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Histopathological Grading and a Role of IHC in Grading of Phyllodes Tumor of Breast

Rajarajan S.

1. Introduction

Phyllodes tumor of breast are rare fibroepithelial neoplasms. It accounts for <1% of all breast tumors[1]. In 1827, Chelius described this tumor for the first time. Johanner muller was the first person to use the term Cystosarcoma phyllodes (1838) [2]. Phyllodes tumor was considered as benign till 1943. In 1943, the malignant potential of this tumor was reported by Cooper and Ackerman. In 1981, the term phyllodes tumor was adopted by WHO. It exhibit a varying biological behavior ranging from benign to highly aggressive malignant sarcomas. Malignant phyllodes tumor has a five year survival rate of 50 -60%. It has 65% chance of local recurrence and 10% chance of distant metastasis if treated conservatively. Phyllodes tumor has been classified as benign, borderline and malignant phyllodes based on histological features. The conventional histological features has many overlapping features resulting in difficult to grade the phyllodes tumor accurately. Hence there is definite need of immunohistochemical markers such as CD117, Ki67 and P53 for accurately differentiating phyllodes tumor into benign, borderline and malignant category, so that appropriate management is given. C KIT is a protooncogene involved in cell proliferation and survival. It encodes tyrosine kinase receptor (CD117). Overexpression of CKIT leads to increased cell proliferation and malignancy. The finding of CKIT over expression in phyllodes tumor led to development of targeted therapy with imatinib. P53 is a tumor suppressor gene. The mutation of P53 is one of the most common genetic abnormalities in cancer. MIB-1 is a monoclonal antibody against Ki 67. Ki 67 is a nuclear antigen expressed in non Go proliferating cells.

Aim of the Study

- To study the role of immunohistochemical markers CD117, Ki 67 and P53 in phyllodes tumor - Benign, borderline and malignant categories.
- To compare the expression of immunohistochemical markers CD117, Ki 67 and P53 with histopathological grading of phyllodes tumor.
- The immunohistochemical study with these markers CD117, P53, Ki67was done to highlights the accurate differentiation of phyllodes tumor.

Objectives of the study

1) To find out the incidence of phyllodes tumor in our institution.
2) To evaluate the histological grading of phyllodes tumor.
3) To access the significance of IHC markers such as CD117, Ki67 and P53 in grading of phyllodes tumor of breast.

2. Materials and Methods

The study was both retrospective and prospective study. The retrospective study period was between JAN 2017 - DEC 2018 and prospective study period was between JAN 2019 - AUG 2020. Ethical clearance for the study was obtained from the Ethics Committee of KAPV Government medical college, Trichy. This study consists 30 cases of phyllodes tumor.
Study was conducted in pathology department, KAPV Government medical college, Trichy.

Selection Criteria:

Inclusion Criteria:
1) Age group - 20-70 years
2) Breast lumpectomy / mastectomy specimens which are histopathologically diagnosed as phyllodes tumor.

Exclusion Criteria:
1) Male patients
2) Autolysed or ill fixed specimens
3) Over fixed specimens

Histopathological Grading:
Breast lumpectomy / mastectomy specimens fixed in 10% formalin and embeeded in paraffin. Serial 3µm thick sections were placed and stained with hematoxylin and eosin. The initial diagnosis and grading of phyllodes tumor done by conventional histological features. They are stromal cellularity, stromal overgrowth, tumor margins, nuclear pleomorphism and mitosis. The threshold for number of mitosis required for classification into each subgroup were <5/10 HPF (Benign), 5-9/ 10 HPF (Borderline), >/= 10/ 10 HPF (Malignant). Mitotic activity is common adjacent to the epithelial component of all phyllodes tumors. Therefore, increased activity away from the ducts is most significant. Stromal overgrowth was considered when there is marked stromal proliferation to the point where the epithelial component is absent in at least 1 low-power field (4x).

These histopathologically diagnosed and graded phyllodes tumors are then taken up for immunohistochemistry. Immunohistochemical staining with P53, Ki67 and CD117 was done to demonstrate the stromal expression in phyllodes tumor.

