Original Article

Immunohistochemical Expression of KI-67 and P53 in Different Grades of Phyllodes Tumors of the Breast and its Association with Clinicopathological Characteristics

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Abstract

Objective: This study aimed to investigate the immunoreactivity of Ki67 and p53 in Phyllodes tumors (PTs) of the breast and assess their correlation with clinicopathological features, including PT grade and clinical parameters.

Methodology: A comparative, analytical cross-sectional design was employed to evaluate Ki67 and p53 immunoreactivity in the Phyllodes tumors. The research was conducted at the Histopathology Department, DDRRL/DUHS, Karachi, over an 8-month period. Ethical approval was also obtained from DUHS's board. Purposive nonprobability sampling was used to select the patients. Immunohistochemistry, performed on FFPE tissue blocks, involved evaluating Ki67 and p53 staining. Statistical analysis (descriptive, chi-squared, and Kruskal Wallis tests) was carried out using IBM SPSS version 26, and a p-value <0.05 was considered statistically significant.

Results: The research involved 50 patients, comprising benign 19 (38%), borderline 23 (46%), and malignant 08 (16%) PTs. Malignant tumors exhibited significantly higher expression of Ki67 and p53 compared to benign and borderline tumors ($p < 0.005$). Clinicopathological parameters, such as tumor mobility, skin ulceration, tumor borders, leaf-like architecture, stromal overgrowth, necrosis, and surgical margins, showed significant associations with Ki67 and p53 expression ($p < 0.005$). These results recommend that Ki67 and p53 may serve as valuable diagnostic and prognostic markers in PTs.

Conclusion: The study's results indicate that the immunohistochemical evaluation of Ki67 and p53 could be beneficial in the diagnosis of PTs. The association between Ki67 and p53 expression and various clinicopathological features, particularly PT grade, highlights their potential clinical significance. Further research could contribute to the advancement of standardized diagnostic and prognostic criteria for PTs, improving patient management and outcomes.

Keywords: Phyllodes Tumor, p53 and Ki67

Introduction

Phyllodes tumors (PTs) of the breast are neoplasms characterized by a unique biphasic histological composition, encompassing benign epithelial elements and fibrosarcomatous stroma. This distinctive morphology was first documented in 1838 by Johannes Müller, laying the foundation for subsequent research into these rare breast tumors.[1,2]

Despite their infrequent occurrence, PTs have garnered significant attention in the medical community due to their unpredictable clinical behavior and the diagnostic challenges that they pose in the confirmation of diagnosis in phyllode tumors that have overlapping morphological features.[3,4] While they represent a small fraction of all breast neoplasms (approximately 0.3-1.5%), they present a unique clinical conundrum, straddling the boundary between benign and malignant tumors.[5]

PTs exhibit variations in clinical behavior, particularly among different ethnic groups. Asian populations tend to develop these tumors at a younger age and have a relatively higher relapse rate in comparison to non-Asian populations.[6,7] The complex histogenesis of PTs, arising de novo from ductal and lobular stromal components and driven by intricate interactions between epithelial and stromal elements, adds another layer of complexity to their diagnosis and management.[6]

While the histological criteria for PT classification—benign, borderline, and malignant—have served as the primary diagnostic framework, distinguishing between these categories can remain challenging.[8] This challenge is further compounded by the overlap in sonographic features between PT and the more common fibroadenoma, often necessitating histopathological examination for definitive diagnosis.[1] Differentiating between benign PT and cellular fibroadenoma, which share similar microscopic features except for minimal stromal cellularity in the former, has proven to be a diagnostic challenge.[9]

Malignant transformation in PTs, though not considered a diagnostic criterion, is often associated with features such as the presence of heterologous elements and necrosis.[10] To provide more clarity, the World Health Organization (WHO) classifies PTs into three primary categories based on five major histological features: stromal cellular atypia, stromal hypercellularity, stromal overgrowth, mitotic count, and margin characteristics.[10] These distinctions are crucial in guiding therapeutic decisions and predicting outcomes.[8,10]

