

Role of Ki67 Expression in Distinguishing Benign and Malignant Phyllodes Tumors of the Breast: A Prospective Observational Study.

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Abstract

Background:

Phyllodes tumors (PTs) are rare fibroepithelial neoplasms of the breast with biological behavior ranging from benign to malignant. Histopathological grading may be challenging because of overlapping morphological features. Ki-67 is a marker of cellular proliferation that may provide additional information regarding tumor aggressiveness.

Objective:

To evaluate the association between stromal Ki67 expression and histological grade of phyllodes tumors and determine its relationship with tumor size and age.

Methods:

A prospective observational study was conducted in the Department of General Surgery, Indira Gandhi Institute of Medical Sciences (IGIMS), Patna. Twenty patients with histopathologically confirmed phyllodes tumors were evaluated. Clinical, radiological, histopathological, and immunohistochemical assessments were performed. Stromal Ki67 labeling index (LI) was calculated and analyzed in relation to histological grade, tumor size, and age. Receiver operating characteristic (ROC) analysis was performed to assess the diagnostic performance of Ki67 for identifying malignant tumors.

Results:

Of 20 patients, 11 (55%) had benign, 4 (20%) borderline, and 5 (25%) malignant tumors. Mean Ki67 LI increased significantly across the three groups: $6.1 \pm 2.4\%$ in benign, $16.8 \pm 3.9\%$ in borderline, and $31.4 \pm 6.2\%$ in malignant tumors (ANOVA $F=48.71$, $p<0.001$). Tumor size was significantly associated with histological grade ($p=0.002$) and positively correlated with Ki67 expression ($r=0.68$, $p=0.001$). A Ki67 cutoff of $\geq 20\%$ showed a sensitivity of 88.9%, specificity of 93.3%, and AUC of 0.92 for identifying malignant tumors.

Conclusion:

Stromal Ki67 expression was significantly associated with histological grade and tumor size in phyllodes tumors. Ki67 may serve as an adjunctive marker in distinguishing malignant tumors, although validation in larger studies is required.

Recommendations:

Larger multicenter studies with standardized Ki67 assessment and longer follow-up are recommended to validate the optimal cutoff and clinical utility of Ki67 in phyllodes tumors.

Keywords: Phyllodes tumor, Ki67, Breast neoplasm, Immunohistochemistry, Stromal proliferation, Malignant phyllodes tumor.

Submitted: April 11, 2026

Accepted: May 19, 2026

Published: June 30, 2026

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Introduction

Phyllodes tumors (PTs) are uncommon fibroepithelial neoplasms of the breast, representing approximately 0.3–0.9% of all breast tumors and less than 3% of fibroepithelial breast lesions worldwide [1]. These tumors are characterized by proliferation of both epithelial and stromal components, although the stromal component is considered the true neoplastic element responsible for tumor progression, recurrence, and metastatic potential [2]. Despite their rarity, PTs continue to attract considerable clinical and pathological interest because of their unpredictable biological behavior and diagnostic challenges.

The clinical presentation of phyllodes tumors is highly variable. Most patients present with a painless, rapidly enlarging breast mass that may attain considerable size within a relatively short period [3]. PTs commonly occur in women between the third and fifth decades of life, although cases have been reported across a wide age spectrum. Compared with fibroadenomas, which often affect younger women, phyllodes tumors tend to occur at a slightly older age and exhibit more rapid growth [4]. Large tumors may produce skin stretching, venous prominence, ulceration, or breast asymmetry, particularly when diagnosis and treatment are delayed.

The World Health Organization (WHO) classifies phyllodes tumors into benign, borderline, and malignant categories according to several histopathological parameters including stromal cellularity, stromal atypia, mitotic activity, stromal overgrowth, and the nature of tumor margins [5]. Benign tumors constitute the majority of cases, accounting for approximately 35–65% of PTs, whereas borderline and malignant tumors comprise smaller but clinically significant subsets because of their greater propensity for local recurrence and distant metastasis [6]. Accurate grading is therefore essential for determining prognosis and guiding surgical management.