IHC Scoring Criteria
For CD117, membrane and cytoplasmic expressions of stromal cells was considered. For p53 and ki67 nuclear expression of stromal cells was considered. Graduation of p53, CD117 was done by immunoreactivity score. Immunoreactivity score was obtained by multiplying the expression intensity by the marked cell score. Expression

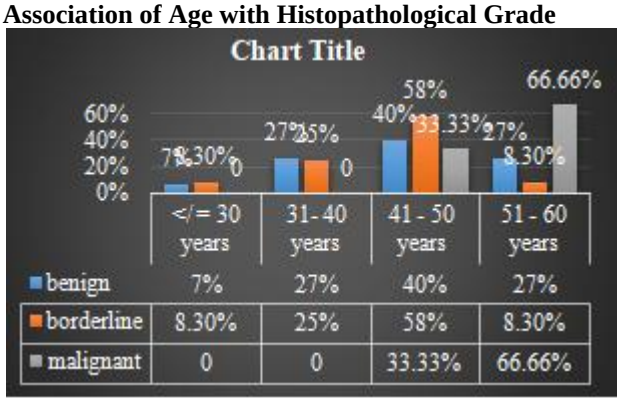
intensity was classified as0-no expression, 1-weak, 2 - moderate, 3-strong. Marked cell score: 0-no positive cells.1- < 10% of positive cells, 2-10-50 % of positive cells, 3-51-80 % of positive cells, 4->80 % of positive cells.

IRS classification: IRS 0 - 1-negative, IRS 2 - 3-positive weak expression, IRS 4-8 - positive moderate expression, IRS 9-12 - positive strong expression. Specifically ki-67 graduation done by percentage of marked cell score alone. The 10% cutoff point for Ki-67 has the best balanced sensitivity with specificity, with area under the curve of 0.98 (95% confidence interval [CI]: 0.95-1) that results in high specificity (96.4%), sensitivity (88.9%), accuracy (94.5%) for borderline or malignant PTs and also has very good concordance coefficient kappa of 0.85 ($p < 0.001$). For ki 67 expression analysis, if more than 10% of the total neoplastic cell nuclei stained, the tumor was considered positive[3].

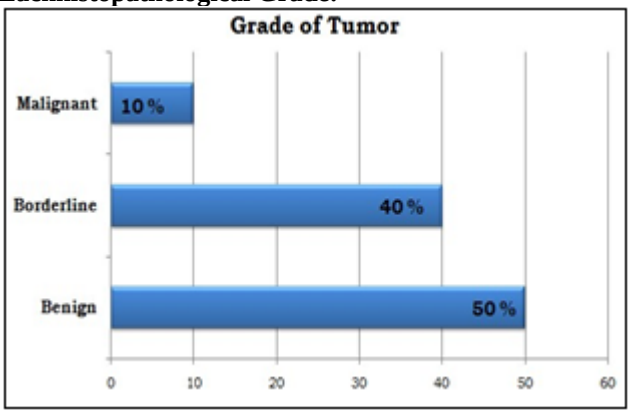
Immunohistochemistry Procedure:
Paraffin sectioned slides are incubated in hot air oven at 60°c for 30 mins. Deparaffinise the tissue sections in xylene I and II for 30 mins - each 15 minutes. Wash in isoprophyl alcohol I and II for 10 minutes - two changes. Put the slides in distilled water for 15 minutes. Add distilled water in cooker and preheat (sim) - 5 minutes. Place slides inside the TRIS EDTA buffer and keep it inside cooker - wait for 2 minutes. Cool down in wash basin till the pressure lessens. Cool down under fan for 10 - 15 minutes. Distilled water - 5 minutes - 2 changes. Wash buffer 1minute - 2 changes. Wipe the slides without disturbing the sections. Put the slides in H2O2 for 5 minutes. Wash buffer 5 minutes-each 2 changes. Wipe the slides and keep the slides on wet tissue. Add primary antibody on sections for ½ hour. Cover the slides with another slide tray. Wash buffer 5 minutes - 2 changes. Add target binder for 15 minutes. Wash buffer II 5 minutes - 2 changes. Add HRP-15 minutes. Wash buffer 5 minutes - 2 changes. Add DAB - 3 minutes. Wash with distilled water - 5 minutes (once). Hematoxylin - 20 seconds. Wash with tap water - Blueing. Xylene dip and Mount with DPX.

3. Observations and Results

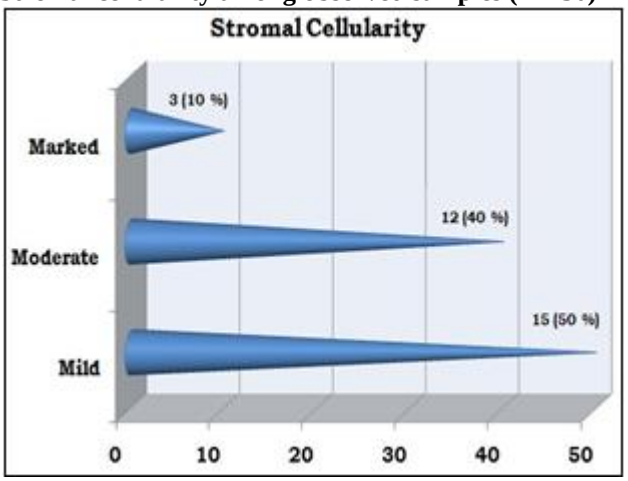
Association of Age with Histopathological Grade



