

Comparison of histopathological and immunohistochemical features of fibroadenoma and low-grade phyllodes tumor

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ABSTRACT

Objective: To evaluate the utility of immunohistochemical (IHC) markers-CD10, CD117, Ki-67, and p53-in differentiating fibroadenoma (FA) from low-grade phyllodes tumors (LGPTs) in breast biopsies.

Methods: This cross-sectional study was conducted at Dow Diagnostic Reference and Research Laboratory (DDRRL), Karachi, from July to December 2023, following ethical approval (IRB-3052/DUHS/Approval/2023/222). A total of 94 breast biopsies (47 FA and 47 LGPTs) were included. Samples with invasive carcinoma, incomplete data, or lack of consent were excluded. Histological diagnosis was made on H&E-stained paraffin sections, followed by IHC analysis using CD10, CD117, Ki-67, and p53 markers. Expression was evaluated using an immunoreactivity score based on staining intensity and percentage of positive cells. Expression intensity was categorized into four levels: +3 (strong), +2 (moderate), +1 (weak), or 0 (no expression). Statistical analysis was performed using SPSS version-26.0.

Results: Mean age was 31.7 ± 9.2 years for FA and 35.2 ± 10.8 years for LGPTs, with significantly larger tumor size in LGPTs (6.4 ± 3.8 cm) than FA (3.3 ± 1.9 cm). Morphologically, stromal hypercellularity, nuclear atypia, and leaf-like architecture were predominantly noted in LGPTs. Immunohistochemically, LGPTs showed higher moderate-to-strong expression of p53 (17 vs. 8 cases, $p=0.043$), Ki-67 (13 vs. 4 cases, $p=0.050$), CD117 (19 vs. 8 cases, $p=0.024$), and CD10 (8 vs. 1 case, $p=0.046$), compared to FA. LGPTs consistently exhibit higher proportions of moderate to strong stromal expression across all evaluated markers.

Conclusion: Immunohistochemical markers CD10, CD117, Ki-67, and p53 demonstrate significant differential expression between FA and LGPTs. Their combined use can enhance diagnostic accuracy in distinguishing fibroepithelial breast lesions.

Keywords: Fibroadenomas (MeSH); Phyllodes Tumors (MeSH); Tumor Suppressor Protein p53 (MeSH); Ki-67 Antigen (MeSH); CD117 (MeSH); CD10 (MeSH); Nephrilysin (MeSH); Immunohistochemistry (MeSH).

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consist of well-defined ductal structures with minimal cellular atypia. Microscopically, FA are well-circumscribed and encapsulated, with a balanced stromal-to-epithelial ratio.³ In contrast, LGPTs demonstrate distinct features, including hypercellular stroma, elongated ducts arranged in a characteristic leaf-like pattern, mild to moderate cytologic atypia, increased mitotic activity, and pushing borders. Although LGPTs are less aggressive than high-grade PTs, accurate diagnosis and management remain critical due to their recurrence potential.⁴

Recent advancements in immunohistochemistry have significantly improved diagnostic accuracy for fibroepithelial lesions, enabling differentiation between FA and LGPTs and offering insights into their biological behavior. Key immunohistochemical markers include CD10, CD117, Ki-67, and p53.^{1,5-7} CD10, a cell surface neutral endopeptidase also known as the common acute lymphoblastic leukemia antigen (CALLA), has been investigated for its utility in distinguishing FA from LGPTs. CD10 expression is often higher in the stromal component of LGPTs, correlating with higher histological grades. Its ability to highlight stromal differences between these lesions makes it a valuable diagnostic adjunct.⁸⁻¹⁰ CD117, or c-kit, is a membrane-bound tyrosine kinase receptor that has garnered attention for its potential role in LGPTs. Research indicates that CD117 is frequently expressed in the stromal cells of malignant PTs and may be associated with disease recurrence.^{11,12} Additionally, its therapeutic relevance, as demonstrated

INTRODUCTION

Fibroepithelial lesions of the breast, encompassing fibroadenomas (FA) and phyllodes tumors (PTs), present diagnostic challenges due to overlapping histopathological features. Fibroadenomas are benign and represent the most common breast tumors in women, whereas low-grade phyllodes tumors (LGPTs) are rare, accounting for approximately 0.3% to 1% of all breast tumors.¹ A key clinical challenge lies in distinguishing these entities accurately,

as their therapeutic approaches differ significantly. FA generally require conservative management or excision for symptomatic relief, while LGPTs, particularly those of higher grades, necessitate more aggressive treatment due to their potential for local recurrence.²

