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NEURAL EPIDERMAL GROWTH FACTOR-LIKE 1 (NELL1) EXPRESSION AND THE NOTTINGHAM PROGNOSTIC INDEX IN BREAST CARCINOMA: A CROSS-SECTIONAL STUDY FROM PAKISTAN

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ABSTRACT

Breast carcinoma remains a major cause of cancer-related morbidity and mortality, particularly in resource-limited settings where prognostic assessment relies largely on histopathology and immunohistochemistry. Neural epidermal growth factor-like 1 (NELL1) is a secreted glycoprotein with emerging but incompletely defined relevance in tumour biology. This cross-sectional observational study evaluated NELL1 protein expression in breast carcinoma and assessed its association with the Nottingham Prognostic Index (NPI) and selected clinicopathological parameters. The study was conducted at the Institute of Pathology and Diagnostic Medicine, Khyber Medical University, Peshawar, Pakistan, from May 2025 to October 2025. A total of 128 histologically confirmed breast carcinoma cases were included using consecutive sampling. NELL1 immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections, and staining was interpreted using the Allred scoring system; scores of 0–2 were considered negative, whereas scores of 3–8 were considered positive. NELL1 immunoreactivity was observed in 108 cases (84.4%), while 20 cases (15.6%) were negative. Mild, moderate, and strong staining intensities were observed in 37 (28.9%), 37 (28.9%), and 34 (26.6%) cases, respectively. NELL1 expression showed significant associations with tumour stage ($P<0.001$), lymph node involvement ($P=0.002$), and NPI category ($P<0.001$). No significant association was observed with tumour size, histological grade, surgical margin status, lymphovascular invasion, or distant metastasis. These findings suggest that NELL1 expression may be associated with adverse clinicopathological features and poorer NPI categorization in breast carcinoma, especially where expanded molecular testing is not routinely available. Further validation with molecular subtyping and outcome-based data is required.

KEYWORDS: Breast carcinoma; NELL1; immunohistochemistry; Nottingham Prognostic Index; Allred score; prognostic biomarker.

INTRODUCTION

Breast carcinoma remains one of the most frequently diagnosed malignancies among women and a leading cause of cancer-related mortality worldwide [1,2]. Although contemporary breast cancer classification increasingly integrates histomorphology with immunohistochemical and molecular features, conventional clinicopathological parameters, including tumour size, histological grade, lymph node status, and receptor profile, continue to provide essential prognostic information [3-5]. However, variability in disease behaviour among tumours with similar clinicopathological profiles highlights the need for additional biomarkers that may refine risk stratification and improve prognostic assessment. The Nottingham Prognostic Index (NPI), which incorporates tumour size, lymph node stage, and histological grade, remains a clinically relevant prognostic model for invasive breast carcinoma and has continued to be evaluated alongside newer prognostic tools and molecular assays [6-8]. Because NPI reflects key

determinants of tumour aggressiveness, identifying immunohistochemical markers associated with NPI categories may provide useful insight into tumour biology and prognostic heterogeneity.

Neural epidermal growth factor-like 1 (NELL1) is a secreted glycoprotein initially recognized for its role in osteogenesis, chondrogenesis, skeletal development, and broader disease-associated biological pathways [9,10]. Recent cancer-related studies suggest that NELL1 may have context-dependent functions, with epigenetic silence associated with advanced renal cell carcinoma and increased expression implicated in extracellular matrix remodeling and osteosarcoma progression [11,12]. In breast carcinoma, however, evidence regarding NELL1 expression remains limited, and data from South Asian populations are particularly scarce. This gap is important in Pakistan, where resource-limited pathology settings often rely on affordable, reproducible immunohistochemical markers and established clinicopathological tools for prognostic assessment. Therefore, the present study aimed to evaluate NELL1 protein expression in breast carcinoma and determine its association with NPI and other clinicopathological parameters in a resource-limited Pakistani setting.

METHODS

Study Design and Setting

This cross-sectional observational study was conducted in the Institute of Pathology and Diagnostic Medicine (IPDM), Khyber Medical University. The study duration was from May 2025 to October 2025.

Study Population and Sampling Technique

A total of 128 histologically confirmed cases of breast carcinoma were included using a non-probability consecutive sampling technique. The sample size was duration-based, and all eligible cases diagnosed during the study period were consecutively enrolled after applying the predefined inclusion and exclusion criteria.