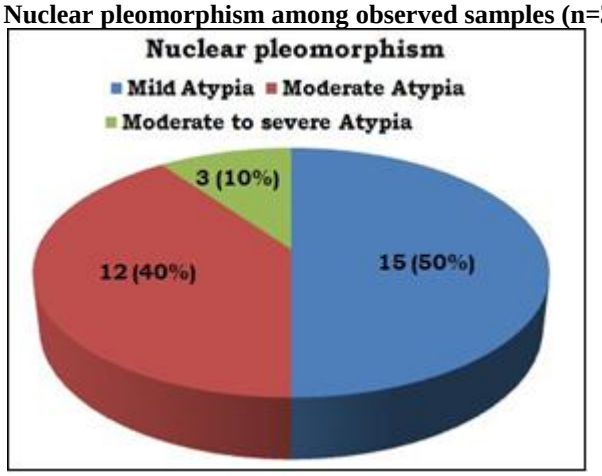
Percentage of Phyllodes Tumors in Eachhistopathological Grade:



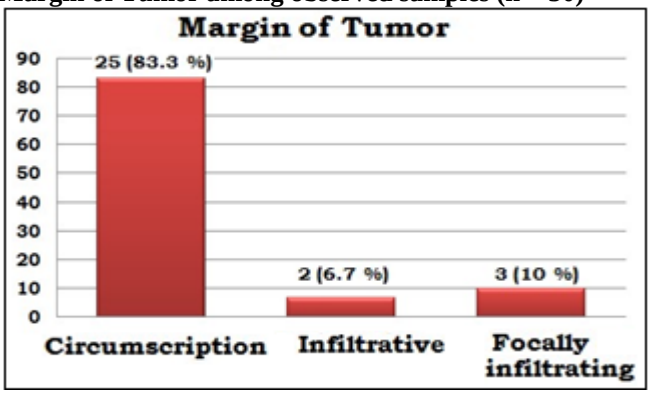
Stromal cellularity among observed samples (n = 30)



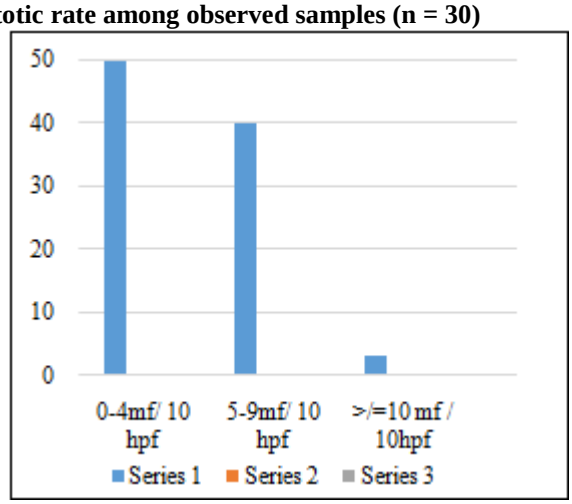
Nuclear pleomorphism among observed samples (n=30)



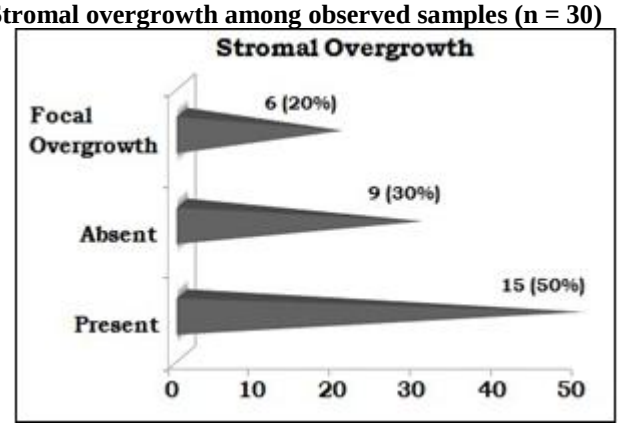
Margin of Tumor among observed samples (n = 30)



