Content available at: https://www.ipinnovative.com/open-access-journals

IP Journal of Diagnostic Pathology and Oncology

Journal homepage: https://www.jdpo.org/

Original Research Article

Assessment of Ki-67 expression in Phyllodes tumor of breast: An Indian study

Chatura Ramakantha Kasimsetty[1], **Thingujam Deeparani**[2]*

[1]*Dept. of Pathology, J.J.M Medical College, Davangere, Karnataka, India*
[2]*Dept. of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur, India*

ARTICLE INFO

Article history:
Received 20-11-2023
Accepted 20-12-2023
Available online 31-01-2024

Keywords:
Ki67 labelling index
Phyllodes
WHO grading

ABSTRACT

Background: Phyllodes tumor (PT) accounts for 0.3-1% of all breast tumors and classified into benign (BP), borderline (BLP) and malignant (MP). However, grading system is somewhat subjective and diagnosis remains challenging. Ancillary techniques may help in classification and prognostication of the tumor.
Aim & Objective: Evaluation of the proliferative activity by Ki-67 index in Indian scenario.
Materials and Methods: 50 PTs received from January 2014 to December 2018 were graded using WHO criteria 2019. Ki-67 labelling index (LI) was calculated and graded 1+ (1-35%), 2+ (35-70%), 3+ (70-100%). Relevant statistical analysis was applied. P value of <0.05 was considered significant.
Results: 70% (35) were BP, 26% (13) BLP and 4% (2) MP. Infiltrative margin, increased stromal cellularity, overgrowth, nuclear atypia and mitotic index were significantly associated with higher grade (P<0.000). The proportion of Ki-67 positive stromal cells in MP (mean76%) was higher than in BLP (mean 56.2%) and BP (mean 23.1%). Increasing Ki-67 index was significantly associated with infiltrative margin, presence of stromal overgrowth, increasing stromal cellularity, nuclear atypia, mitotic index and histological grade (P<0.000). Ki-67 statistically distinguished BP from BLP and MP (P<0.000, P<0.001 respectively), but not between BLP and MP (P<0.329). As a prognostic feature, high Ki-67 index expression did not predict local recurrence and due to fewer recurrent cases, the statistical correlation with any parameter could not be done.
Conclusion: Histopathologic characteristics correlated with Ki-67 index. However, it was uncertain whether Ki-67 could provide independent prognostic information beyond histopathological typing. Continued follow-up with greater number of significant episodes may yield more informative correlations.

1. Introduction

Phyllodes tumor (PT) is a rare fibroepithelial neoplasm of the breast and presents a morphologic continuum from benign to malignant.[1] It is classified as benign, borderline, or malignant according to the WHO classification of 2019 and is based on a combination of several histologic features.[2] There are no defined criteria or clear cutoffs for individual histologic parameters. Like all morphologic grading systems, this grading scheme is somewhat subjective, especially at the cut points between grades. Histologic features have not always been found to be predictive of clinical behavior in individual patients. Thus, the diagnosis of PTs based on the integration of morphology remains challenging.[1]

Inadequate treatment may necessitate repeat surgery and worry to patients as the application of guidelines are weighed down by ambiguity even though they are straight forward.[3]

* *Corresponding author*.
E-mail address: drdeeparanithingujam@gmail.com (T. Deeparani).

https://doi.org/10.18231/j.jdpo.2024.001

Ancillary diagnostic tools may help to identify tumors with potentially aggressive behavior.[4] Several biomarkers have been reported to be associated with histologic grades and show some prognostic value, of these, p53 expression and Ki-67 index were reported to be significantly associated with disease-free (DFS) and overall survivals (OS).[1]

2. Materials and Methods

50 cases of PTs received in 5 years from 2014 to 2018 were included in the study. Representative areas were sampled, H and E staining was done and the standard criteria of WHO was used for grading PTs.

Ki-67 staining on representative paraffin blocks was done according to the manufacturer's specification and staining intensity was evaluated in the stromal component and correlated with various grades of PTs.

Ki-67 was considered to be positive if unequivocal nuclear staining of stromal cells was present. Ki-67 was scored in the area with maximal immunoreactivity. Ki-67 index was calculated as the percentage (%) of nuclei showing positive staining after counting 1,000 neoplastic cells per slide. The percentage of immunoreactive cells was then graded using a 3-point system: 1+, 1–35%; 2+, 35–70%; 3+, 70-100%.

