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Study of IHC Expression of Ki-67 and P53 in Phyllodes Tumor in Correlation with Its Histological Grading

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Abstract

The incidence of phyllodes tumor is 1.5% and it is said to be 1 in 100,000 women which makes it a rare fibroepithelial neoplasm, as per other olden studies. The peak of phyllodes tumor occurs in women aged 45 to 49 years. Phyllodes tumor (PT) is basically a biphasic breast tumor that is composed of cellular spindle stroma with epithelial elements but the stromal element is regarded as the neoplastic component. Accurate and reproducible grading of these tumors into benign, borderline and malignant is challenging, and by the virtue of the importance of assessment of multiple histological parameters, certain markers contribute to a streamlined diagnosis.

The aims and objectives are to study the IHC expression of Ki-67 and p53 in phyllodes tumor in correlation with its histological grading, calculate the frequency of benign, borderline and malignant lesion of phyllodes tumor. The study can also determine the association of Ki67 and p53, grade and stage the tumors for therapeutic and prognostic purpose. This is retrospective and a prospective study of breast lesions conducted in a tertiary care hospital from 2017 to 2022 over a period of 60 months with sample size of 30 cases. The study included 11 benign cases, 9 borderline cases, and 10 malignant cases. Expressions of Ki-67 and p53 were evaluated on all the cases and compared in these three categories. We found the mean p53 and Ki67 increased significantly from benign to malignant lesions. In our study, we observed a wide range in age distribution which was between 20-64 years with mean age of 46.4±10.8years. Majority of phyllodes tumor patients fell between 41 to 50 years. A positive correlation was noted between cases with high p53, high Ki 67 and rate of recurrence in the lesion.

Keywords: Immunohistochemistry, phyllodes tumor, benign, borderline, malignant

1. Introduction

Regardless of its rarity which accounts to less than 1% of all breast neoplasm, Phyllodes tumor has a malignant fatal streak. The tumor is rarely found in adolescents and the elderly. The histogenesis of phyllodes tumor is believed to arise de novo, basically from intralobular stroma, involving the interaction between epithelial and stromal component of the breast tissue. Phyllodes tumor (PT) is basically a biphasic breast tumor that is composed of cellular spindle stroma with epithelial elements but the stromal element is regarded as the neoplastic component and, consequently, as the determinant of clinical behavior (Tan *et al*. 2016; Feakins *et al*. 1999).

Accurate and reproducible grading of these tumors is challenging, and by the virtue of the importance of assessment of multiple histological parameters, certain markers contribute to a streamlined diagnosis.

The interobserver variability in the diagnosis of borderline phyllodes tumor is high. The marker of proliferation, Ki67 has emerged as an important marker due to its important role in neoadjuvant radiotherapy in addition to its prognostic value. Staining for p53 was statistically significant in differentiating between benign, borderline and malignant phyllodes tumor. Both these markers showed a higher expression in the stromal component emphasizing that it is the stroma which undergoes a malignant change.

This study will help clinicians to plan the treatment protocol i.e. targeted therapy. Complete surgical resection is the established treatment for breast PT, since residual PT at the excision margins is a strong predictor of local recurrence; however there is a consensus that benign PTs benefit from more conservative treatment.

Primary objectives are to study of IHC expression of Ki-67 and p53 in phyllodes tumor in correlation with its histological grading. The aims and objectives of this study are:

- To explore the correlation of histopathological grade with IHC result.
- To calculate the frequency of benign, borderline and malignant lesion of phyllodes tumor.
- To determine the Ki67 protein expression in phyllodes tumor
- To determine the p53 protein expression in phyllodes tumor
- To determine the association of Ki67 and p53, grade and stage the tumors for therapeutic and prognostic purpose.

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This is retrospective and a prospective study of breast lesions conducted in a tertiary care hospital from 2017 to 2022 over a period of 60 months with sample size of 30 cases.

2. Materials and Methods

During this period the surgically resected specimens were included in the study.

Inclusion Criteria: All the mastectomy, lumpectomy, wide local excision and biopsy specimens received in department of pathology for histopathology.