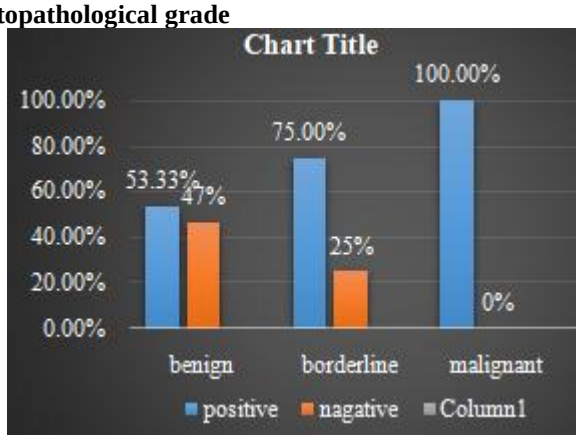
Mitotic rate among observed samples (n = 30)



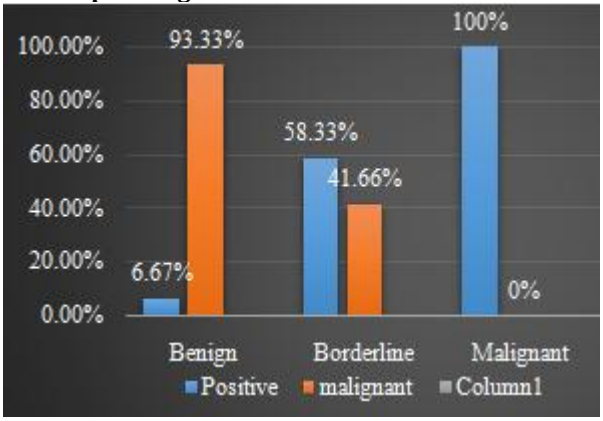
Stromal overgrowth among observed samples (n = 30)



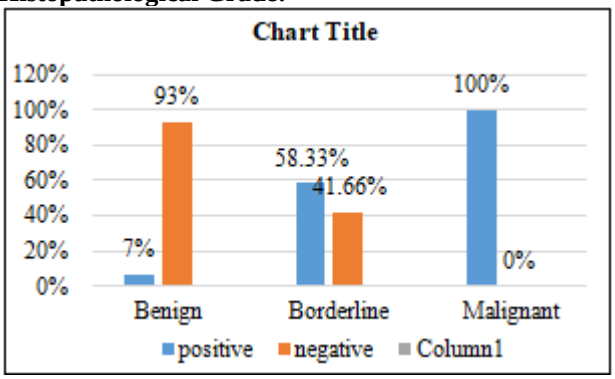
Association of stromal expression of P53 with histopathological grade



Association of CD117 Expression in Stromal Component with Histopathological Grade



Association of Ki67 Stromal Expression with Histopathological Grade:



Correlation between p53 IRS interpretation and Grade of tumor (n = 30)

Grade of tumor	p53 IRS interpretation			Test of significance (χ^2; df; p)
	Negative	Positive Mild Expression	Positive Moderate Expression	
Benign	7	7	1	12.86; 4; **0.01***
Borderline	3	6	3	
Malignant	0	0	3	
Total	10	13	7	

*p value <0.05 was considered to be statistically significant

Correlation between CD117-IRS interpretation & Grade of tumor:

Grade of tumor	CD117-IRSinterpretation			Test of significance (χ^2; df; p)
	Negative	Positive Mild Expression	Positive Moderate Expression	
Benign	14	1	0	28.86; 4; **0.0001***
Borderline	5	7	0	
Malignant	0	1	2	
Total	19	13	7	

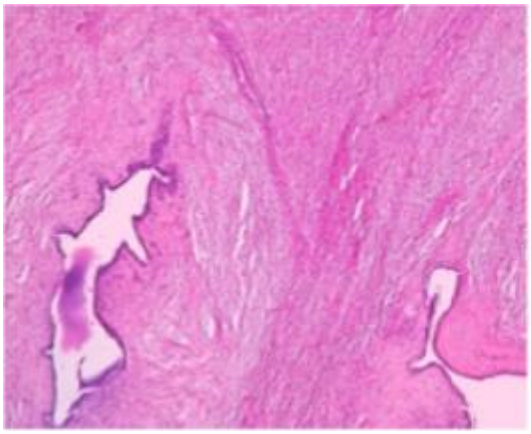
*p value <0.05 was considered to be statistically significant

Correlation between Ki67-Labelling Index% interpretation & Grade of tumor (n = 30)