2.1. Statistical analysis

Qualitative data was represented in the form of frequency and percentage. Association between qualitative variables was assessed by Chi Square test. Quantitative data was represented using mean & SD. Analysis between two groups was done using unpaired 't' test. Comparison of mean values between three groups was done with ANOVA and multiple comparisons were done with Tukey's Post Hoc Analysis. P<0.05 was considered statistically significant.

3. Results

Of the 50 cases of PTs, histologically, 35(70%) were benign, 13(26%) borderline and 2(4%) malignant based on the WHO criteria. All were females, with an age range from 15 to 60 years and mean age of 36.4 years and median age of 35 years. A maximum of 16 cases (32%) belonged to the age group of 31-40 years. Pathological parameters of 50 cases stratified according to histological grade are in (Table 1).

Size of PTs ranged from 2-15 cms. 16 (45.7%) BP, 6 (46.2%) BLP and 1(50%) MP were between 6-10cms. Tumor size did not correlate with increasing grade of the tumor (P<0.894). Grossly, on cut-surface 24 BP (68.6%) had leaf-like processes. 9 BLP (69.2%) and 2 MP (100%) showed solid surface. Leaf-like processes were observed more in BP and solid surface was evident as tumor increased in grade (P<0.01).

34 BP (97.1%) had pushing margin whereas 1 BP (2.9%), 8 BLP (61.5%) and 2 MP (100%) showed infiltrative margin, either focally or diffusely, confirming that infiltrative margin is associated with increased tumor grade (P<0.000). 21 BP (60%) had 1+ stromal cellularity, 12 BLP (92.3%) had 2+ and both cases of MP (100%) had 3+, hence stromal cellularity increased with tumor grade (P<0.000). 33 BP (94.3%) showed mild/no nuclear atypia, all BLP cases showed moderate atypia and all MP had marked nuclear atypia. Hence, nuclear atypia increased with grade (P<0.000). Stromal overgrowth was absent in BP, however, present in 9 BLP (69.2%) and both MPs. Hence, stromal overgrowth was associated with higher grade (P<0.000). Mitotic index/count ranged from 1-4/10 HPF in BP, 3-8/10 HPF in BLP, and ≥10/10 HPF in MP, signifying that tumor grade was directly proportional to mitotic count (P<0.000).

The stromal component of PT showed nuclear staining of Ki-67. Epithelial cell staining was not taken into consideration. Ki-67 index/ score was calculated and graded as 1+ (1-35%), 2+ (35-70%) and 3+ (70-100%) (Figure 1).

Ki-67 index of 50 cases ranged from 2% - 82%. BP showed mean of 23.1%, BLP mean of 56.2%, and MP mean of 76% .The proportion of Ki-67 positive stromal cells in MP was higher than in BLP and BP.30 BP (85.7%) showed 1+ Ki-67 grade, 9 BLP (69.2%) 2+ and one MP 2+ and the other MP 3+ grade, so, Ki-67 grade increased with tumor grade (P<0.000).

The correlation of Ki-67 index with tumor size, gross findings, margin status, stromal overgrowth, stromal cellularity, nuclear atypia, mitotic index and histological grades were analysed applying appropriate statistical analysis (Tables 2 and 3).

Increasing Ki-67 index was significantly associated with macroscopic finding (P<0.03), infiltrative microscopic margins (P<0.000), presence of stromal overgrowth (P<0.000), increasing stromal cellularity (P<0.000), increasing stromal cell nuclear atypia (P<0.000), high mitotic index (P<0.000) and increasing histological grade (P<0.000). However there was no difference in the expression of Ki-67 in relation to tumour size. Hence, Ki-67 index was differentially expressed as PTs progressed from benign to malignant. It statistically distinguished BP from BLP and MP (P<0.000, P<0.001 respectively), however, did not differentiate between BLP and MP (P<0.329).

In addition, as a prognostic feature, high Ki-67 index expression did not predict local recurrence. 3 BP cases recurred within a time period of one year from the initial lumpectomy, of whom 2 cases then underwent mastectomy. Up-gradation was seen in one case to BLP. One case had positive infiltrative margin at original resection. All 3 cases had stromal cellularity of 2+, nuclear atypia of 1+ to 2+, mitotic index 2-3/10 HPF. Ki-67 index in initial and recurrence was 28% and 30% in one, 62% and 64% in another and in the upgraded case was 80% and 82%. However, due to few cases, correlation of recurrence with

Table 1: Pathological parameters of 50 cases stratified according to histological grade