Recurrence, a significant concern in the management of PTs, is defined by the reappearance of a tumor of the same histological type in the same quadrant. Positive margins or incomplete surgical excisions are recognized as contributors to recurrence.[11] High mitotic activity has also been linked to an increased risk of recurrence, while metastatic capability appears to be associated with stromal hypercellularity rather than stromal overgrowth, cytological atypia, tumor necrosis, or the presence of heterologous elements.[1]

In the quest to improve the diagnostic accuracy of PTs and refine prognostication, researchers have explored the role of immunohistochemistry (IHC). Among the various biomarkers examined, two have emerged predominantly: Ki67 and p53.[12-14]

Ki67, a non-histone nuclear protein, plays a pivotal role in cell proliferation. It serves as a nuclear antigen expressed during the cell cycle, except in the resting phase (G0). Ki-67 is vital in determining the rate of cell division and is particularly significant in cancer research. Its expression in cancer cells helps differentiate rapidly dividing cancer cells from non-cancerous or slow-growing ones.[15] Elevated Ki67 indices correlate with increased tumor aggressiveness as rapidly dividing cells are typically associated with malignancy, aiding histological grading and prognostication.

On the other hand, p53, encoded by the TP53 gene, serves as a critical tumor suppressor gene situated on chromosome 17p13.1. This gene encodes a nuclear phosphoprotein with a molecular weight of approximately 53 kDa. In normal cells, p53 is present at low levels and serves essential roles in regulating the normal cell cycle and the process of apoptosis, which is programmed cell death. Through its functions, p53 helps maintain the integrity of the genome and prevent the uncontrolled proliferation of damaged or abnormal cells, making it a key factor in safeguarding against the development of cancer. When p53 is mutated or dysfunctional, it can lead to the loss of these regulatory mechanisms, contributing to the formation and progression of various types of cancer. Serves as a potent tumor suppressor, regulating cellular responses to stress and DNA damage. When p53 is functioning normally, it helps prevent the development of cancer by halting the growth of cells with damaged DNA. However, mutations or alterations in the p53 gene can lead to the loss of this suppressor function, allowing abnormal cells to proliferate unchecked.[12,16] This study aimed to find the immunoreactivity of Ki67 and p53 in the context of PTs and to correlate their expression in relation to clinicopathological features.

Methodology

The study employed a comparative analytical cross-sectional design to investigate the immunoreactivity of Ki67 and p53 in relation to clinicopathological features of Phyllodes tumors (PT) of the breast. The research was conducted at the Histopathology Department, Dow diagnostic, Research reference lab, DUHS, Karachi.The study was conducted over a period of 8 months.

Purposive nonprobability sampling was the chosen technique for sample selection. The study variables in-

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cluded a dependent variable, which was the presence of Phyllodes tumor in patients, and independent variables, such as age and the nature of surgery. Ethical approval was obtained from the Institutional Review Board (IRB) of DUHS, Karachi, (Ref #IRB-2887/DUHS/Approval/2023/71)ensuring the protection of patients' identities and confidentiality.

Sample collection:

The sample collection process adhered to ethical standards, beginning with obtaining informed consent from female patients following ethical approval taken from DUHS. Inclusion criteria encompassed female patients with Phyllodes tumors who underwent resection procedures (excluding core biopsies) and willingly agreed to participate in the study. Exclusion criteria included poorly fixed tissues, inadequately sectioned or stained histological slides, requisition forms lacking essential clinical information, benign lesions such as fibroadenomas, patients diagnosed with breast carcinoma, and those who had undergone core biopsies. Breast specimens were grossed according to international grossing protocols. Hematoxylin and Eosin (H&E) stained slides were reviewed by consultant pathologists from the institution, who were devoid of knowledge regarding the pathological report or any clinical data. The cases were diagnosed as benign, borderline, and malignant PTs according to WHO classification (17), based on histopathologic parameters, including stromal cellularity and atypia, stromal overgrowth, mitotic count, and tumor border characteristics. A diagnosis of benign PT case is made when the tissue shows low cellularity, no stromal overgrowth, mild pleomorphism, a rounded margin, and a mitotic count of ≤2/10 high-power fields. Malignant PT is diagnosed when the mitotic count is ≥5/10 high-power fields, along with stromal overgrowth and an infiltrative margin. Borderline PT is identified when the criteria for malignant PT are not fully met. (17-19) The findings were matched with the previous reports of the patients for subsequent analyses. After a thorough review, IHC was performed.