Inclusion Criteria

All histopathologically diagnosed cases of breast carcinoma with adequate formalin-fixed paraffin-embedded tissue blocks and complete clinicopathological records were included in the study. Cases were included irrespective of patient age, histological type, tumour grade, tumour stage, lymph node status, and receptor profile, provided that representative tumour tissue was available for immunohistochemical analysis.

Exclusion Criteria

Recurrent breast carcinoma cases, post-neoadjuvant therapy specimens, poorly preserved tissue blocks, cases with insufficient tumour tissue, and cases with incomplete clinical or pathological records were excluded from the study

Clinicopathological Evaluation

Clinicopathological data, including patient age, tumour size, histological type, histological grade, tumour stage, lymph node involvement, lymphovascular invasion, surgical margin status, and distant metastasis status, were retrieved from pathology records. Tumours were staged according to the TNM classification system. Histological grading was performed using the Nottingham modification of the Bloom-Richardson grading system.

Nottingham Prognostic Index Assessment

The Nottingham Prognostic Index was calculated using the standard formula:

NPI = (0.2 × tumour size in cm) + lymph node stage + histological grade

Based on NPI scores, cases were categorized into good prognosis, moderate prognosis, and poor prognosis groups. Cases with NPI scores <3.4 were classified as good prognosis, those with scores between 3.4 and 5.4 as moderate prognosis, and those with scores >5.4 as poor prognosis.

Immunohistochemistry for NELL1

Immunohistochemical staining for NELL1 was performed on representative formalin-fixed paraffin-embedded tumour tissue sections. Sections of approximately 3-4 µm thickness were cut, mounted on poly-L-lysine-coated slides, deparaffinized in xylene, and rehydrated through graded alcohols. Heat-induced epitope retrieval was carried out using retrieval solution at pH 9.0 for 35 minutes, after which endogenous peroxidase activity was blocked.

Sections were then incubated for 20 minutes with a rabbit polyclonal anti-NELL1 primary antibody (Invitrogen, Thermo Fisher Scientific; catalogue no. PA5-98721) at a working dilution of 1:100. An HRP-based secondary detection system (Thermo Fisher Scientific) was applied, and immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) as the chromogen, followed by hematoxylin counterstaining, dehydration, and mounting.

A kidney tissue section served as the positive control, and an identically processed section with omission of the primary antibody served as the negative control. Both controls were included in every staining run. The primary antibody

manufacturer and catalogue number, dilution, retrieval conditions, and detection system are reported to support reproducibility; no breast-specific, externally standardized NELL1 scoring assay is currently established.

Evaluation of NELL1 Expression

NELL1 immunostaining was evaluated in tumour cells by assessing both staining intensity and the proportion of positive cells. Immunohistochemical interpretation was performed using the Allred scoring system. Staining intensity and proportion scores were combined to obtain the total Allred score. Total Allred scores of 0-2 were considered negative, whereas scores of 3-8 were regarded as positive for NELL1 expression. Evaluation was performed independently by two histopathologists who were blinded to the clinicopathological data and Nottingham Prognostic Index categories. Any discrepant interpretations were resolved by joint review and consensus.

Statistical Analysis

Data were analyzed using SPSS version 25. Categorical variables were presented as frequencies and percentages, whereas continuous variables were summarized as mean ± standard deviation or median with range, as appropriate. Associations between NELL1 expression and clinicopathological parameters, including Nottingham Prognostic Index categories, were assessed using the Chi-square test. A P-value <0.05 was considered statistically significant.

RESULTS

Demographic and Clinicopathological Characteristics

A total of 128 cases of breast carcinoma were evaluated. Most patients were aged 50 years or older (84/128, 65.6%), with the highest frequency observed in the >60-year age group (43/128, 33.6%). Right-sided tumours were slightly more common than left-sided tumours (67/128, 52.3% vs. 61/128, 47.7%). Most tumours measured >2 cm but <5 cm (67/128, 52.3%).

Stage II and III tumours constituted the majority of cases (91/128, 71.1%), with stage III being the most frequent stage (49/128, 38.3%). Histologically, grade II and grade III tumours predominated, together accounting for 90 cases (70.3%). Lymph node assessment showed that 65 cases (50.8%) were node-negative, while advanced nodal involvement categorized as N3 was present in 43 cases (33.6%).