Pathological parameters		BP (35)	BLP (13)	MP (2)	P value
Size in cms	1-5 (n=19) (%)	14 (73.6)	4 (21.1)	1 (5.3)	χ^2 =1.09, df = 4, P < 0.894
	6-10 (n=23) (%)	16 (69.6)	6 (26.1)	1 (4.3)	
	11-15 (n=8) (%)	5 (62.5)	3 (37.5)	0 (0)	
Macroscopic features	Leaf-like (n=28) (%)	24 (85.7)	4 (14.3)	0 (0)	χ^2 = 8.149, df = 2, P < 0.01
	Solid (n=22) (%)	11 (50)	9 (40.9)	2 (9.1)	
Tumor margin	Pushing (n=39) (%)	34 (87.2)	5 (12.8)	0 (0)	χ^2 = 36.408, df = 2, P< 0.000
	Infiltrative (n=11) (%)	1 (9.1)	8 (72.7)	2 (18.2)	
Stromal cellularity	1+ (n=21) (%)	21 (100)	0 (0)	0 (0)	χ^2 = 46.686, df = 4, P< 0.000
	2+ (n=26) (%)	14 (53.8)	12 (46.2)	0 (0)	
	3+ (n=3) (%)	0 (0)	1 (33.3)	2 (66.7)	
Nuclear atypia	1+ (n=33) (%)	33 (100)	0 (0)	0 (0)	χ^2 = 64.686, df = 4, P< 0.000
	2+ (n=16) (%)	2 (12.5)	13 (81.2)	1 (6.3)	
	3+ (n=1) (%)	0 (0)	0 (0)	1 (100)	
Stromal overgrowth	Present (n=11) (%)	0 (0)	9 (81.8)	2 (18.2)	χ^2 = 33.862, df = 2, P< 0.000
	Absent (n=39) (%)	35(89.7)	4 (10.3)	0 (0)	
Mitotic index (/10 HPF)	0-4 (n=36) (%)	35 (97.2)	1 (2.8)	0(0)	χ^2 = 94.87, df = 4, P< 0.000
	5-9 (n=12) (%)	0 (0)	12 (100)	0 (0)	
	≥10 (n=2) (%)	0 (0)	0 (0)	2 (100)	
Ki-67 grade	1+ (n=30) (%)	30 (100)	0 (0)	0 (0)	χ^2 = 33.189, df = 4, P< 0.000
	2+ (n=14) (%)	4 (28.6)	9 (64.3)	1 (7.1)	
	3+ (n=6) (%)	1 (16.7)	4 (66.6)	1 (16.7)	

Table 2: Expression of Ki-67 in correlation with pathological parameters

Parameters		Ki-67 index (%)			Unpaired t test & P Value
		N	Mean	SD	
Gross	Leaf-like process	28	27.32	23.94	t=-2.176, P<0.03
	Solid	22	42.00	23.33	
Margin	Pushing	39	26.59	21.66	t=-4.645, P<0.000
	Infiltrative	11	59.27	15.98	
Stromal overgrowth	Present	11	66.00	13.91	t=6.864, P<0.000
	Absent	39	24.69	18.48	
Nuclear atypia	Grade 1+	33	20.64	16.46	t=-7.575, P<0.000
	Grade 2+	16	57.88	15.43	

Table 3: Correlation of expression of Ki67 index by ANOVA one way test and Post Hoc Tukey's Multiple Comparisons

Parameters		Ki-67 index (%)			ANOVA & P Value	Post Hoc Tukey's Multiple Comparisons	P value
		N	Mean	SD			
Stromal cellularity	Grade 1+	21	11.95	5.12	F= 46.16 P<0.000	Grade 1+vs 2+	P<0.000
	Grade 2+	26	46.54	19.36		Grade 1+ vs 3+	P<0.001
	Grade 3+	3	76.00	6.00		Grade 2+ vs 3+	P<0.005
Mitotic index (/10 HPF)	0-4	36	23.47	18.72	F=21.842 P<0.000	0-4 Vs 5-9	P<0.000
	5-9	12	57.67	16.44		0-4 Vs ≥10	P<0.001
	≥10	2	76.00	8.49		5-9 Vs ≥10	P<0.386
Histological diagnosis	BP	35	23.06	18.83	F=21.43 P<0.000	BP Vs BLP	P<0.000
	BLP	13	56.15	16.66		BP Vs MP	P<0.001
	MP	2	76.00	8.49		BLP Vs MP	P<0.329