Exclusion Criteria: Benign, inflammatory and malignant epithelial lesions of the breast will be excluded.

Grossing Steps for a Mastectomy Specimen

i). Mastectomy specimens may be orientated by the surgeon, e.g. by placing a suture in the axillary tail. If not oriented anatomically orient the specimen by placing axillary contents laterally.

ii). Measurements: Note the dimensions of the specimen, skin and breast parenchyma. Describe the skin surface for previous scars, puckering, peau d'orange, ulceration, ipsilateral satellite nodules etc. Nipple and areola are carefully examined for retraction, inversion or ulceration. These findings are important to note since tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules) changes the stage of the tumor as pT4.

iii). Fixation: Mastectomy specimens should not be allowed to fix intact without incision of the tumor as the loss of tissue antigenicity and autolysis in a poorly fixed mastectomy is profound due to presence of skin. The time of fixation should be no less than 6 hours and not more than 72 hours before processing as underfixation of breast tissue may lead to false-negative oestrogen receptor (ER) results and over fixation may lead to false positive HER2neu results. Sectioning: Ink the deep resection plane (i.e.) the base of resection. The breast is dissected by a series of parallel incisions through posterior Breast surface into 1cm slices. Identify the tumor.

iv). Tumor examination: Document tumor dimensions, quadrant, consistency, borders, necrosis, hemorrhage and distance from overlying skin/nipple and also from the base of resection.

v). Take a disc of nipple and serially section in longitudinal or coronal sections.

vi). Adjacent breast examination: Examine all the quadrants of the breast to look for satellite nodules, multifocality, multicentricity and fibrocystic changes. Carefully look for micro calcifications in and around the main tumor. Sample any grossly seen abnormality. There are no margins in a mastectomy except base.

vii). Axillary node dissection

Principle for immunohistochemistry staining is localization of antigens in tissue sections using specific primary antibodies. Once the antibody-antigen binding occurs it is recognized by a universal secondary antibody formulation conjugate to an enzyme-labeled polymer. The polymer complex is then visualized with an appropriate substrate/chromogen. Sample type taken for IHC is paraffin embedded tissue sections and cell block.

3. Results & Discussion

Table 1: Distribution of age of study subjects.

Age group	Frequency	Percentage
< 30 years	3	10%
31 to 40 years	6	20%
41 to 50 years	11	36.7%
51 to 60 years	8	26.7%
61 to 70 years	2	6.7%
Total	30	100%

Table 2: Distribution of laterality of breast lesions

Laterality	Frequency	Percentage
Left	21	70%
Right	9	30%
Total	30	100%

Table 3: Symptoms presentation among study groups.

Symptoms	Frequency	Percentage
Lump	23	76.7%
Recurrence	2	6.7%
Others	5	16.7%
Total	30	100%

Table 4: Presence of pain in our study.

Pain	Frequency	Percentage
Present	10	33.3%
Absent	20	66.7%
Total	30	100%

Table 5: Duration of disease in our study.

Duration	Frequency	Percentage
< 5years	7	23.3%
5 to 10 years	8	26.7%
10-15 years	3	10%
>15 years	12	40%
Total	30	100%

Table 6: Radiodiagnosis of breast lesions in our study.

Radiodiagnosis	Frequency	Percentage
Fibroadenoma	13	43.3%
Neoplastic etiology	3	10%
Multilobulated lesion	1	3.3%
Phyllodes	13	43.3%
Total	30	100%

Table 7: Biopsy findings among study population.

	Frequency	Percentage
Benign	14	46.7%
Borderline	1	3.3%
Malignant	10	33.3%
Total	25	100%

Table 8: Specimen type distribution

Specimen type	Frequency	Percentage
L	11	36.7%
M	15	50%
WLE	4	13.3%
Total	30	100%

Table 9: Distribution of size of breast lesion

Size (Cms.)	Frequency	Percentage
<5 cms.	8	26.7%
5 to 10 cms.	5	16.7%
10 to 15 cms.	7	23.3%
15 to 20 cms.	4	13.3%
>20 cms.	6	20%
Total	30	100%

Table 10: Distribution of tumor borders in our study.