Lymphovascular invasion was identified in 53 cases (41.4%), involved surgical margins in 48 cases (37.5%), and distant metastasis in 51 cases (39.8%). On the basis of the Nottingham Prognostic Index, most tumours were classified in the moderate prognostic category (79/128, 61.7%), followed by the poor (39/128, 30.5%) and good prognostic categories (10/128, 7.8%). Detailed demographic and clinicopathological characteristics are summarized in Table 1.

Table 1. Demographic and clinicopathological characteristics of breast carcinoma cases (n=128)

Variable	Category	Frequency, n	Percentage, %
Age group	20-30 years	3	2.3
	30-40 years	12	9.4
	40-50 years	29	22.7
	50-60 years	41	32.0
	>60 years	43	33.6
Breast side	Right	67	52.3
	Left	61	47.7
Tumour size	≤2 cm	39	30.5
	>2-<5 cm	67	52.3

	≥5 cm	22	17.2
Tumour stage	Stage I	17	13.3
	Stage II	42	32.8
	Stage III	49	38.3
	Stage IV	20	15.6
Histological grade	Grade I	38	29.7
	Grade II	45	35.2
	Grade III	45	35.2
Lymph node status	N0	65	50.8
	N1	9	7.0
	N2	11	8.6
	N3	43	33.6
Lymphovascular invasion	Present	53	41.4
	Absent	75	58.6
Surgical margin	Involved	48	37.5
	Not involved	80	62.5
Distant metastasis	Present	51	39.8
	Absent	77	60.2
Nottingham Prognostic Index	Good	10	7.8
	Moderate	79	61.7
	Poor	39	30.5

Footnote: Data are presented as frequency (n) and percentage (%). NPI indicates Nottingham Prognostic Index.

NELL1 Expression Pattern

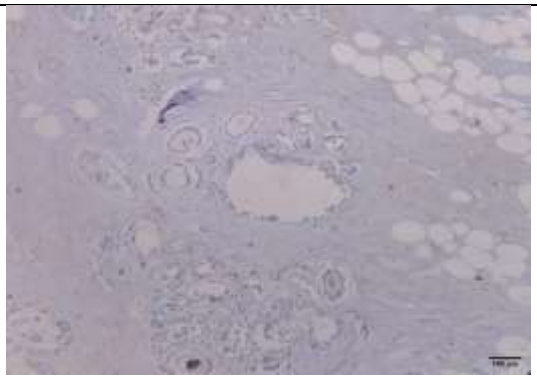
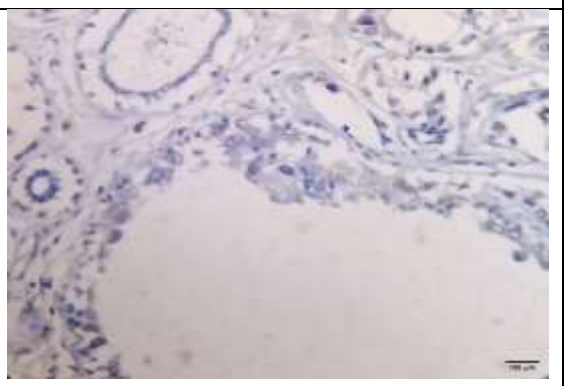
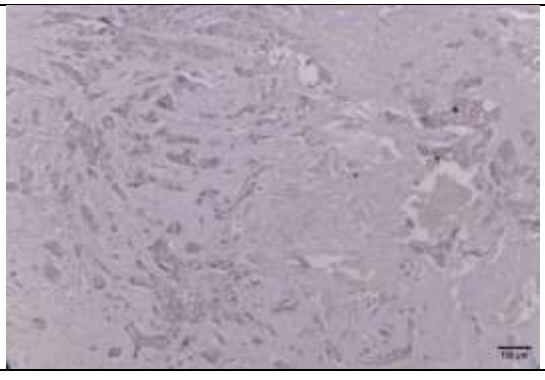
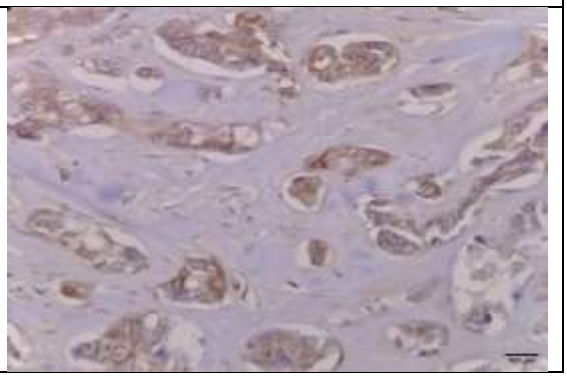
NELL1 immunoreactivity was observed in 108 of 128 breast carcinoma cases (84.4%), whereas 20 cases (15.6%) were negative. Mild and moderate staining intensities were each observed in 37 cases (28.9%), while strong staining was seen in 34 cases (26.6%) (Table 2). Representative photomicrographs showing negative, mild, moderate, and strong NELL1 immunostaining patterns at 100× and 400× magnification are shown in Figure 1.