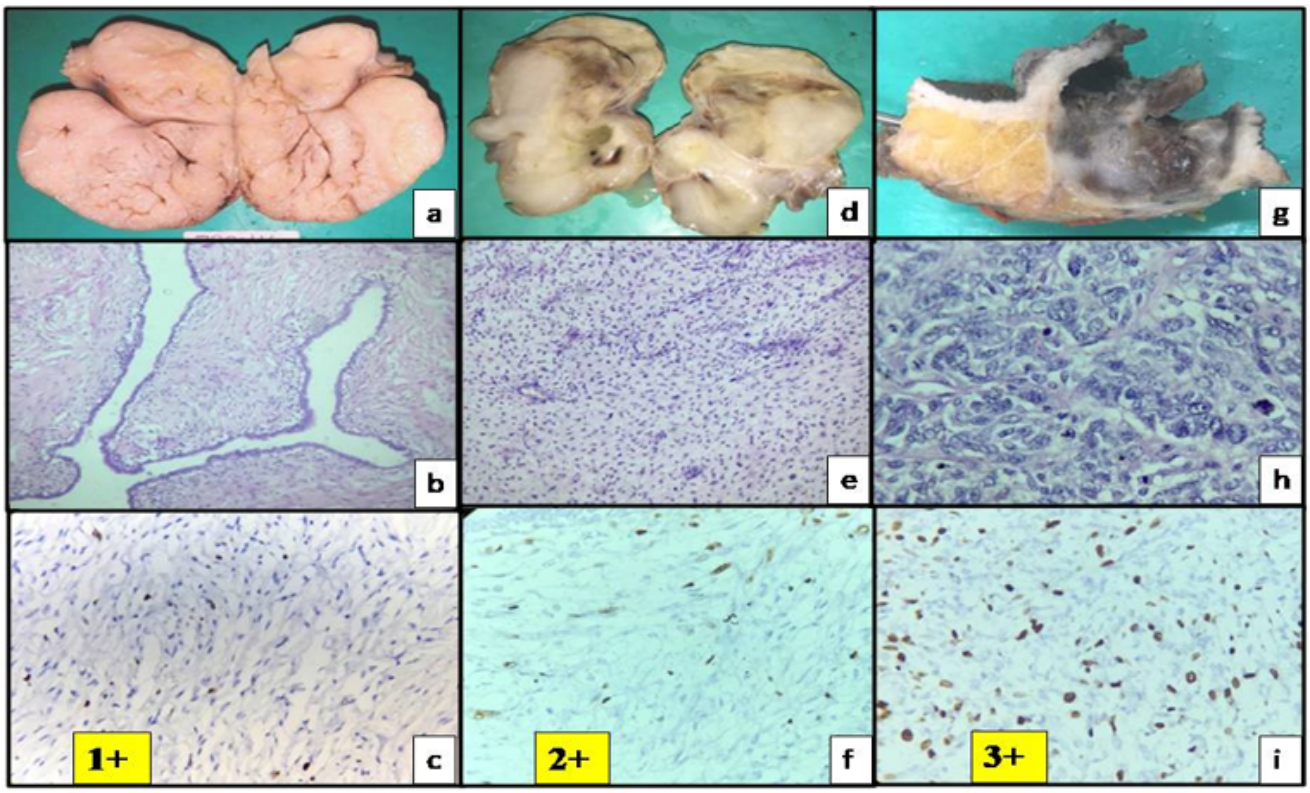

Figure 1: (a,b,c) Benign phyllodes, **a.** Leaf-like processes on cut-section; **b:** Histological benign grade; **c:** Ki-67 index grade 1+. (**d,e,f**) borderline phyllodes**, d:** Solid surface on cut-section; **e:** Histological borderline grade; **f:** Ki-67 index grade 2+. (**g,h,i**) malignant phyllodes; **g:** Solid surface with infarction on cut-section, h. Histological malignant grade; **i:** Ki-67 index grade 3+.

any parameter could not be done.

4. Discussion

PTs account for 2.5% of all fibroepithelial tumors of the breast.[2] In Asian countries, they appear to be more frequently encountered, with younger age of occurrence.[5] Ours is the only study from India with Ki67 index of these rare neoplasms, that typically behave in a benign fashion.[4]

Most PTs (70%) were benign, as in other studies 72.7%,[5] and 75.9%.[6] MP (4%) was the least common as with others 8.9%,[5] and 7.7%.[6]

They occur predominantly in middle-aged women (average of 40-50 years) about 15-20 years later than for fibroadenomas (FA).[2] In our study, 16 cases were in 31-40 years with mean of 36.4, but in others mean was 42,[5] and 45,[7] years. Many benign cases were below the mean and all malignant cases were above the mean. However more borderline cases were below mean age unlike other studies.[5,7]