Tumor Borders	Frequency	Percentage
Infiltrating	10	33.4%
Pushing	16	53.3%
Irregular	4	13.3%
Total	30	100%

Table 11: Distribution of various gross appearances in our study.

Surface	Frequency	Percentage
Nodular	15	50%
Leaf like	8	26.7%
Smooth	5	16.7%
Ulcerative	2	6.7%
Total	30	100%

Table 12: Distribution of mitosis in our study.

Mitosis (per 10 hpf)	Frequency	Percentage
< 5	11	36.7%
5 to 10	10	33.3%
>10	9	30%
Total	30	100%

Table 13: Distribution of diagnosis in our study.

Diagnosis	Frequency	Percentage
Benign	11	36.7%
Borderline	9	30%
Malignant	10	33.3%
Total	30	100%

Table 14: Association of various factors with diagnosis of breast lesions.

		Benign (n=11)	Borderline (n=9)	Malignant (n=10)	P value
Age in years	< 30	2 (18.2%)	0	1 (10%)	0.085
	31 to 40	3 (27.3%)	1 (11.1%)	2 (20%)	
	41 to 50	2 (18.2%)	7 (77.8%)	2 (20%)	
	51 to 60	4 (36.4%)	1 (11.1%)	3 (30%)	
	61 to 70	0	0	2 (20%)	
Laterality	Left	6 (54.5%)	7 (77.8%)	8 (80%)	0.418
	Right	5 (45.5%)	2 (22.2%)	2 (20%)	
Symptoms	Lump	10 (90.9%)	8 (88.9%)	5 (50%)	0.123
	Recurrence	0	0	2 (20%)	
	Others	1 (9.1%)	1 (11.1%)	3 (30%)	
Pain		1 (9.1%)	4 (44.4%)	5 (50%)	0.115
Discharge		1 (9.1%)	0	1 (10%)	1
Duration in years	< 5	2 (18.2%)	2 (22.2%)	3 (30%)	0.888
	5 to 10	2 (18.2%)	3 (33.3%)	3 (30%)	
	10-15	2 (18.2%)	1 (11.1%)	0	
	>15	5 (45.5%)	3 (33.3%)	4 (40%)	
Size (Cms.)	<5	5 (45.5%)	2 (22.2%)	1 (10%)	0.322
	5 to 10	1 (9.1%)	2 (22.2%)	2 (20%)	
	10 to 15	4 (36.4%)	1 (11.1%)	2 (20%)	
	15 to 20	1 (9.1%)	2 (22.2%)	1 (10%)	
	>20	0	2 (22.2%)	4 (40%)	

Table 15: Association of IHC expression of p53 and Ki67 with WHO grades.

WHO grading	P53	Ki67
Benign	1.64±3.6	4±2.9
Borderline	58.11±4.6	15.3±3.4
Malignant	83.2±4.7	43.6±15.3
P value	<0.001	<0.001