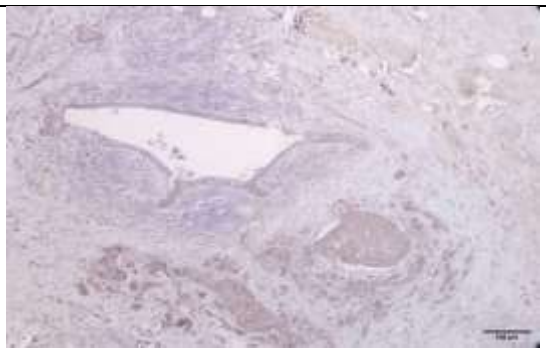
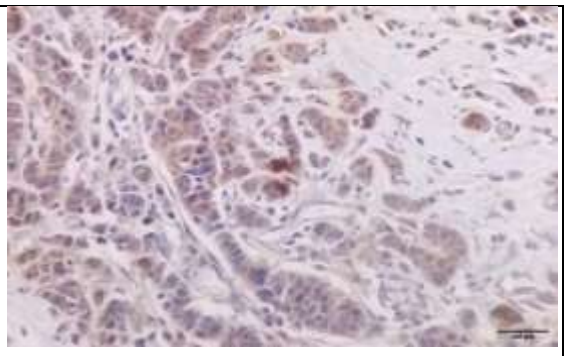
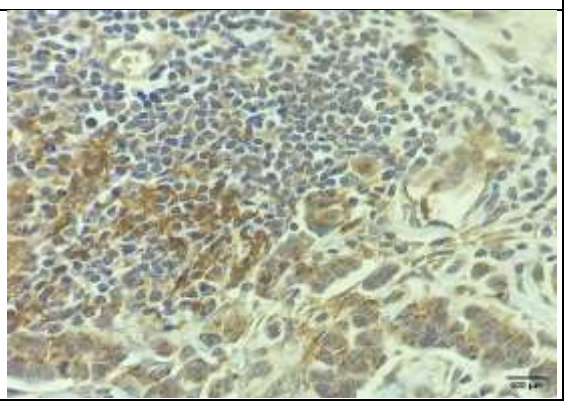
Table 2. NELL1 immunohistochemical expression pattern in breast carcinoma cases (n=128)

NELL1 staining intensity	Frequency, n	Percentage, %
Negative	20	15.6
Mild	37	28.9
Moderate	37	28.9
Strong	34	26.6
Total	128	100.0

Footnote: *NELL1 expression was evaluated by immunohistochemistry. Negative staining corresponds to Allred total scores of 0–2, whereas mild, moderate, and strong staining categories represent positive NELL1 expression with Allred total scores of 3–8. NELL1 indicates neural epidermal growth factor-like 1.*

Figure 1. Representative photomicrographs of NELL1 immunohistochemical expression in breast carcinoma showing negative staining, mild staining, moderate staining, and strong staining at 100× and 400× magnification.

Staining	Power (100x)	Power (400x)
Negative Staining		
Mild Staining		

Moderate Staining		
Strong Staining		

Association of NELL1 Expression with Clinicopathological Parameters

NELL1 expression showed no statistically significant association with tumour size (P = 0.581), histological grade (P = 0.407), surgical margin status (P = 0.355), lymphovascular invasion (P = 0.256), or distant metastasis (P = 0.513) (Table 3). In contrast, a statistically significant association was observed between NELL1 expression and tumour stage (P < 0.001). NELL1 positivity increased with advancing stage and was most frequent in stage III and stage IV tumours. A significant association was also observed between NELL1 expression and lymph node involvement (P = 0.002), with the highest frequency of positivity noted in N3 disease, where 39 of 43 cases showed positive expression (Table 3).