Immunohistochemical analysis and scoring:

Immunohistochemistry was performed on the recent cases of PT using 4-µm tissue sections from formalin-fixed paraffin-embedded (FFPE) blocks containing the highest density of tumor cells, covering an area of at least 1x1cm for each PT case. The process involved tissue preparation, antigen retrieval, and blocking, utilizing Ready-to-Use primary antibodies (DAKO Omnis platform), specifically FLEX Monoclonal Mouse Anti-Human p53 Protein (Clone DO-7) and FLEX Monoclonal Mouse Anti-Human Ki67 Antigen (Clone MIB-1). Subsequently, a secondary antibody (HRP powder) was applied, followed by visualization of the results using chromogenic substrates. Skilled medical technologists managed these steps to ensure accuracy. Additionally, positive as well as negative controls were included in each batch to maintain quality standards.

Stained sections were considered positive based on the presence of unequivocal nuclear staining, irrespective of staining intensity. The Ki-67 index was scored according to the International Ki67 in Breast Cancer Working Group (IKWG), low index (<5% positive cells) and high index (>30% positive cells). (20) For the evaluation of p53 IHC staining, the intensity of neoplastic stromal nuclear staining was scored, ranging from negative (0), weak staining (1+), moderate staining (2+), and strong staining (3+). The percentage of positive cells was analyzed in 10 microscopic HPF. The categorization of the distribution of nuclear-positive cells included sporadic (positive cells < 10%), focal (positive cells ranging from 11% to 50%), and diffuse (positive cells ≥50%). Positive expression of p53 was attributed to IHC scores of 2+ and 3+ with focal to diffuse distribution, which is in line with the criteria reported previously. (21, 22) The data was entered and analyzed by using IBM SPSS version 26.0. Descriptive statistics, such as means, standard deviations, and frequencies, were calculated to summarize the clinicopathological variables of the study. The chi-squared test was run to examine the associations between the immunoreactivity of Ki67 and p53 and categorical clinicopathological features. The Kruskal-Wallis analysis reinforced the comparison of the means of p53 and Ki67 expressions with PTs since the data was non-parametric. A statistically significant result was determined by a p-value of less than 0.05.

Results

The study included a total of 50 patients with fibroepithelial breast tumors; the benign PTs accounted for 19 (38.0%) cases, the borderline category constituted the largest proportion, encompassing 23 (46.0%) samples, and the malignant cases comprised 8 (16.0%) tumors. The clinicopathological characteristics of the patients and tumors are presented in Table 1. Patients with malignant tumors exhibited the highest mean age (42.5 ± 15.3 years), older than those with benign (29.5 ± 10.8 years) and borderline (38.3 ± 11.1 years) tumors. Tumor size, measured in centimeters, was observed to increase with malignancy, with malignant cases having the largest mean size (7.6 ± 1.4 cm), compared to borderline (6.3 ± 1.5 cm) and benign (4.0 ± 0.86 cm) cases. Regarding laterality, the distribution of tumor laterality varied: 36.8% of benign, 56.5% of borderline, and 50% of malignant PTs were on the right side, while the remaining cases were found on the left side.

The clinical and histopathological characteristics are shown in Table 2. Significant associations were observed between tumor grade and the presence of mobility (p 0.006), skin ulceration (p < 0.001), tumor borders (p = 0.013), leaf-like architecture (p < 0.001), stromal overgrowth (p < 0.001), necrosis (p < 0001), and the presence of heterologous elements (p = 0.037). However, non-significant associations were found between tumor grade, the absence of pain (p = 0.317),

with Esophageal Variceal Grade.

and the completeness of surgical margins (p = 0.087).

Expressions of p53 and Ki67 across various clinicopathological parameters complemented by corresponding p-values were shown in Table 3. Tumors presenting with skin ulceration, absence of leaf-like architecture, stromal overgrowth, and necrosis exhibited elevated expression levels of p53 ($p < 0.05$). In terms of Ki67, a significant association was observed solely with tumors featuring stromal overgrowth (p=0.019).