Histologically, FA are characterized by a biphasic architecture comprising stromal and epithelial components. The stromal component exhibits varying proportions of collagenous and myxoid stroma, while the epithelial elements

in gastrointestinal stromal tumors (GISTs), underscores its potential utility in prognostic assessments and treatment planning for LGPTs.¹³ Ki-67, a nuclear protein linked to cell proliferation, has been widely studied for its correlation with LGPT grade. High-grade PTs exhibit elevated Ki-67 indices, reflecting increased stromal proliferation. This marker serves as a valuable adjunct in grading PTs, particularly when histological features are inconclusive.^{5,14,15} The p53 protein, a tumor suppressor gene product, has been extensively studied in breast PTs. While its exact role remains debated, some studies suggest that p53 expression in stromal cells may be associated with malignant histological features.^{5,6,16}

However, despite these advances, there remains a significant diagnostic challenge in differentiating cellular fibroadenomas from LGPTs due to overlapping morphological features and variability in marker expression. Many studies have assessed these markers individually or in small sample sizes, and regional data, particularly from South Asian populations are limited. Therefore, there is a need for comprehensive, marker-based evaluation of these lesions in a single study setting to improve diagnostic accuracy and support histopathological grading.

This study aimed to address these gaps by evaluating the immunohistochemical expression of CD10, CD117, Ki-67, and p53 in distinguishing FA from LGPTs, thereby enhancing diagnostic precision and contributing to better-informed clinical management.

METHODS

This cross-sectional study was conducted at the Histopathology Department of Dow Diagnostic Reference Research Laboratory (DDRRL) in Karachi, Pakistan, for 6 months (July-Dec 2023) following ethical approval (IRB-3052/DUHS/Approval/2023/222) from the Institutional Review Board (IRB) at DUHS, Ojha Campus.

The inclusion criteria for sample selection in this study encompassed specimens that were clinically suspected to exhibit fibroepithelial lesions, including surgically excised

breast biopsies reported in DDRRL. Furthermore, the study considered females of all age groups as eligible candidates for inclusion. Conversely, exclusion criteria comprised patients diagnosed with invasive breast carcinoma, requisition forms lacking essential patient particulars, and cases where patient consent was not obtained.

All female breast biopsies showing suspicion of fibroepithelial lesions underwent a macroscopic examination in accordance with standard International Grossing Protocols. Subsequently, initial diagnoses were established on paraffin section slides stained with hematoxylin and eosin (H & E). Cases diagnosed as FA and LGPTs were selected for further analysis through immunostaining with CD10, CD117, Ki67, and p53 antibodies (DAKO, Denmark). These slides were subsequently observed under a bright field microscope.

Cases meeting the inclusion criteria were simultaneously reviewed by two pathologists, applying the World Health Organization criteria for the diagnosis and grading of PT. The diagnosis of FA was made based on the presence of fibroepithelial lesions with unaltered stromal cellularity and the absence of well-formed leaf-like projections. Immunohistochemistry procedures were conducted on formalin-fixed, paraffin-embedded tissue sections, which underwent deparaffinization and rehydration before epitope retrieval and inactivation of endogenous peroxidase. Staining procedures were performed by a professional medical technologist using a DAKO Omnis platform.

The assessment of stained sections in this study was conducted utilizing an Olympus microscope, with detailed microscopic examination. For the immunohistochemical markers an immunoreactivity score was employed to quantify the expression levels. This score was derived by multiplying the assigned expression intensity by the corresponding marked cell score. Furthermore, the assessment of CD10, CD34, and CD117 involved a thorough examination of both membrane and cytoplasmic expressions. In contrast, the analysis of p53 and Ki-67 was

specifically focused on the nuclear compartment. The evaluation of Ki-67, p53, and CD117 extended beyond the cell type, encompassed both stromal and epithelial expressions. However, CD10 was only assessed for stromal expression. The expression intensity was categorized into four levels: +3 (strong), +2 (moderate), +1 (weak), or 0 (no expression). Simultaneously, the marked cell score ranged across four categories: 0 (<4%), +1 (5% to 33%), +2 (34% to 66%), and +3 (>66%).¹⁹

The statistical analyses for this study were performed using SPSS 26.0 software. Continuous variables, specifically the age of patients and the size of the tumor, were expressed as means along with their respective standard deviations to provide a measure of central tendency and variability. On the other hand, categorical data, which include variables such as different lesions and immunological markers, underwent analysis using the Chi-square test. A significance level of p -value < 0.05 was considered statistically significant.