Table 3. Association of NELL1 expression with clinicopathological parameters in breast carcinoma cases (n=128)

Variable	Category	NELL1 negative, n	NELL1 positive, n	Total, n	P-value
Tumour size	≤2 cm	4	35	39	0.581
	>2-<5 cm	13	54	67	
	≥5 cm	3	19	22	
Tumour stage	Stage I	6	11	17	<0.001
	Stage II	14	28	42	
	Stage III	0	49	49	
	Stage IV	0	20	20	
Histological grade	Grade I	3	35	38	
	Grade II	10	35	45	0.407
	Grade III	7	38	45	
Lymph node status	N0	11	54	65	
	N1	2	7	9	0.002
	N2	3	8	11	
	N3	4	39	43	
Surgical margin	Involved	10	38	48	0.355
	Not involved	10	70	80	
Lymphovascular invasion	Present	6	47	53	0.256
	Absent	14	61	75	
Distant metastasis	Present	8	43	51	0.513
	Absent	12	65	77	

Footnote: *NELL1 expression was categorized as negative for Allred total scores of 0–2 and positive for Allred total scores of 3–8. P-values were calculated using the Chi-square test or Fisher's exact test, as appropriate. A P-value <0.05 was considered statistically significant. LVI indicates lymphovascular invasion.*

Association of NELL1 Expression with Nottingham Prognostic Index

A statistically significant association was identified between NELL1 expression and Nottingham Prognostic Index categories ($P < 0.001$) (Table 4). NELL1 positivity increased across prognostic groups, being observed in 7 of 10 cases (70.0%) with good NPI, 62 of 79 cases (78.5%) with moderate NPI, and all 39 cases (100.0%) with poor NPI. These findings indicate that NELL1 expression is significantly associated with poorer prognostic categorization in breast carcinoma.

Table 4. Association of NELL1 expression with Nottingham Prognostic Index categories in breast carcinoma cases (n=128)

NPI category	NELL1 negative, n	NELL1 positive, n	Total, n	NELL1 positivity, %	P-value
Good NPI <3.4	3	7	10	70.0	<0.001
Moderate NPI 3.4-5.4	17	62	79	78.5	
Poor NPI >5.4	0	39	39	100.0	
Total	20	108	128	84.4	

Footnote: *NPI categories were defined as good prognosis, NPI <3.4; moderate prognosis, NPI 3.4–5.4; and poor prognosis, NPI >5.4. NELL1 expression was categorized as negative for Allred total scores of 0–2 and positive for Allred total scores of 3–8. P-value was calculated using the Chi-square test. NPI indicates Nottingham Prognostic Index; NELL1, neural epidermal growth factor-like 1*

DISCUSSION

Breast carcinoma remains a major global malignancy and continues to contribute substantially to cancer-related morbidity and mortality among women [1,2]. In low- and middle-income countries, including Pakistan, delayed presentation, variable access to screening, and limited availability of advanced molecular assays may increase reliance on histopathology, immunohistochemistry, and cost-effective prognostic tools for risk stratification [3,4]. In the present study, most patients were aged 50 years or above, and a considerable proportion of tumours belonged to stage II or III disease, reflecting a clinicopathological pattern in which conventional prognostic assessment remains highly relevant. Established parameters such as tumour size, histological grade, lymph node involvement, stage, and receptor profile continue to guide prognostic evaluation and treatment planning in breast carcinoma [5-8]. The Nottingham Prognostic Index remains clinically useful because it combines three routinely available variables—tumour size, lymph node stage, and histological grade—and has continued to show relevance even when compared with newer prognostic tools and computational models [9-12]. In this context, evaluating NELL1 expression in relation to NPI may be useful as an exploratory approach, particularly in resource-limited settings where additional low-cost immunohistochemical markers could potentially support conventional prognostic assessment.