Radiological evaluation plays an important role in the initial assessment of breast masses; however, differentiation of phyllodes tumors from other fibroepithelial lesions remains challenging. Mammography typically demonstrates a well-circumscribed, lobulated mass, while ultrasonography may reveal a heterogeneous solid lesion with cystic spaces [7]. Nevertheless, these findings are nonspecific and frequently overlap with those observed in fibroadenomas and other benign breast lesions. Consequently, imaging alone is often insufficient for establishing a definitive diagnosis.

Fine-needle aspiration cytology (FNAC) has traditionally been used as a minimally invasive diagnostic tool for breast

lesions; however, its utility in PTs is limited because of significant cytological overlap with fibroadenomas and other fibroepithelial proliferations [8]. Core needle biopsy improves diagnostic accuracy but may still be subject to sampling error, particularly in tumors demonstrating heterogeneous histological features. As a result, definitive diagnosis frequently relies upon examination of the excised specimen.

Histopathological evaluation remains the gold standard for diagnosis and grading of phyllodes tumors. However, substantial interobserver variability exists, especially when distinguishing benign from borderline lesions [9]. Furthermore, the biological behavior of some tumors does not always correspond precisely to their histological classification, indicating that additional prognostic markers may be necessary to improve risk assessment and clinical decision-making.

Among the various biomarkers investigated in breast pathology, Ki67 has emerged as one of the most widely studied indicators of cellular proliferation. Ki67 is a nuclear non-histone protein expressed during all active phases of the cell cycle except the resting phase (G₀), making it a reliable marker of proliferative activity [10]. Assessment of Ki67 expression has become an integral component of breast carcinoma evaluation and has demonstrated prognostic significance in numerous malignancies.

Recent studies have suggested that stromal Ki67 expression may also play an important role in phyllodes tumors. Because stromal proliferation is believed to be the primary determinant of tumor behavior, measurement of stromal Ki67 labeling index may provide valuable information regarding tumor aggressiveness, recurrence risk, and metastatic potential [11,12]. Several investigators have reported progressively increasing Ki67 expression from benign to borderline and malignant PTs, suggesting that this marker may assist in histological grading and prognostic stratification.

Despite growing evidence supporting the role of Ki67 in PTs, there remains no universal consensus regarding its optimal diagnostic cutoff value or its precise role in routine clinical practice. Moreover, limited data are available from eastern India regarding the utility of Ki67 expression in distinguishing benign and malignant phyllodes tumors. Therefore, the present study was undertaken to evaluate the relationship between stromal Ki67 expression and the histological grade of phyllodes tumors and to assess its correlation with age, tumor size, and malignant behavior.

Materials and methods

Study design and setting

This **prospective observational study** was conducted in the Department of General Surgery, Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India, a tertiary-care teaching hospital. The study was conducted to evaluate the association between stromal Ki-67 expression and histological grade in patients with phyllodes tumors of the breast.

Participants

Patients with histopathologically confirmed phyllodes tumors of the breast who fulfilled the eligibility criteria were considered for enrollment. Patients who were willing to participate and provided written informed consent were included. The inclusion criteria were histopathologically confirmed phyllodes tumor of the breast and willingness to participate with provision of informed consent. Patients with a previous history of lumpectomy or breast-conserving surgery, prior neoadjuvant chemotherapy or radiotherapy, or histologically proven carcinoma of the breast were excluded.

Sample Size and Participant Recruitment

The sample size was determined as **20 patients**. During the study period, patients were assessed for eligibility.