The expressions and association of p53 and Ki67 across different tumors were presented in Table 4 and Figure 1 (A-I). The p53 revealed a predominance of sporadic staining (68.42%) within benign cases, while malignant were primarily observed with diffuse staining (>50% cells) at 62.50%. Similarly, for Ki67 expression according to IKWG protocol, benign tumors mainly had a low index (68.42%), while borderline (82.6%) and malignant tumors (75%) displayed a higher percentage of cases with a high index. Both p53 and Ki67 expressions showed statistically significant differences across PTs ($p < 0.05$).

Table 1. Child-Pugh Turcotte Class

Age in years (Mean± S.D)		**35.64 ± 12.55**
Size in cm (Mean± S.D)		**5.74 ± 1.94**
Laterality (n, %)	Right	24 (48%)
	Left	26 (52%)
Grades of tumor (n, %)	Benign tumor	19 (38%)
	Borderline tumor	23 (46%)
	Malignant tumor	8 (16%)

Table 2. Association of Clinicopathological Characteristics of Lesions with Phyllodes Tumor

Clinicopathological Characteristics		Benign PT	Borderline PT	Malignant PT	P-Value
Mobility of tumor	Present	18	17	3	0.006
	Absent	1	6	5	
Skin Ulceration	Present	0	3	5	0.000
	Absent	19	20	3	
Pain	Present	8	15	4	0.317
	Absent	11	8	4	
Tumor borders	Circumscribed	18	20	4	0.013
	Permeative	1	3	4	
Leaf-like architecture	Present	19	22	4	0.000
	Absent	0	1	4	
Stromal overgrowth	Present	0	14	7	0.000
	Absent	19	9	1	
Heterologous elements	Present	1	0	2	0.037
	Absent	18	23	6	
Necrosis	Present	0	0	8	0.000
	Absent	19	23	0	
Surgical margins	Completely excised	17	16	8	0.087
	Not completely excised	2	7	0	

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Table 3. Association of p53 and Ki67 with Clinicopathological characteristics of Tumors

		p53				Ki67		
		Sporadic (0%-10% cells)	Focal (11%-50% cells)	Diffuse (>50% cells)	p-value	Low index (<5% cells)	High index (>30% cells)	p-value
Mobility of tumor	Present	18	15	5	0.206	15	23	0.490
	Absent	3	5	4		4	8	
Skin Ulceration	Present	1	2	5	0.002	4	4	0.351
	Absent	20	18	4		15	27	
Pain	Present	10	13	4	0.438	8	19	0.152
	Absent	11	7	5		11	12	
Tumor borders	Circumscribed	18	18	6	0.273	17	26	0.342
	Permeative	3	2	3		2	5	
Leaf-like architecture	Present	21	19	5	0.001	17	28	0.638
	Absent	0	1	4		2	3	
Stromal over-growth	Present	5	9	7	0.022	4	17	0.019
	Absent	16	11	2		15	14	
Heterologous elements	Present	1	2	0	0.549	1	2	0.680
	Absent	20	18	9		18	29	
Necrosis	Present	1	2	5	0.002	2	6	0.342
	Absent	20	18	4		17	25	
Surgical margins	Completely excised	18	16	7	0.836	18	23	0.068
	Not completely excised	3	4	2		1	8	

Table 4. Pattern of p53 and Ki67 in Phyllodes tumor of Breast

		Benign PT	Borderline PT	Malignant PT	P-Value
p53	Sporadic (0%-10% cells)	13	7	1	0.001
	Focal (11%-50% cells)	5	13	2	
	Diffuse (>50% cells)	1	3	5	
Ki67	Low index <5% cells	13	4	2	0.002
	High index >30% cells)	6	19	6	

Discussion

Phyllodes tumors constitute a distinctive category of breast neoplasms characterized by their fibroepithelial composition. The stromal element plays a pivotal role in determining the clinical characteristics and behavior of these tumors. The disruption of the equilibrium between the epithelium and stroma is believed to be a critical factor in both the development and malignant transformation of PTs.