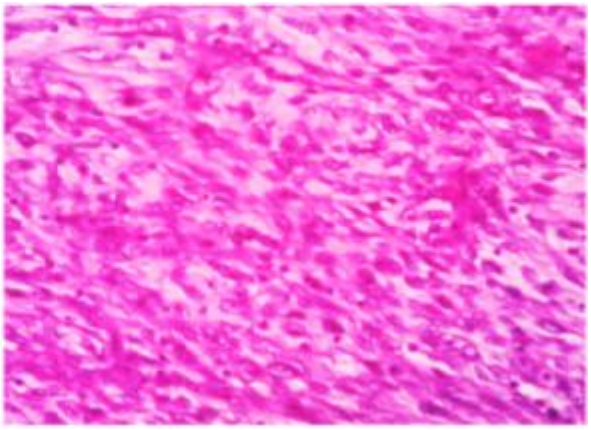
Grade of tumor	Ki67-Labelling Index%		Test of significance (c^2; df; p)
	Negative	Positive	
Benign	14	1	13.42; 2; **0.001***
Borderline	5	7	
Malignant	0	3	
Total	19	11	

*p value <0.05 was considered to be statistically significant

Colour plates



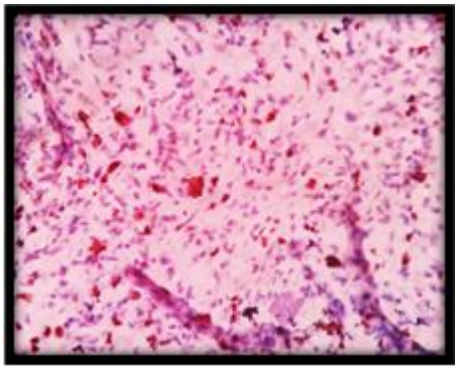
Benign phyllodestumor



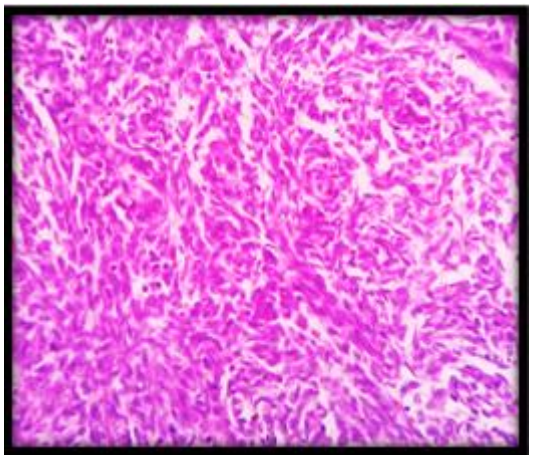
Borderline phyllodestumor

Malignant phyllodesphyllodestumor

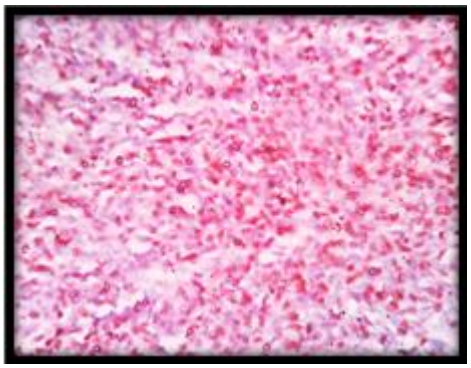
Immunohistochemistry of stromal P53 in malignant phyllodestumor (40X)



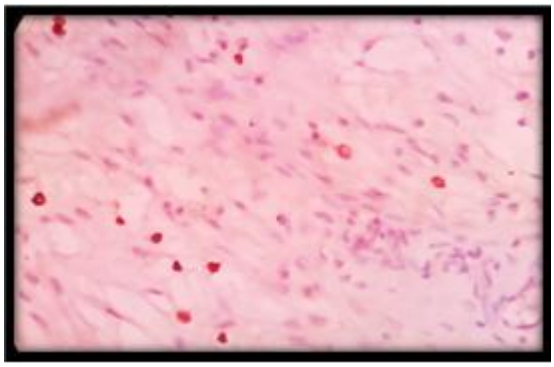
Immunohistochemistry of stromal P53 in malignant phyllodestumor (40X)



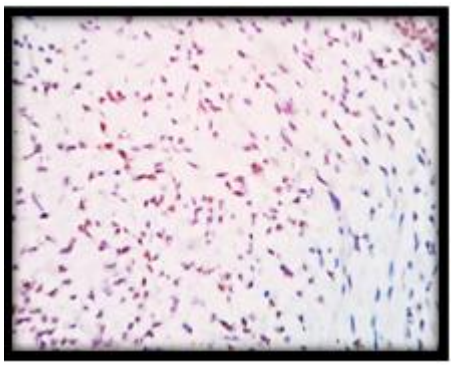
Immunohistochemistry of stromal Ki 67 in malignant phyllodestumor