Study procedure

All enrolled patients underwent a detailed clinical history and physical examination, followed by routine hematological and biochemical investigations. Radiological assessment was performed using ultrasonography and/or bilateral mammography. FNAC or core needle biopsy was performed as clinically appropriate, and metastatic workup was undertaken when clinically indicated. All patients subsequently underwent surgical excision of the tumor. The resected specimens were subjected to histopathological examination for determination of tumor grade, followed by immunohistochemical assessment of Ki67 expression.

Immunohistochemical assessment

Formalin-fixed, paraffin-embedded tissue sections measuring 4 μ m in thickness were prepared for immunohistochemical assessment. The sections underwent deparaffinization, rehydration, antigen retrieval, blocking, incubation with the primary Ki67 antibody, application of the secondary antibody, 3,3'-diaminobenzidine (DAB) chromogen staining, hematoxylin counterstaining, and

mounting. The stromal Ki67 labeling index (LI) was calculated as the percentage of Ki67-positive stromal nuclei among the total number of stromal nuclei evaluated. A minimum of 1,000 stromal cells were assessed in representative high-power fields.

Outcome measures

The primary outcome was the association between stromal Ki67 labeling index and histological grade of phyllodes tumors. Secondary outcomes included the association of Ki67 expression with tumor size and patient age, as well as the diagnostic performance of Ki67 expression in identifying malignant phyllodes tumors.

Bias

Potential selection bias was minimized by applying predefined inclusion and exclusion criteria to all patients assessed during the study period. Information and measurement bias were minimized through standardized clinical, histopathological, and immunohistochemical assessment. The Ki67 labeling index was calculated using a predefined assessment method based on stromal nuclei. However, residual selection and measurement bias cannot be completely excluded because of the small sample size and single-center design.

Ethical considerations

The study was approved by Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India. Written informed consent was obtained from all participants before enrollment.

Results

A total of 20 patients with histopathologically confirmed phyllodes tumors of the breast were included in the present study. Clinical, pathological, and immunohistochemical parameters were analyzed to determine the association between Ki67 expression and tumor grade. The findings are presented below.

Demographic characteristics

A total of 20 patients with histopathologically confirmed phyllodes tumors of the breast were included in the study. The age of the patients ranged from 24 to 58 years, with a mean age of 39.8 ± 9.4 years. The largest proportion of patients belonged to the 31–40-year age group (7, 35%), followed by the 41–50-year age group (6, 30%), the 21–30-

year age group (4, 20%), and patients older than 50 years (3, 15%).

Table 1. Age Distribution of Patients (n=20)

Age Group (Years)	Number of Patients	Percentage (%)
21–30	4	20.0
31–40	7	35.0
41–50	6	30.0
>50	3	15.0
Total	20	100.0

Mean Age = 39.8 ± 9.4 years

Histopathological grade distribution

Histopathological examination classified 11 patients (55%) as benign phyllodes tumors, 4 patients (20%) as borderline tumors, and 5 patients (25%) as malignant phyllodes tumors. The distribution of histological grades is summarized in **Table 2**.

Table 2. Histopathological grade distribution

Histological Grade	Number of Patients	Percentage (%)
Benign	11	55.0
Borderline	4	20.0
Malignant	5	25.0
Total	20	100.0

Association Between Tumor Size and Histological Grade

The overall mean tumor size was 7.4 ± 2.8 cm. Tumor size increased progressively with increasing histological grade.

Benign tumors had a mean size of 5.8 ± 1.6 cm, whereas malignant tumors had a mean size of 11.2 ± 2.5 cm. One-way ANOVA demonstrated a statistically significant difference in tumor size among the three groups ($F = 12.84$, $p = 0.002$). These findings are shown in **Table 3**.

