217 |
| Negative | 5 (7.5%) | 54 (80.6%) | 8 (11.9%) | 67 (100.0%) | |
| Borderline | 1 (20.0%) | 3 (60.0%) | 1 (20.0%) | 5 (100.0%) | |
| Unknown | 1 (33.3%) | 1 (33.3%) | 1 (33.3%) | 3 (100.0%) | |

# Discussion

Our study confirms a significant association between Ki-67% and the histological grade of breast cancer, suggesting that higher Ki-67 levels are predominantly observed in higher-grade tumors. This finding aligns with prior studies, such as Trihia et al., where high Ki-67 expression was also correlated with increased tumor grade, specifically in Grades II and III [18]. Similarly, Kanyılmaz et al. identified a clear link between high Ki-67 and more aggressive tumor grades, reinforcing the role of Ki-67 as an indicator of tumor proliferation and aggressiveness [6].

The relationship between Ki-67 and cancer grade in our findings also mirrors observations in other international studies, which have reported a trend of higher Ki-67 expression in tumors of higher histological grade. For instance, Brown et al. demonstrated that in early hormone receptor-positive breast cancer, patients with Grade-3 tumors had significantly higher Ki-67 levels (over 20%), supporting its utility as a prognostic marker for aggressive disease [19]. Moreover, in a retrospective cohort by Stathopoulos et al., patients classified under the basal-like subtype, typically associated with higher grade, frequently exhibited elevated Ki-67 levels [20].

The significance of Ki-67 as a proliferation marker is further underscored in studies focusing on molecular subtypes of breast cancer. Yip et al. and Pai et al. reported that in luminal subtypes, particularly in luminal B, Ki-67 was frequently elevated, contrasting with lower levels in luminal A cases, which tend to have a better prognosis [21, 22]. This aligns with our study, where high-grade tumors with elevated Ki-67 likely represent more aggressive molecular subtypes such as luminal B or HER2-enriched types. Additionally, Stathopoulos et al. highlighted that the triple-negative subtype also presented with consistently high Ki-67, further linking it with poor prognosis [20].

Beyond histological grading, our study found no statistically significant association between Ki-67 and histological type, tumor size, axillary lymph node involvement, or TNM staging. This finding, while consistent with some studies, contrasts with those indicating a correlation between tumor size and Ki-67 levels, as noted by Kanyılmaz et al. [6]. These discrepancies may stem from population heterogeneity, sample size variations, or differences in assessment methods for Ki-67. The diversity in findings reinforces the need for standardized assessment protocols in Ki-67 testing, as advocated by Brown et al. [19].

Other studies have also illustrated the variability in Ki-67 association with TNM stage and nodal involvement. For example, Fasching et al. demonstrated that while Ki-67 could predict five-year disease-free survival in specific subgroups, it had limited prognostic value in isolation [23]. This underscores the importance of combining Ki-67 with other biomarkers to enhance prognostic accuracy, particularly for cases with intermediate risk.

Finally, HER2 status did not exhibit a significant association with Ki-67 in our study, a finding consistent with Yip et al., who noted that HER2 overexpression does not necessarily correspond to higher Ki-67 levels [21]. However, this is somewhat contradictory to studies like Trihia et al., which showed a positive relationship between HER2 positivity and elevated Ki-67, emphasizing that HER2's influence on proliferation may vary based on additional tumor characteristics [18].

# Conclusion

In conclusion, our study highlights the strong association between Ki-67 and histological grade in breast cancer, supporting its role as a marker of tumor proliferation and aggressiveness. While Ki-67's relationship with other clinical parameters remains

less definitive, this study reinforces its utility as a complementary biomarker in evaluating breast cancer prognosis, particularly when used alongside other established indicators.

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