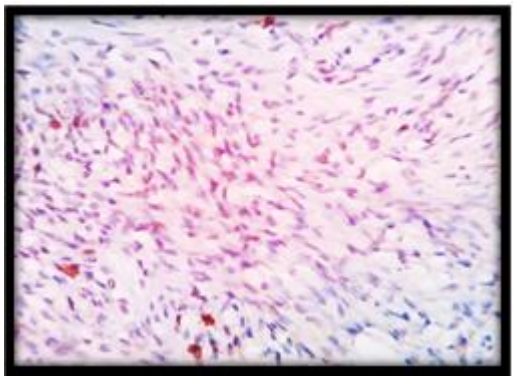
Immunohistochemistry of stromal Ki 67 in malignant phyllodestumor



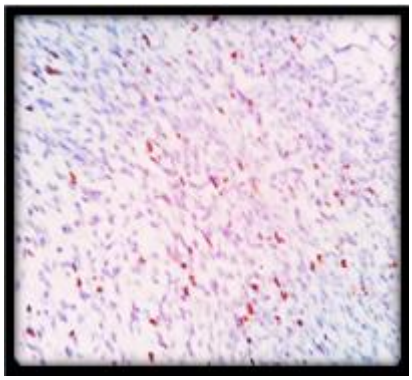
Immunohistochemistry of stromal CD117 malignant phyllodestumor (40X)



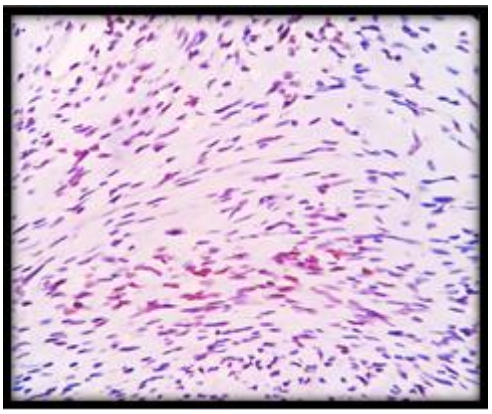
Immunohistochemistry of stromal P53 in borderline phyllodestumor (40X)



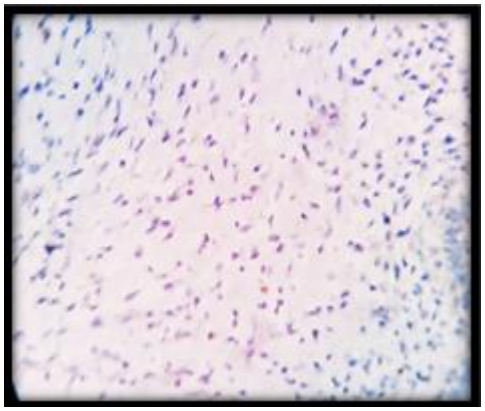
Immunohistochemistry of stromal CD117 in borderline phyllodestumor (40X)



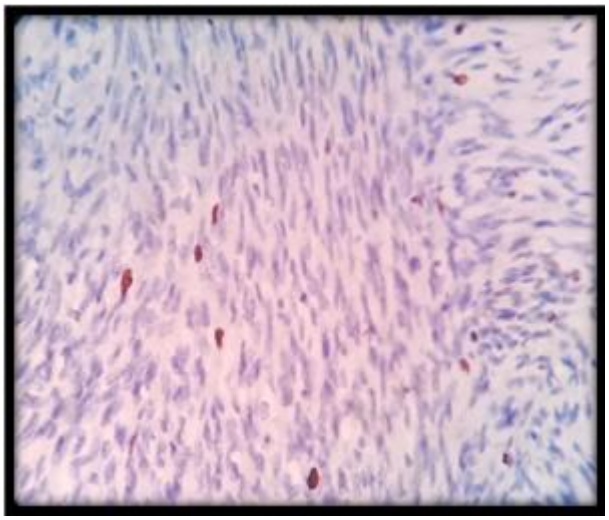
Immunohistochemistry of stromal ki67 in borderline phyllodestumor



Immunohistochemistry of stromal P53 in benign phyllodestumor (40X)



Immunohistochemistry of stromal CD117 in benign phyllodestumor (40X)



Immunohistochemistry of stromal Ki67 in benign phyllodestumor (40X)

4. Discussion

The phyllodes tumor accounts for 0.4% of all neoplasms and 4% of all breast neoplasms reported in department of pathology, KAPV government medical college, Trichy.

The age group of phyllodes tumor in the present study was between 20-60 years.