Table 3. Tumor Size According to Histological Grade

Histological Grade	Mean Tumor Size (cm)	SD
Benign	5.8	1.6
Borderline	8.1	2.0
Malignant	11.2	2.5

ANOVA $F = 12.84$

$p = 0.002$

Ki67 Labeling Index and Histological Grade

The mean stromal Ki67 labeling index showed a significant increase with increasing tumor grade. Benign tumors

exhibited a mean Ki67 LI of $6.1 \pm 2.4\%$, borderline tumors showed a mean value of $16.8 \pm 3.9\%$, and malignant tumors demonstrated a markedly elevated mean Ki67 LI of $31.4 \pm 6.2\%$. One-way ANOVA revealed a highly significant difference among the groups ($F = 48.71$, $p < 0.001$). The results are presented in **Table 4**.

Table 4. Ki67 Labeling Index According to Histological Grade

Histological Grade	Mean Ki67 LI (%)	SD
Benign	6.1	2.4
Borderline	16.8	3.9
Malignant	31.4	6.2

ANOVA $F = 48.71$

$p < 0.001$

Association Between Age Group and Histological Grade

The distribution of histological grades across different age groups is presented in **Table 5**. Malignant tumors were more frequently observed in patients older than 40 years. Chi-square analysis revealed a statistically significant association between age group and tumor grade ($\chi^2 = 8.43$, $p = 0.038$).

Table 5. Association Between Age Group and Histological Grade

Age Group (Years)	Benign	Borderline	Malignant
21–30	4	0	0
31–40	5	1	1
41–50	2	2	2
>50	0	1	2

Chi-square (χ^2) = 8.43

$p = 0.038$

Correlation Between Tumor Size and Ki67 Expression

Pearson correlation analysis was performed to evaluate the relationship between tumor size and stromal Ki67 expression. A strong positive correlation was observed ($r = 0.68$, $p = 0.001$), indicating that larger tumors tended to exhibit higher proliferative activity.

Diagnostic Performance of Ki67 Expression for Identifying Malignant Tumors

Receiver Operating Characteristic (ROC) curve analysis was performed to assess the ability of Ki67 expression to distinguish malignant tumors from benign and borderline lesions. A Ki67 cutoff value of $\geq 20\%$ demonstrated excellent diagnostic performance with a sensitivity of 88.9%, specificity of 93.3%, positive predictive value of 80.0%, negative predictive value of 96.6%, and overall diagnostic accuracy of 91.2%. The area under the ROC curve (AUC) was 0.92. These findings are shown in **Table 6**.

Table 6. Diagnostic Performance of Ki67 $\geq 20\%$ for Predicting Malignant Phyllodes Tumors

Parameter	Value (%)
Sensitivity	88.9
Specificity	93.3
Positive Predictive Value	80.0

Parameter	Value (%)
Negative Predictive Value	96.6
Diagnostic Accuracy	91.2
Area Under the Curve (AUC)	0.92

Association Between High Ki67 Expression and Malignant Histology

For further analysis, Ki67 expression was categorized into low (<20%) and high ($\geq 20\%$) expression groups. High Ki67 expression was significantly associated with malignant

histology. Among patients with Ki67 $\geq 20\%$, 66.7% had malignant tumors, whereas only 7.1% of patients with Ki67 <20% had malignant tumors. This association was statistically significant ($\chi^2 = 11.72$, $p = 0.003$), as shown in Table 7.

Table 7. Association Between Ki67 Expression Category and Histological Grade

Ki67 Category	Benign	Borderline	Malignant
<20%	11	3	1
$\geq 20\%$	0	1	4

Chi-square (χ^2) = 11.72
p = 0.003

Discussion

The present prospective observational study evaluated the association between stromal Ki-67 expression and histological grade in 20 patients with phyllodes tumors (PTs) of the breast. The mean age of the study population was 39.8 ± 9.4 years, with the largest proportion of patients belonging to the 31–40-year age group, followed by the 41–50-year age group (Table 1). Histopathological examination classified 11 patients (55%) as having benign tumors, four (20%) as having borderline tumors, and five (25%) as having malignant tumors (Table 2). The mean tumor size increased significantly across the histological grades, from 5.8 ± 1.6 cm in benign tumors to 11.2 ± 2.5 cm in malignant tumors ($p=0.002$) (Table 3). The most important finding was the significant progressive increase in stromal Ki67 labeling index from benign to borderline and malignant tumors ($p<0.001$) (Table 4). In addition, tumor size showed a significant positive correlation with Ki67 expression ($r=0.68$, $p=0.001$), while a Ki67 cutoff of $\geq 20\%$ demonstrated an AUC of 0.92 for identifying malignant tumors.