Size at presentation was often larger than for FA,[8,9] although increased breast awareness and the impact of screening has resulted in a trend towards presentation at smaller tumor sizes.[9] Tumors of 2-3 cms diameter are becoming more common, but the average size remains around 4-5 cms.[2]

The mean tumor size in our study was 7cms, and in others 4cms,[5] and 5.5cms.[7] Many BP were lesser than but BLP more than mean. However the two MP had sizes lesser than mean, unlike the other two studies.[5,7]

PTs present distinct challenges relating to their diagnosis, classification, predicted behaviour and clinical management.[3] According to the WHO, classification into categories is based on a constellation of histological parameters that primarily focuses on stromal features of atypia, mitoses, cellularity, overgrowth and nature of tumor margins. The attention on stromal features is premised on the view that the stroma of PT represents the neoplastic component that progresses and which is responsible for clinical behavior.[5] The grading should be based on the areas of highest cellular activity and most florid architectural pattern.[10] As each microscopic parameter has two to three tiers of stratification, there are significant challenges in accurate and reproducible categorization.[3]

Pushing margin was observed often in benign and infiltrative margin in malignant PTs. Few borderline PTs had pushing and few had infiltrative margins. These findings were comparable with previous studies.[5–7] Margins were helpful in predicting the grades of the PTs (P<0.000).

Mild stromal cellularity (1+) was often with BP, moderate (2+) with BLP and marked (3+) with MP (P<0.000). Stromal cellularity increased with increase in tumor grades in other studies also.[5–7]

Mild stromal nuclear atypia (1+) was often in BP, and moderate atypia (2+) in BLP. MPs had moderate (2+) and marked atypia (3+) (P<0.000). Others also state that nuclear atypia increased as the tumor grade progressed.[5–7]

PTs are biphasic tumors,[1] with prominent stromal component and can show morphologic patterns that range from fibroadenoma-like to frankly sarcomatous.[4] The epithelium promotes stromal growth. Once the stroma acquires specific, as yet unknown, mutations and becomes malignant, the stromal proliferation becomes autonomous and no longer requires a mitogenic stimulus from the epithelium. As a result, the stromal compartment grows in excess of the epithelial compartment.[11] However, the notion of the epithelial component acting as a bystander was challenged recently with reports of both epithelial and stromal components harbouring different sets of genetic changes on clonality and cytogenetic analyses.[12]

Stromal overgrowth was absent in all BP while all MPs showed overgrowth. Some BLPs showed stromal overgrowth and some did not (P<0.000). Many authors have observed that stromal overgrowth was associated with higher grade.[5–7]

Mitotic index increased with increased tumor grade, (P<0.000), as in others.[5,7]

Multivariate analysis revealed stromal atypia, overgrowth and surgical margins to be independently predictive of clinical behavior, with mitoses achieving near significance (P=0.058). Univariate analysis showed that mitotic rate, grade, surgical margin, atypia, hypercellularity, overgrowth and borders affected recurrent free survival significantly.[5]

Biological and genetic markers may assist in categorising PT for prognostic purposes, but the classification of PTs remains problematic in terms of standardised universal application. How the assessment of each histological parameter combines together to assign a specific grade, and whether there are histological features that are individually more important in predicting recurrent behaviour, have not been resolved.[5]

A study focussed on pathological criteria that could help to separate PTs identified according to the genetic data and suggests that only two types of PTs could be distinguished on a genomic basis: BP and MP (which includes the borderline and malignant categories). Univariate analysis identified two significant pathological criteria, namely, nuclear size and mitotic activity as helpful adjuncts to differentiate the genetic groups of PTs.[13]

The issue with grade, however, is with inherent challenges to reproducibility. Assessment of atypia and cellularity may be hampered by interobserver variability. Evaluation of mitoses, too, may have concerns about the number of fields to be analysed and whether it should be a maximal or average mitotic count. Stromal overgrowth, while appearing the least contentious, may possibly vary due to differences in the size of the low-power microscopic field. Many of these perceived problems are related to the lack of clear definition. While these can be rectified through use of more clearly defined histological criteria, there is the additional difficulty of how various parameters are integrated into a final microscopic grade. Surgical margins have been regarded as a critical element in impacting the likelihood of PT recurrence.[5]

At the extreme ends of the spectrum, the diagnosis of grade is mostly straightforward. In the intermediate range, however, it is difficult to accurately assign a grade, and it may become a judgement call based on the parameter(s) believed to have stronger biological emphasis[5] How the proposed histological criteria are amalgamated to fit an individual tumor into a specific grade is not universally established?[7]