p53, located on chromosome 17p13.1, is a critical tumor suppressor gene regulating cell cycle and apoptosis, guarding against cancer development. Mutations disrupt its functions, promoting cancer formation.[23] Ki-67, expressed during active cell cycle phases, aids in determining cell division rates, which is crucial in cancer research for distinguishing rapidly dividing cancer.[15]

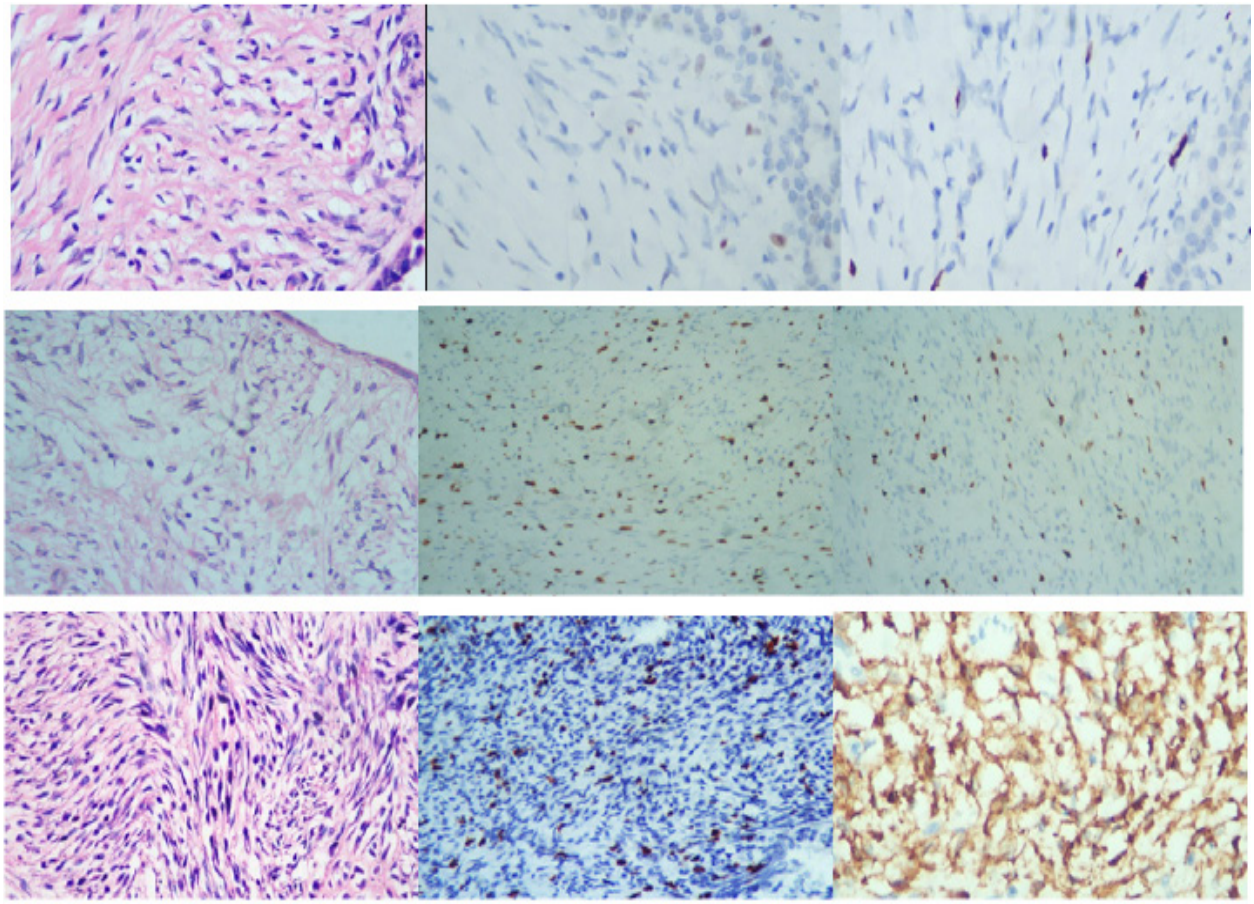

Figure 1: Photomicrograph of Phyllodes Tumor at 40x magnification

A. Benign phyllodes tumor (hematoxylin-eosin staining)

B. Sporadic p53 staining in benign phyllodes tumor (1%-10% cells positive)

C. Low index Ki67 staining in benign phyllodes tumor (<5% cells positive)

D. Borderline phyllodes tumor (hematoxylin-eosin staining)

E. Focal p53 staining in borderline phyllodes tumor (11%-50% cells positive)

F. High index Ki67 staining in borderline phyllodes tumor (>30% cells positive)

G. Malignant phyllodes tumor (hematoxylin-eosin staining)

H. Diffuse p53 staining in malignant phyllodes tumor (>50% cells positive)

I. High index Ki67 staining in malignant phyllodes tumor (>30% cells positive)

The literature showed inconsistent findings regarding the age of the patients and the grades of PTs. Some studies consistently linked older age with malignant PTs, while others, such as those by Alkushi et al.[24], Atalay et al.[25], and Cabioğlu et al.[26], reported occurrences of malignant PTs in younger patients. Our study aligned with previous research, as the mean ages of our patients - 42.5 years for malignant PTs, 29.5 years for benign, and 38.3 years for borderline tumors - were consistent with these previous findings.[14,22,27,28] Our study findings showed an association between tumor size and the specific subtypes of Phyllodes tumors (PTs). Malignant PTs were consistently found to be larger in size compared to benign and borderline PTs, a pattern supported by previous research.[28-31] However, few studies haven't shown these observations, possibly due to differences in sample sizes and patient characteristics.[26,32]

While the distribution of tumor laterality in this study showed variability, with benign, borderline, and malignant tumors distributed across both sides, most of the previous literature hasn't emphasized laterality as a key factor in PT. The primary focus in the literature has been on age, tumor size, and histopathological characteristics.[12,14,22,24]

Our findings regarding p53 expression in different PTs align with some aspects of the existing literature on