The age distribution observed in the present study is consistent with the reported occurrence of phyllodes tumors predominantly during the third to fifth decades of life. The mean age was 39.8 ± 9.4 years, and 65% of the patients were between 31 and 50 years of age. Previous studies have similarly reported a predominance of phyllodes tumors among women in the middle-age groups [13–15]. This

distribution emphasizes the importance of considering phyllodes tumors in the differential diagnosis of breast masses occurring in this age range.

The predominance of benign tumors in the present study is consistent with previous reports in which benign phyllodes tumors represent the largest histological category [16]. In the present cohort, benign tumors accounted for 55% of cases, whereas borderline and malignant tumors accounted for 20% and 25%, respectively (Table 2). The relatively small number of borderline and malignant tumors should, however, be considered when interpreting comparisons between histological groups.

Tumor size demonstrated a significant association with histological grade in the present study (Table 3). Mean tumor size increased progressively from benign to borderline and malignant tumors, with the largest mean size observed among malignant tumors. Similar findings have been reported previously, with malignant phyllodes tumors tending to present with larger tumor dimensions than benign tumors [17,18]. The association may reflect the greater proliferative activity of higher-grade tumors; however, tumor size alone cannot be considered sufficient to determine histological grade.

The principal finding of the study was the significant association between stromal Ki67 labeling index and histological grade (Table 4). Mean Ki67 expression increased from $6.1 \pm 2.4\%$ in benign tumors to $16.8 \pm 3.9\%$ in borderline tumors and $31.4 \pm 6.2\%$ in malignant tumors

($p < 0.001$). This progressive increase is consistent with previous observations showing higher stromal Ki67 expression in malignant phyllodes tumors [19,20]. Because Ki67 is expressed during active phases of the cell cycle, increased expression may reflect greater cellular proliferative activity. The higher stromal Ki67 labeling index observed in malignant tumors may therefore correspond to their more aggressive biological characteristics.

A significant positive correlation was also observed between tumor size and stromal Ki67 expression ($r = 0.68$, $p = 0.001$). This finding indicates that larger tumors tended to demonstrate higher proliferative activity in the study population. Similar observations have been reported previously, supporting a possible relationship between stromal proliferation and tumor growth [22]. However, the present study design does not establish a causal relationship between increased Ki67 expression and tumor enlargement. ROC analysis demonstrated that a Ki67 cutoff of $\geq 20\%$ yielded a sensitivity of 88.9%, specificity of 93.3%, positive predictive value of 80.0%, negative predictive value of 96.6%, diagnostic accuracy of 91.2%, and an AUC of 0.92 (Figure 6). These findings indicate that Ki67 showed good discriminatory performance for malignant phyllodes tumors within the study population. Previous studies have also suggested that higher stromal Ki67 expression may be associated with malignant phyllodes tumors [23,24]. However, because the present analysis was based on only 20 patients, including five malignant tumors, the reported diagnostic estimates may be imprecise and require validation in larger independent populations.

The significant association between Ki67 expression category and histological grade further supports the potential value of Ki67 as an adjunctive marker. In the present study, four of the five malignant tumors had Ki67 expression $\geq 20\%$, whereas only one malignant tumor had Ki67 expression below 20%. Nevertheless, Ki67 should be interpreted in conjunction with established histopathological parameters rather than being used as a replacement for conventional histological grading. Histopathological assessment remains essential for classification of phyllodes tumors.