RESULTS

A total of 94 cases of breast lesions were analyzed in this study, comprising an equal distribution of fibroadenomas (FA) and low-grade phyllodes tumors (LGPTs), with 47 cases each. The patients' ages ranged from 20 to 61 years. The mean age for patients with FA was 31.7 ± 9.2 years, while those with LGPTs had a mean age of 35.2 ± 10.8 years. Tumor sizes ranged from 1.5 to 15 cm overall. The mean tumor size for FA was 3.3 ± 1.9 cm, whereas LGPTs had a mean size of 6.4 ± 3.8 cm.

Regarding the characteristics, both the lesions exhibited the circumscribed borders. While stromal growth and necrosis were completely absent among samples of both lesions. The presence of a leaf-like architecture was mainly observed in LGPTs (91.5%) compared to FA (0%). Nuclear atypia, stromal hypercellularity, and mitotic activity were higher in LGPTs compared to FA. Nuclear atypia was present in 89.3% of LGPTs, with mild atypia being the most common, however, it is completely absent in FA. Stromal hypercellularity was also entirely absent

Table I: Association of immunohistochemical markers with fibroadenoma and low-grade phyllodes tumor

Immunohistochemical markers		Fibroadenoma	Low-Grade Phyllodes Tumor	Total	p-value
p53	Weak (0-2)	39	28	67	0.043
	Moderate (3-4)	7	17	24	
	Strong (≥ 5)	1	2	3	
Ki67	Weak (0-2)	43	34	77	0.050
	Moderate (3-4)	3	8	11	
	Strong (≥ 5)	1	5	6	
CD117	Weak (0-2)	39	28	47	0.024
	Moderate (3-4)	8	16	24	
	Strong (≥ 5)	0	3	3	
CD10	Weak (0-2)	46	39	85	0.046
	Moderate (3-4)	1	6	7	
	Strong (≥ 5)	0	2	2	

p53: Tumor Protein p53; Ki-67: Kiel-67 Antigen; CD117: Cluster of Differentiation 117; CD10: Cluster of Differentiation 10 (Neprilysin)

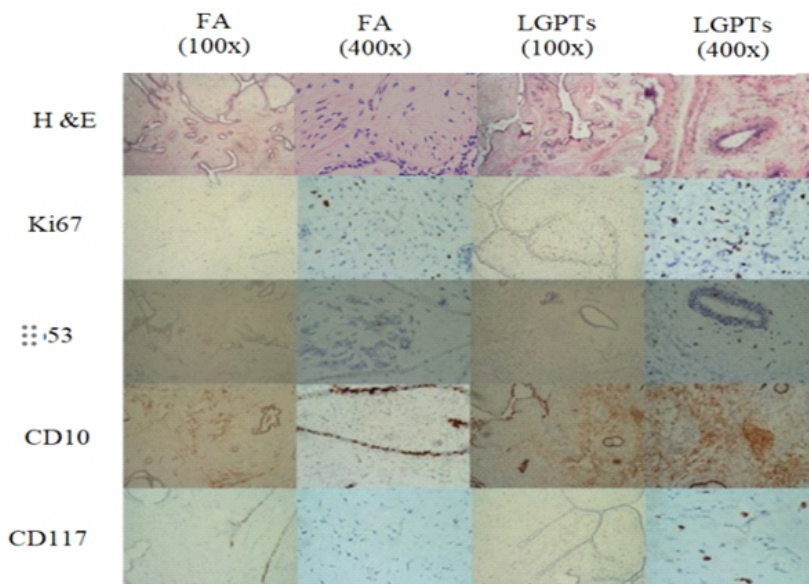


Figure 1: Histopathological and immunohistochemical features of fibroadenomas (FAs) and low-grade phyllodes tumors (LGPTs) demonstrated by H&E and IHC staining (Ki-67, p53, CD10, CD117) at 100x and 400x magnifications

in FA, although a moderate degree of hypercellularity was observed in 95.7% of LGPTs. Moreover, higher mitotic activity (5-10 mitotic cells) was detected in only 2.1% of LGPTs, whereas FA exhibited lower mitotic activity (0-4 mitotic cells). The presence of heterologous elements was only seen

2.1% of LGPTs displaying this feature, while it was absent in FA. Regarding surgical margins, LGPTs were more frequently presented with complete excision (95.7%) compared to FA (18.1%). For the histopathological marker p53, weak staining was observed in 39 cases (82.9%) of

fibroadenomas (FAs) and 28 cases (59.5%) of low-grade phyllodes tumors (LGPTs), showing a statistically significant difference ($p = 0.043$). In the case of Ki-67, weak staining was noted in 43 FAs (91.4%) and 34 LGPTs (72.3%), also demonstrating statistical significance ($p = 0.050$).