On univariate analysis of pathologic and immunohistochemical features associated with DFS and OS, all pathologic parameters were associated with shorter DFS and OS (P<0.001). However, multivariate Cox's proportional hazard model analysis showed a significant correlation of stromal overgrowth (P<0.001,) and stromal mitosis (P<0.013) with DFS.[6]

Interpretive subjectivity, overlapping histological diagnostic criteria, suboptimal correlation between histological classification and clinical behaviour and the lack of robust molecular predictors of outcome make further investigation of pathogenesis of these fascinating tumors a matter of active research.[3]

PT is well- known for its unpredictable behaviour in terms of local recurrences and distant metastases. There is now a clear consensus that histopathological appearence and biological behavior in PT may poorly correlate, and histopathological features alone are of relatively limited value in discriminating BP and MP.[14]

Distinction of PT from FA on FNAC also becomes difficult due to overlapping features. Number and cellularity of epithelial and stromal components and dispersed stromal cells in the background were reevaluated in FNAC of 27 proved PTs. Accuracy rate in pre-operative smears was 47.82% and reached 78.26% after a review. The Chi-square statistic was 4.023 and significant p = 0.0448. These cytological parameters individually may not be promising, but, taken together, can be used effectively in distinguishing the two groups, as a pre-operative diagnosis is crucial to plan

for surgical treatment.[15]

Local recurrences can occur in all PTs, at an overall rate of 21%.[2] Recurrence rates in the literature are 10-17%, 14-25% and 23-30% and in a large Asian series 10.9%, 14.4% and 29.6% for BP, BLP and MPs, respectively.[3] Interestingly, in a clinicopathological analysis, there was a suggestion that Asian patients experienced a higher recurrence rate than those of non-Asian ethnicity.[16] While grade has predictive utility across cohorts, with quoted recurrence of 17%, 25% and 27% in BP, BLP and MPs, information on likely recurrent behaviour in an individual patient is uncertain.[5]Adverse events are, in general, rare for all forms of PTs when they are subjected to complete local excision.[3]

These recurrences may mirror the microscopic pattern of the original tumor or show dedifferentiation with microscopic upgrading. Many histological features have been reported to possess predictive value for local recurrences in PT, and status of surgical margins at previous excision appears to be the most reliable. A recent study found that, apart from surgical margins, histological parameters that had an independent impact on recurrence were stromal overgrowth and atypia, with mitotic activity being almost significant.[2]

Grade progression during local recurrence of PTs can occur. There have been several suggestions regarding why this happens, including a lack of representative sampling of the initial tumor, tumor heterogeneity with the presence of stromal subclones, and loss of stromal–epithelial interdependency.[3]

Surgical excision is usually the preferred procedure. What constitutes a sufficient margin for PTs is yet another unresolved dilemma.[3] In MPs, standard treatment includes mastectomy or wide local excision. Compounding treatment decisions is the lack of reliable histologic indicators that predict recurrence.[4]

Different studies have regarded stromal overgrowth, infiltrating margins, high mitotic rate and degree of stromal atypia as important predictors of recurrence and/or prognosis, while others have disagreed with these findings. A positive margin status is the most consistent indicator of local recurrence, and a multivariate analysis showed negative margins reduced recurrence hazards by 51.7%. Such conflicting results highlight the need for markers that can more reliably predict aggressive behaviour and patient outcome.[4]

Multiple biological markers have been evaluated for their prognostic value, and many have shown associations with histological grade. However, none has been able to demonstrate independent prognostic value and provide an improvement over current histological grading[7] and their use in defining grade and potential clinical behaviour in specific cases remains limited.[3] Follow-up studies to determine the behaviour of PTs have demonstrated the inadequacy of histological criteria alone in predicting biological behaviour and hence led to various immunohistochemical markers being evaluated to more reliably predict patient outcome.[10]

It is with this knowledge of existing deficiencies in PT classification and behavior prediction that we made an effort to assess the prognostic value of Ki-67 LIs with grades of PTs in 50 cases as an adjunct to morphological diagnosis.

Many studies have shown increased Ki-67 expression with increasing histological grade of PT. However, they deferred in their conclusions as to the utility of Ki-67 in predicting outcome.[10]

Our findings of a wider range of Ki-67 index 2- 80%, 38- 82%, and 70- 82% unlike others studies 1-30%, 1-10%, 10-90%;[17] <1-10%, 5-60%, 20-40%;[4] for BP,BLP and MPs respectively, might be because of the larger sample size as compared to others. As Ki-67 index presented a large range given the number of tumors analyzed, an evaluation in yet a large number of PTs is needed for validation. It would be of interest to perform Ki-67 index on an external set of PTs to correlate with the pathological categories.