Histopathological grading of phyllodes tumor was done based on stromal cellularity, stromal overgrowth, nuclear pleomorphism, mitotic rate / 10 hpf and margin of tumor.

The common incidence of benign phyllodes tumors was in the age group 41-50 years. The occurrence of borderline phyllodes tumors was common in the age group 41-50 years, malignant phyllodes tumors wascommon in the age group between 51-60 years.

The mean age of phyllodes tumor was 46 years. The most frequent mean occurrenceof benign phyllodes tumors was 46 years, borderline phyllodes tumors was 44 years and malignant phyllodes tumors was 54 years.

In our study, the incidence of benign phyllodes tumor was 53%, borderline phyllodes tumor was 37% and malignant phyllodes tumor was 10%.

P53 immunohistochemistry comparision with various studies:

The results of various studies have been summarized and compared with our findings in the table that follows

Study	No. of cases	Benign phyllodes tumor	Borderline phyllodes tumor	Malignant phyllodes tumor
Kleer et al.4	20	2/7 (29%)	4/7 (57%)	3/6 (50%)
Kucuk, Ulku, et al.5	26	2/15 (13%)	NA	4/9 (44%)
Rivero et al.3	146	5/ 110 (4.5%)	8 /16 (50%)	15/ 20 (75%)
Our study	**30**	**8/15 (53.33%)**	**9 /12 (75%)**	**3/3 (100%)**

As shown in above table, studies done by Kleer et al[4], Kucuk et al[5] and Rivero et al[3] have shown that there is increasing P53 expression is associated with increasing grades of phyllodes tumor. Similarly, in our study also there is increasing expression of P53 as a grade increases from benign to borderline and malignant categories.

Grade of tumor	p53 IRS interpretation			Test of significance (χ^2; df; p)
	Negative	Positive Mild Expression	Positive Moderate Expression	
Benign	7	7	1	12.86; 4; **0.01***
Borderline	3	6	3	
Malignant	0	0	3	
Total	10	13	7	

*p value <0.05 was considered to be statistically significant

As shown in above table, in our study there is statistically significant difference in expression between borderline and malignant phyllodes tumor compared to benign phyllodes tumor and also between malignant and borderline phyllodes tumor (P value = 0.01).

C117 Immunohistochemistry comparision with various studies:

Study	No. of cases	Benign hyllodes tumor	Borderline phyllodes tumor	Malignant phyllodes tumor
Chen et al[6]	19	1/7 (14.3%)	NA	9/12 (75%)
Sawyer et al[7]	30	1/20 (5%)	NA	5/10 (50%)
Tse et al[8]	179	17/101 (17%)	12/50 (24%)	13/28 (46%)
Carvalho et al[9]	19	6/13 (46.2%)	NA	6/6 (100%)
Tan et al[10]	273	7/206 (3.4%)	4/41 (9.8%)	6/26 (23.1%)
Esposito et al[11]	30	2/16 (13%)	5/8 (63%)	4/6 (67%)
Our study	30	1/15 (6.67%)	7/12 (58.33%)	3/3 (100%)

As shown in above table, studies done by chen at al[6], Sawyer et al[7], Tse et al[8], Carvalho et al[9], Tan et al[10], Espsito et al[11] have shown that increasing CD117 expression is associated with increasing grades of phyllodes tumor. Similarly, in our study also there is increasing expression of CD117 as a grade increases from benign to borderline and malignant categories.

Grade of tumor	CD117-IRSinterpretation			Test of significance (c^2; df; p)
	Negative	Positive Mild Expression	Positive Moderate Expression	
Benign	14	1	0	28.86; 4; **0.0001***
Borderline	5	7	0	
Malignant	0	1	2	
Total	19	13	7	

*p value <0.05 was considered to be statistically significant

As shown in above table, in our study there is statistically significant difference in expression between borderline and malignant phyllodes tumor compared to benign phyllodes tumor and also between malignant and borderline phyllodes tumor (P value = 0.0001).

Ki 67 expression is > 10% in one benign case (6.67%) and negative in 14 benign cases. In borderline phyllodes tumor, 7 out of 12 cases have >10 % expression (58%).

In malignant phyllodes tumor, 3 out of 3 cases have more than 10% ki67 expression.

Our study showed that increasing Ki 67 labelling index was associatedwith increasing grades of phyllodes tumor. Studies done by Kocova et al.1[2]showed high Ki67-positivity in malignant phyllodes than in benign phyllodes tumor. Elaborate studies done by Ridgway et al.1[3] showed significantly lower mean Ki67 index in benign Phyllodes tumors (33.31 ± 6.73) than in malignant Phyllodes tumors (76.42 ± 38.55) (p = 0.007) and our findings correlated with them.