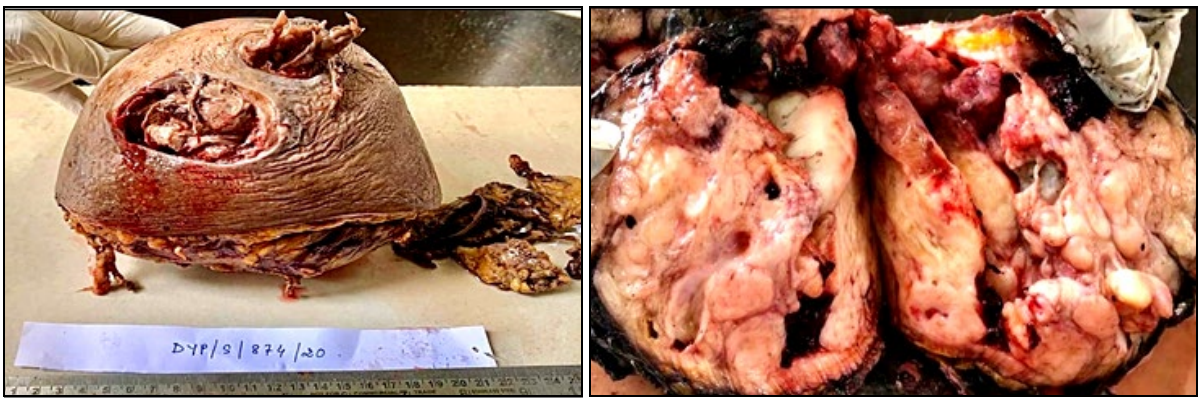

Fig 1: Skin ulceration in a malignant phyllodes specimen and cut section

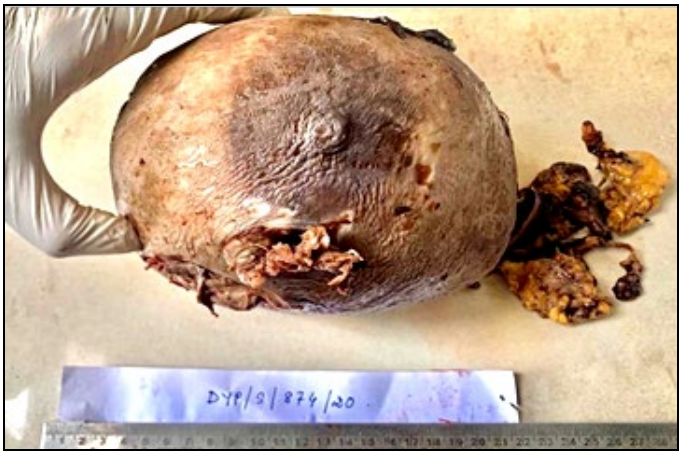

Fig 2: Characteristic whorled pattern with curved clefts resembling leaf buds

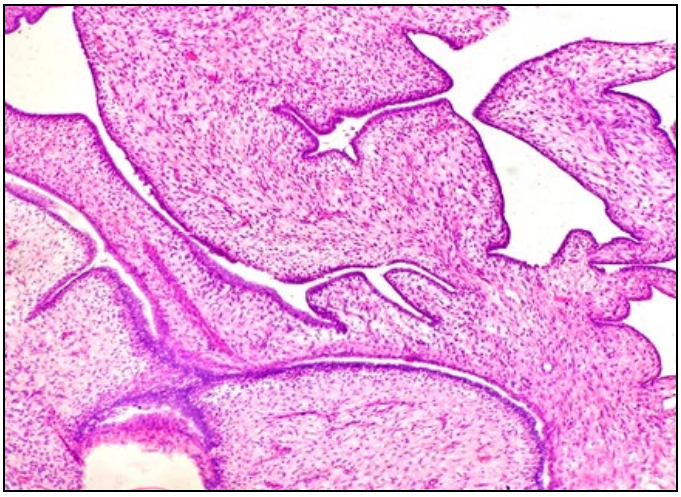

Fig 3: Stromal atypia in a malignant phyllodes tumor with atypical mitosis

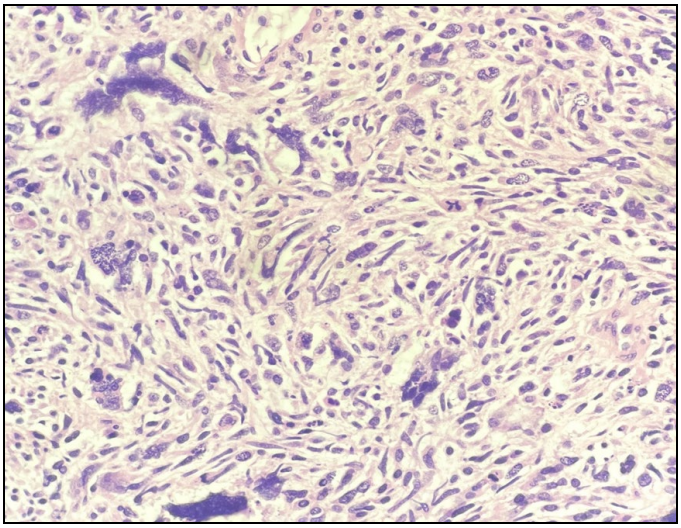

Fig 4: Stromal atypia in a malignant phyllodes tumor with tumor giant cells

Discussion

The most frequent cancer among Indian women is now breast carcinoma. In addition to having a high prevalence, these cases also have a higher mortality rate than the general western population. The study participants' average age was 46.4 ±10.8 years. The majority of patients (36.7%) were between the ages of 41 and 50. Sarrio *et al*. studies indicated that 53 years was the average age of breast cancer patients. 71 These results were comparable to those of the Sweeny *et al*.72 study, which included a larger number of patients in their fifth to seventh decade of life. According to Swasti Shubham *et al*.73, individuals with benign, borderline, and malignant PT had mean ages of 36.0 years, 38.5 years, and 48 years, respectively. According to Vani *et al*.74, the age range was 24-64 years, with a mean age of 38.2 years. In their investigation, Rivero *et al*.6 used a mean age of 44.7 years. The bulk of our patients were benign (36.7%), followed by malignant (33.3%), and 30% were borderline. 50% of Shubham, *et al*. 73 study's lesions were benign, and 50% were cancerous. Ali *et al*.76 relationships were 73.7% benign, 15.3% borderline, and 10.5% malignant. Compared to Moore *et al*.77 study's which revealed that most of the instances in

their study had a more significant infiltrating margin as compared to the pushing margins, our study had roughly 53.3% pushing borders, 33.4% infiltrating borders, and 13.3% irregular borders. We had majority with 10 in 30% cases. Ali *et al*.76 had 77.2% 9 mitosis per 10hpf. Tumor sizes in the current study ranged from 2 to 38 centimetres, with an average of 13.1 centimetres, which is consistent with average sizes reported in the literature. 78 In benign, borderline, and malignant tumours, the average tumour size is 8.03 cm, 13.8 cm, and 18 cm, respectively. According to Vani *et al*.74, the average tumour size ranged from 2-26 cm, and the mean tumour sizes for benign, borderline, and malignant tumours were 6.9 cm, 9.3 cm, and 19.7 cm, respectively. The biological behaviour of PT is unpredictable and has the potential to proceed to malignancy as well as reoccur. These tumors exhibit a diverse spectrum of clinicopathological characteristics, from benign lesions that blend in with fibroadenomas to malignant forms that overlap with