Mean Ki-67 index reported in our study was 23.1%±18.8% for BP, 56.2%±16.7% for BLP and 76%±8.5% for MP. Ki-67 index increased with tumor grade (P<0.000) similar to others. Higher Ki-67 index observed in respective grades as compared to other previous studies[4,9,17–19] may be due to our larger sample size. Ki-67 index could significantly differentiate BP from BLP (P<0.001) and MP (P<0.001) but not between BLP and MP (P=0.42),[4] as also seen in our study (P<0.000, P<0.001, P<0.329 respectively).

A study of 118 cases of PTs concluded that histologically MPs with a Ki-67 index (< 11.2%) had a clinical outcome similar to that of histologically BP or BLP, whereas high Ki-67 indices in MP (>11.2%) correlated with poor clinical outcomes. The results of multivariate analysis suggest that Ki-67 index might be a prognostic factor in terms of OS, significantly related with DFS as well as with OS.[20]

A study reported that three BP patients with an MIB-1 index >10% had recurred and progressed to MP.[21] This indicated that immunohistochemical analysis might be useful for identifying patients at high risk of systemic recurrence and death from disease.[17] Others found that Ki-67 expression was not useful in predicting recurrence.[4,10] A study of 23 cases divided PTs into low-grade and high-grade categories only and the average Ki-67 was 43% for low-grade and 89% for high-grade PTs.[22]

As the follow-up period was relatively short in our series, only 3 recurrences were encountered. Although Ki-67 index was high in all these at initial presentation, these findings should not be over-interpreted, owing to the small number of events and also the recurrences had similar high Ki-67 indexes. Our findings support the conclusions of others that Ki-67 index is differentially expressed in PTs and aid in

distinguishing benign from borderline or malignant PTs. However, this index did not significantly correlate with tumor behaviour.

Ki-67 LI in the recurrent tumors ranged from 28% - 80%, but in other studies range was from <1% to 60%[4] and 0.2% - 19%.[20] Thus, proliferative fraction of cells as assessed by Ki-67 did not appear to be a reliable predictor of tumor recurrence.

Microscopic tumor margin status evaluated in the recurrent cases was infiltrative in one and pushing in two cases in the first instance of lumpectomy. This emphasises the importance of surgical free margins in recurrence rather than the nature of the tumor margins.[5,7]

We demonstrated a significant association between increasing Ki-67 index expression with increasing histological grade, tumor margin, stromal hypercellularity, stromal nuclear atypia, stromal overgrowth and increased mitotic count. Limitations of our study may relate to the relatively few cases in malignant category. Since this study investigated only a small number of patients and recurrence was not observed in patients with BLP or MP, it is uncertain whether Ki-67 could provide independent prognostic information beyond histopathological typing.