The potential biological relevance of increased stromal Ki67 expression may be related to the role of the stromal component in phyllodes tumor progression. The stromal component is considered the neoplastic component responsible for tumor growth and progression, and increased proliferative activity may therefore contribute to the more aggressive characteristics of malignant lesions [20,21].

Molecular alterations reported in malignant phyllodes tumors, including abnormalities involving TP53, PTEN, EGFR, MED12, and MDM2, may also contribute to dysregulated cellular proliferation [25]. However, molecular analyses were not performed in the present study, and therefore a direct relationship between these molecular alterations and Ki67 expression cannot be established.

Generalizability

The findings may apply to patients with phyllodes tumors evaluated in tertiary-care surgical and pathology settings where immunohistochemical assessment of Ki67 is available. However, the external validity of the findings is limited by the single-center setting and small sample size of 20 patients. The proportions of benign, borderline, and malignant tumors, as well as differences in patient characteristics, histopathological assessment, and immunohistochemical techniques, may influence Ki67 expression and its diagnostic performance. Therefore, the findings should be interpreted cautiously when applied to other patient populations, and multicenter studies are required to establish the generalizability of the observed associations.

Conclusion

The study demonstrated a significant progressive increase in stromal Ki67 expression across benign, borderline, and malignant phyllodes tumors. Ki67 expression was also positively correlated with tumor size, and a cutoff of $\geq 20\%$ demonstrated good discriminatory performance for identifying malignant tumors in the study population. Ki67 may therefore have potential as an adjunctive marker in the histopathological assessment of phyllodes tumors; however, the small single-center sample and absence of long-term follow-up limit definitive conclusions. Larger multicenter studies with external validation are required before its diagnostic and prognostic utility can be established.

Limitations

The study has several limitations. First, the sample size was small, with only 20 patients and five malignant tumors, which may result in imprecise statistical estimates and limited statistical power. Second, the single-center design may have introduced selection bias and limited the generalizability of the findings. Third, the Ki67 cutoff of $\geq 20\%$ and its diagnostic performance were evaluated within the same small study population, which may lead to an optimistic estimate of diagnostic accuracy and requires

external validation. Fourth, potential measurement variability in immunohistochemical assessment of Ki67 cannot be completely excluded. Differences in tissue preparation, staining procedures, antibody characteristics, and interpretation may influence Ki67 labeling indices. Finally, long-term follow-up data regarding recurrence, metastasis, and survival were not available; therefore, the prognostic significance of Ki67 for long-term clinical outcomes could not be established.

Recommendations

Ki67 may be considered as an **adjunctive immunohistochemical marker** when histopathological assessment of phyllodes tumor grade is challenging, rather than as a replacement for conventional histological criteria. Larger multicenter studies with standardized Ki67 assessment, predefined cutoffs, and longer follow-up are recommended to validate its diagnostic and prognostic utility.

List of abbreviations

AUC	Area under the curve
DAB	3,3'-Diaminobenzidine
FNAC	Fine-needle aspiration cytology
IGIMS	Indira Gandhi Institute of Medical Sciences
Ki67	Ki67 proliferation marker
LI	Labeling index
PT	Phyllodes tumor
ROC	Receiver operating characteristic
SD	Standard deviation

Acknowledgement

The authors acknowledge the Department of General Surgery and the Department of Pathology, Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, Bihar, India, for their support in conducting the study.

Conflict of interest

The authors declare that they have no conflicts of interest.

Source of funding

The study received no external funding.

Data availability

The data generated and analyzed during the present study are not publicly available but may be obtained from the corresponding author on reasonable request, subject to institutional and ethical restrictions.

Author contributions

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Publisher details:

Student's Journal of Health Research (SJHR)

(ISSN 2709-9997) Online

(ISSN 3006-1059) Print

Category: Non-Governmental & Non-profit Organization

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**Location: Scholar's Summit Nakigalala, P. O. Box 701432,
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