fibrosarcomas. Malignant PT is defined by PT's unpredictable biological activity, which has the potential for both malignant progression and recurrence. These tumors exhibit a diverse spectrum of clinicopathological characteristics, from benign lesions that blend in with fibroadenomas to malignant forms that overlap with fibrosarcomas. Epithelial interaction is expected to have a significant role in the growth and malignant transformation of PT in malignant PT. Therefore, the pathophysiology of PT is reflected in the parameters used to evaluate malignancy. It has been discovered that borderline and malignant PT have a stronger correlation with a number of clinical variables, including older age and larger lesions. The use of IHC markers in PT for subtyping and diagnosis has been investigated. The stroma, which is hypothesised to develop from periductal stromal cells, is the neoplastic component of PT. This is corroborated by the observations that the stroma of PT reacts positively to vimentin, inconsistently to actin, CD34, and desmin, and negatively to S-100 protein. The stromal cells of PT are constituted of cells with characteristics of both fibroblasts and myofibroblasts, according to their ultrastructure. These stromal cells mimic the typical stromal cells in the mammary gland. Proliferating cell nuclear antigen and Ki 67 immunostaining did not demonstrate any appreciable distinctions between PT and fibroadenoma in the investigation by Kaya *et al*. Some writers have claimed that the markers Ki 67, p53, and c-kit are helpful in separating benign from malignant PT in circumstances when the diagnosis is challenging. The grade of PT was observed to correspond with the cellular proliferation marker Ki 67 in the current investigation. Other studies also published findings that were comparable. The Ki 67 labelling index in the literature is 5%-25% in benign and 15%-100% in malignant PTs. This score ranged from 1 to 9% in benign PT patients, 12% to 22% in borderline PT cases, and 32% to 73% in malignant PT cases in our study, indicating an overlap between malignant and borderline instances. Kleer *et al*. detected a minor overlap at both high and low Ki 67 labelling indices, and they reported similar findings. Esposito *et al*. also reported mean Ki 67 labelling indices of 3.6%, 26%, and 32% for benign, borderline, and malignant PTs, respectively. Additionally, both of these groups discovered that the Ki 67 labelling index was unable to predict clinical behaviour or recurrences. According to Vani *et al*., the average Ki 67 expression was 4.5% (range 1-8% and 14% (range 13-15% in BPT and BlPT, respectively. With an average LI of 56%, MPT showed a Ki 67 range of 41-80%. In BPT and MPT, respectively, Gatalica *et al*. reported mean Ki 67 of 7.73 and 23.42.