CD117 showed distinct differences in staining intensity: weak staining in 39 FAs (82.9%) versus 28 LGPTs (59.5%), and moderate staining in 8 FAs (17%) versus 16 LGPTs (34%), with a significant p-value of 0.024. Similarly, CD10 revealed weak staining in 46 FAs (97.8%) and 39 LGPTs (82.9%), and moderate staining in 1 FA (2.1%) versus 6 LGPTs (12.7%), with a p-value of 0.046 (Table I).

DISCUSSION

This study evaluated p53, Ki-67, CD10, and CD117 expressions to distinguish FAs from LGPTs. Significant differences were observed in the staining patterns of all markers, supporting their role in differentiating these morphologically similar breast lesions.

The age of our study participants ranged from 20 to 61 years, with a mean age of 31.7 years for FAs and 35.2 years for LGPTs. In comparison, Tian F, et al.,¹⁷ reported higher mean ages, 43.2 years for FAs and 42.8 years for LGPTs. Other studies have also documented a broader age range for patients with fibroepithelial lesions (FELs).^{5,18} Regarding lesion size, the mean diameter was 3.3 cm for FAs and 6.4 cm for LGPTs in our study, whereas Shubham S, et al.⁵ and Puri V, et al.,¹⁸ reported mean sizes of 7 cm and 5 cm, respectively, for LGPTs.

Our results regarding higher weak staining for all four immunohistochemical markers investigated (Ki67, CD10, CD117, and p53), in FA in comparison to LGPTs were in line with the study by Vilela MHT, et al.,¹⁹ They assessed the diagnostic utility of CD10, CD34, CD117, p53, and Ki-67 in distinguishing FAs from phyllodes tumors (PTs) of the breast. Among these, only CD117 showed statistically significant differences in epithelial and mast cell expression ($p = 0.048$ and $p = 0.001$, respectively) when differentiating FAs from LGPTs, while the remaining markers did not yield significant results.

In contrast, our study demonstrated statistically significant differences in all four markers-CD10, CD117, p53, and Ki-67-highlighting their broader diagnostic potential in distinguishing FAs from LGPTs.

Regarding CD10 expression, studies by Kulkarni MM, et al.,⁹ Puri V, et al.,¹⁸ Al-Masri M, et al.,²⁰ and reported predominantly negative or weak and patchy expression in benign PTs.

Our findings aligned with these previous studies, demonstrating negative CD10 expression in the majority of LGPTs. Similarly, Ali NAM, et al., also reported that the majority (82.4% and 94.1%) of LGPT cases exhibited negative or mild expression of p53, Ki67, and CD117 in LGPTs, which is consistent with our observations. However, it is important to note that they did not include FA in their study, limiting their assessment of these markers in the context of differential diagnosis of PT only.

In contrast to our findings, a recent study by Tian F, et al.,¹⁷ reported strong p53 expression in 66.7% and Ki-67 expression in 58.3% of LGPT cases. In our study, strong expression of p53 and Ki-67 was observed in only 4.25% and 10.6% of cases, respectively. Furthermore, Tian F, et al.,¹⁷ noted strong expression of CD10 and CD117 in 40% and 100% of FA cases, whereas our study did not identify strong expression of these markers in any FA case.

The differences in results may be due to several factors, including variations in sample size, ethnic and geographic differences in patient populations, inter-observer variability in immunohistochemical interpretation, and differences in antibody clones, staining protocols, or scoring systems employed. Additionally, tumor heterogeneity and subjective thresholds for classifying staining intensity may further contribute to these inconsistencies.

Limitations of the study

The relatively small sample size and single-center design may limit the generalizability and population diversity. Only a limited panel of immunohistochemical markers was assessed, and semi-quantitative

evaluation may have introduced inter-observer variability despite standardization efforts.

CONCLUSION

In conclusion, our study provides additional support to the existing body of literature on the immunohistochemical expression patterns of p53, Ki67, CD117, and CD10 in FA and PT of the breast. These findings provide compelling evidence supporting the role of these markers, combined with histopathological features, in improving diagnostic accuracy and patient care. Further research on larger patient cohorts is warranted to refine their clinical application and explore potential therapeutic implications.

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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

FMA, SHS, FD & ST: Acquisition, analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

LA & UB: Conception and study design, analysis and interpretation of data, critical review, approval of the final version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONFLICT OF INTEREST

Authors declared no conflict of interest, whether financial or otherwise, that could influence the integrity, objectivity, or validity of their research work.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request



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