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this subject. Comparable to previous literature such as studies by Ali et al.[12], Shubham et al.[14], Zhang et al. [33], Rivero et al.[22], and Kucuk et al.[34], we observed a strong statistical association between p53 positivity and malignant PTs, emphasizing the diagnostic value of p53 expression in characterizing these tumors. Furthermore, the association between p53 expression patterns and the intensity of the reaction is consistent with previous findings[22,33], underlining the potential diagnostic significance of not only the positivity but also the expression pattern and intensity of p53. Our study demonstrates that similar to p53, Ki67 expression is correlated with PT grading, mirroring the trends observed by Pednekar et al.[35], Yuan et al.[36], Shubham et al.[14] and Ali et al.[12]

The absence of a statistically significant correlation between tumor mobility and p53 expression is consistent with some prior research. However, a significant association with Ki67 expression suggests that cellular proliferation, as indicated by Ki67, may be linked to the tumor's mobility. This aligns with studies that have suggested a relationship between Ki67 and tumor aggressiveness and invasiveness. Statistical associations between leaf-like architecture, stromal growth, and necrosis with the expressions of p53 have also been reported by some researchers similar to our results.[12, 34, 37] Case reports of giant benign phyllodes tumors published by Fernández et al.[38] and Zhang et al.[39] reported the absence of necrosis and stromal overgrowth. Our study also found an absence of the above-mentioned characteristics in the majority of lesions.

In conclusion, the present study shows that p53 and ki67 immunohistochemical stains could be helpful in differentiating overlapping morphological features between benign, borderline, and malignant phyllode tumors. We identified significant associations between tumor subtypes and various clinical and histopathological features, such as larger tumor size and distinct architectural patterns in malignant cases. Ki67 and p53 expressions were associated with tumor subtype, with malignant tumors showing higher and more widespread staining, while benign and borderline tumors exhibited lower or sporadic expression. These findings suggest the relevance of these markers in the diagnosis and management of phyllodes tumors, emphasizing the need for standardized assessment criteria.

LIMITATIONS:

There are few limitations of our study. The study's sample size was relatively small, and it was conducted exclusively in a single center, potentially limiting the generalizability of our findings. Incorporating larger and more diverse centers could offer a broader perspective on the diagnostic utility of Ki-67 and p53 in breast phyllodes tumors.

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