5. Conclusion

PTs of the breast are rare and the diagnosis based on the integration of morphology still remains challenging. We made an effort to analyse the histological parameters to determine grade and to correlate histopathological characteristics with Ki-67 LI. Adequate sampling of the tumor, particularly of grossly heterogeneous areas and meticulous histological assessment, remains the keystone of diagnosis, buttressed by clinical, and immunohistochemical correlation. Continued follow-up with greater number of significant episodes may yield more informative correlations.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Zhang Y, Kleer CG. Phyllodes tumor of the breast histopathologic features, differential diagnosis, and molecular/genetic updates. *Arch Pathol Lab Med*. 2016;140(7):665–71.
2. WHO classification of tumors, 5th edition – Breast tumors. Lyon: International Agency for Research on Cancer; 2019.
3. Tan BY, Acs G, Apple SK, Badve S, Bleiweiss IJ, Brogi E, et al. Phyllodes tumours of the breast: a consensus review. *Histopathology*. 2016;68(1):5–21.
4. Esposito NN, Mohan D, Brufsky A, Lin Y, Kapali M, Dabbs DJ, et al. Phyllodes tumor: a clinicopathologic and immunohistochemical study of 30 Cases. *Arch Pathol Lab Med*. 2006;130(10):1516–21.
5. Tan PH, Thike AA, Tan WJ, Thu MM, Busmanis I, Li H, et al. Predicting clinical behaviour of breast phyllodes tumours: a nomogram based on histological criteria and surgical margins. *J Clin Pathol*. 2012;65(1):69–76.
6. Kwon JE, Jung WH, Koo JS. Molecules involved in epithelial-mesenchymal transition and epithelial-stromal interaction in phyllodes tumors: implications for histologic grade and prognosis. *Tumor Biol*. 2012;33(3):787–98.
7. Ho SK, Thike AA, Cheok PY, Tse GM, Tan PH. Phyllodes tumours of the breast: the role of CD34, vascular endothelial growth factor and β-catenin in histological grading and clinical outcome. *Histopathology*. 2013;63(3):393–406.
8. Yang X, Kandil D, Cosar EF, Khan A. Fibroepithelial tumors of the breast pathologic and immunohistochemical features and molecular mechanisms. *Arch Pathol Lab Med*. 2014;138(1):25–36.
9. Jacklin RK, Ridgway PF, Ziprin P, Healy V, Hadjiminas D, Darzi A, et al. Optimising preoperative diagnosis in phyllodes tumour of the breast. *J Clin Pathol*. 2006;59(5):454–9.
10. Noronha Y, Raza A, Hutchins B, Chase D, Garberoglio C, Chu P, et al. CD117, and Ki-67 expression in phyllodes tumor of the breast: an immunohistochemical study of 33 cases. *Int J Surg Pathol*. 2011;19(2):152–8.
11. Sawyer EJ, Hanby AM, Rowan AJ, Gillett EC, Thomas RE, Poulsom R, et al. The Wnt pathway, epithelial-stromal interactions, and malignant progression in phyllodes tumours. *J Pathol*. 2002;196(4):437–44.
12. Tan WJ, Thike AA, Bay BH, Tan PH. Immunohistochemical expression of homeoproteins Six1 and Pax3 in breast phyllodes tumours correlates with histological grade and clinical outcome. *Histopathology*. 2014;64(6):807–17.
13. Lae´M, Vincent-Salomon A, Savignoni A, Huon I, neaux PF, Sigal-Zafrani B, et al. Phyllodes tumors of the breast segregate in two groups according to genetic criteria. *Mod Pathol*. 2007;20(4):435–44.
14. Kaya R, Pestereli HE, Erdogan G, Gulkesen KH, Karaveli S. Proliferating activity in differential diagnosis of benign phyllodes tumor and cellular fibroadenomas: Is it helpful? *Pathol Oncol Res*. 2001;7(3):213–6.
15. Chatura KR, Deeparani T, Patil SB. A revisit of cytological features in phyllodes tumors. *J Med Radiol Pathol Surg*. 2018;5(6):3–8.
16. Karim RZ, Gerega SK, Yang YH, Spillane A, Carmalt H, Scolyer RA, et al. Phyllodes tumours of the breast: a clinicopathological analysis of 65 cases from a single institution. *Breast*. 2009;18(3):165–70.
17. Yonemori K, Hasegawa T, Shimizu C, Shibata T, Matsumoto K, Kouno T, et al. Correlation of p53 and MIB-1 expression with both the systemic recurrence and survival in cases of phyllodestumors of the breast. *Pathol Res Pract*. 2006;202(10):705–12.
18. Kleer CG, Giordano TJ, Braun T, Oberman HA. Pathologic, immunohistochemical, and molecular features of benign and malignant phyllodes tumors of the breast. *Mod Pathol*. 2001;14(3):185–90.
19. Shpitz B, Bomstein Y, Sternberg A, Klein E, Tiomkin V, Kaufman A, et al. Immunoreactivity of p53, Ki-67, and c-erbB-2 in phyllodes tumors of the breast in correlation with clinical and morphologic features. *J Surg Oncol*. 2002;79(2):86–92.
20. Niezabitowski A, Lackowska B, Rys J, Kruczak A, Kowalska T, Mitus J, et al. Prognostic evaluation of proliferative activity and DNA content in the phyllodes tumor of the breast: immunohistochemical and flow cytometric study of 118 cases. *Breast Cancer Res Treat*. 2001;65(1):77–85.
21. Chan YJ, Chen BF, Chang CL, Yang TL, Fan CC. Expression of p53 protein and Ki-67 antigen in phyllodes tumor of the breast. *J Chin Med Assoc*. 2004;67(1):3–8.
22. Dacic S, Kounelis S, Kouri E, Jones MW. Immunohistochemical profile of cystosarcoma phyllodes of the breast: a study of 23 cases. *Breast J*. 2002;8(6):376–81.

Author biography

Chatura Ramakantha Kasimsetty, Professor https://orcid.org/0000-0001-8070-8348

Thingujam Deeparani, Senior Resident https://orcid.org/0000-0002-1688-9444

Cite this article: Kasimsetty CR, Deeparani T. Assessment of Ki-67 expression in Phyllodes tumor of breast: An Indian study. *IP J Diagn Pathol Oncol* 2024;9(1):1-8.