Comparison of Ki67 with other Studies

In line with other earlier research that found high p53 expression rates were linked to rising tumour grade, p53 expression dramatically increased from benign to malignant tumours. The difference in expression between the malignant/borderline and benign groups was statistically significant, as was the situation with Ki 67. In addition, Tan *et al*. found that a fraction of the luminal epithelium and myoepithelial cells in their series of PTs were p53 immunohistochemistry positive. In other publications, stromal staining and p53 reactivity in the breast epithelium were both mentioned. The discovery of the p53 protein's dual expression in the breast's stroma and epithelium emphasises the potential of a reaction between these two elements.

4. Summary

- The mean age of 46.4 years was noticed with highest distribution between 41 to 50 years (36.7%).
- The tumor was more commonly seen in the left breast (70%) as compared to right breast.
- Majority of the patients presented with lump in the breast (76.7%).
- The lesion was not associated with pain in most of the patients (66.7%).
- 6.7% of the patients complained of discharge associated with the lesion.
- Major part of the patients had this breast lesion chronically, since >15 years.
- On correlation with histopathology, radiodiagnosis showed a just distribution between fibroadenoma and phyllodes tumor (43.3%).
- Biopsy assessment showed correlation in majority of the cases.
- 50% of the specimen types were mastectomy specimens.
- Majority of the breast lesions were
- 53.3% of the tumors had pushing borders while 33.4% had infiltrating border with most of them having grey white appearance.
- 63.3% of the tumors were firm in consistency.
- The tumors predominantly had a nodular surface (50%) followed by leaf like in 26.7% cases.
- Most of the cases (36.7%) showed
- Coinciding with the same, 36.7% of the cases were benign.
- IHC marker, p53 is increased significantly from benign to malignant lesions.
- Similarly, Ki-67 is increased significantly from benign to malignant lesions.

5. Conclusion

In our study, we observed a wide range in age distribution which was between 20-64 years with mean age of 46.4±10.8years. Majority of phyllodes tumor patients fell between 41 to 50 years. Majority of the breast cancer studied were present chronically for more than 15years with predominantly absence of pain and discharge. Based on the histopathological findings, benign phyllodes tumor was more common than the other histological subtypes. On radiological investigations, many histologically proven cases of phyllodes tumor were misdiagnosed as fibroadenoma. Hence, histopathological examination is gold standard in diagnosing

phyllodes tumor and other breast lesions and including multiple histopathological parameters and tumor subclassification prevents misdiagnosis. The biological behavior of phyllodes tumor was noted to be unpredictable with potential to reoccur. We found the mean p53 and Ki 67 increased significantly from benign to malignant lesions. A positive correlation was noted between cases with high p53, high Ki 67 and rate of recurrence in the lesion. On the basis of the above conclusions, we determined that Ki 67 and p53 is important for grading and staging of tumor and can make significant changes in management and prognosis of the disease aiding in better patient care.

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