Grade of tumor	Ki67-Labelling Index%		Test of significance (x^2; df; p)
	Negative	Positive	
Benign	14	1	13.42; 2; **0.001***
Borderline	5	7	
Malignant	0	3	
Total	19	11	

*p value <0.05 was considered to be statistically significant

A shown in above table, in our study there is statistically significant difference in Ki67 expression between borderline and malignant phyllodes tumor compared to benign phyllodes tumor and also between malignant and borderline phyllodes tumor (P value = 0.001).

Expression of IHC markers compared with different histopathological grade

Category	P53 (IRS)		CD117 (IRS)		Ki 67 (LI%)
	Positive, weak expression	Positive, moderate expression	Positive, Weak expression	Positive, moderate expression	Positive (>/=10%)
Benign	7	1	1	0	1/15
Borderline	6	3	7	0	7/12 (<30%)
Malignant	0	3	1	2	3/3 (>/= 30%)

Differentiation between malignant and borderline phyllodes tumor:

As shown in above table, all 3 cases of malignant phyllodes tumor show positive moderate expression of P53.3out of 12 cases of borderline phyllodes tumor also show positive moderate expresion of P53. 2 out of 3 malignant phyllodes tumor cases show positive moderate expression, but none of the borderline cases show positive moderate expression for CD117. Ki67 is positive and >/= 30% in all 3 malignant phyllodes tumor cases.

7 out of 12 cases of borderline phyllodes tumor show positive Ki67 expression and all 7 cases show <30% Ki67 expression.

Based on these findings, a combination of Ki 67 (>/= 30% positivity) and CD117 (positive moderate expression) can be used in conjunction with conventional histopathology to accurately differentiate borderline and malignant phyllodes tuimor in ambiguous cases.

Differentiation between benign and borderline phyllodes tumor:

As shown in above table, 7 out of 12 cases of borderline phyllodes tumor show ki67 positivity, but only one benign phyllodes tumor show Ki67 positivity.

3 out of 12 cases of borderline tumor show positive moderate expression of P53 and 6 out of 12 borderline cases show positive weak expression of p53.

1 out of 15 cases of benign phyllodes tumor show positive moderate expression and 7 out of 15 cases of benign phyllodes tumor cases show positive weak expression of p53.

7 out of 12 cases of borderline phyllodes tumor show positive weak expression of CD117, but only one case of benign phyllodes tumor show positive weak expression of CD117.

Based on these findings, Ki67 alone or in combination with CD117 (positive weak expression) can be used to differentiate benign and borderline tumors in ambiguous cases.

5. Summary and Conclusion

The conventional histopathology is often used to differentiate benign, borderline and malignant phyllodes tumors in many times, but in few cases we unable to conclude definitely the grades especially in borderline and malignant categories. Hence there is definite need of immunohistochemical markers to differentiate phyllodes tumor accurately in ambiguous cases, so that appropriate management can be derived.

- Our results showed a significant association with increased p53 IRS values among the borderline and malignant tumor groups as compared to the benign tumor group. . P53 Immuno-Reactivity Scores (IRS) was significantly higher in the malignant group as compared to the borderline group. (p value 0.01)
- Our results showed a significant association with increasing Ki67-Labelling Index% (LI) values among the borderline and malignant tumor groups as compared to the benign tumor group. Ki67-Labelling Index% (LI) values was significantly higher in the malignant group as compared to the borderline group. (p value 0.001)
- Ours results showed a significant association with increased CD117-IRS values among the borderline and malignant tumor groups as compared to the benign tumor group. CD117-Immuno-Reactivity Scores (IRS) was significantly higher in the malignant group as compared to the borderline group. (P value 0.0001)

The final outcome of our study showed that immunohistochemical markers P53, CD117, Ki 67 were mildly expressed in benign tumors. Malignant and borderline phyllodes tumors showed increased expression of P53, CD117 and Ki 67.

Our study concludes that a combination of Ki 67 (>/= 30% positivity), CD117 (positive moderate expression) and P53 (positive moderate expression) can be used in conjunction with conventional histopathology to accurately differentiate borderline and malignant phyllodes tumor in ambiguous cases.

Ki67 alone or in combination with CD117 (positive weak expression) can be used in grey areas to differentiate benign and borderline tumors.

A larger study of phyllodes tumor would be useful to accurately evaluate the significance of IHC markers with respect to clinical outcome, biological behaviour, tumor progression and targeted therapy in CD117 (CKIT) positive borderline and malignant phyllodes tumors.

References

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