NELL1 is a secreted glycoprotein originally characterized in relation to epidermal growth factor-like repeat-containing proteins, skeletal development, osteogenesis, and craniofacial biology [13-17]. More recent evidence suggests that NELL1 may participate in extracellular matrix regulation and disease-associated biological pathways; however, its role appears to be tissue- and tumour-context dependent rather than uniform across malignancies [16-19]. For example, increased NELL1 expression has been implicated in osteosarcoma matrisome remodelling, whereas NELL1 methylation has been associated with advanced disease in renal cell carcinoma, indicating that altered NELL1 biology may occur through different mechanisms in different tumour types [18,19]. NELL1 has also been studied in diagnostic renal pathology, particularly in NELL1-associated membranous nephropathy, and occasional reports have described NELL1 positivity in tissues from patients with underlying malignancies, including breast cancer [20,21]. Nevertheless, breast carcinoma-specific data remain limited, and NELL1 should not yet be considered an established breast cancer biomarker. In the present study, NELL1 positivity was frequent and showed significant association with tumour stage, lymph node involvement, and NPI category, whereas no statistically significant association was observed with tumour size, histological grade, surgical margin status, lymphovascular invasion, or distant metastasis. These findings suggest that NELL1 expression may overlap with some adverse clinicopathological features, especially nodal burden and composite prognostic categorization, but the lack of association with several individual parameters indicates that its biological relevance in breast carcinoma requires careful interpretation.

The significant association between NELL1 expression and poor NPI category is noteworthy because NPI represents an integrated prognostic construct rather than a single pathological variable. However, this association should be interpreted as hypothesis-generating, not as evidence that NELL1 independently predicts outcome. Breast carcinoma prognosis is influenced by molecular subtype, hormone receptor status, HER2 status, proliferation indices, and treatment-related factors, which may modify or confound associations between immunohistochemical markers and

clinical outcome [22-25]. In addition, immunohistochemical interpretation can be affected by antibody selection, pre-analytical tissue handling, scoring thresholds, and interobserver variability; therefore, standardized scoring and reporting remain essential when assessing novel tissue biomarkers [26,27]. The present study has several limitations, including its cross-sectional design, single-institution setting, duration-based sampling, absence of survival analysis, and lack of multivariable adjustment for molecular subtype and treatment variables. Future studies should validate NELL1 expression in larger independent cohorts, assess its relationship with receptor-defined breast cancer subtypes, and determine whether it adds prognostic information beyond NPI using outcome-based analyses and biomarker reporting standards [28-30]. Until such evidence is available, NELL1 may be considered a promising exploratory immunohistochemical marker associated with adverse prognostic categorization, but not yet a clinically validated prognostic marker in breast carcinoma.

Limitations

This study has some limitations. It was conducted at a single institution with duration-based consecutive sampling, which may limit the generalizability of the findings to other populations and practice settings. Molecular subtyping of breast carcinoma was not included, and survival outcomes were not assessed because of the cross-sectional study design. Therefore, the observed associations should be interpreted cautiously and considered exploratory.

CONCLUSION

NELL1 expression was frequently observed in breast carcinoma in this cohort and showed significant associations with advanced tumour stage, lymph node involvement, and poor Nottingham Prognostic Index category. These findings suggest that NELL1 may be associated with adverse clinicopathological features in breast carcinoma. However, its prognostic relevance requires further validation in larger, multi-institutional studies with molecular subtyping and clinical outcome data.

Recommendations

Future studies should evaluate NELL1 expression in larger and more diverse breast carcinoma cohorts, preferably using standardized immunohistochemical protocols and scoring systems. Correlation with hormone receptor status, HER2 status, molecular subtype, treatment response, recurrence, disease-free survival, and overall survival would help clarify the potential clinical relevance of NELL1 before it can be considered for routine prognostic assessment.

Declarations

Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Ethics Committee of Khyber Medical University, Peshawar, Pakistan (Approval Number DIR/KMU-AS&RB/AN/003172, Dated 14th May, 2025). Written permission for the use of archival formalin-fixed paraffin-embedded tissue blocks and related clinicopathological data was obtained from the Head of the Institute of Pathology and Diagnostic Medicine. As the study involved anonymized archival tissue samples and existing pathology records, with no direct patient contact or intervention, individual patient consent was waived by the Ethics Committee. All procedures were performed in accordance with the Declaration of Helsinki. Patient confidentiality was maintained throughout the study.

Consent for Publication

Not applicable. The manuscript does not contain identifiable individual patient data or images.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

This research received no external funding.

Authors' Contributions

All authors contributed to this work in accordance with ICMJE authorship criteria. Study conceptualization was performed by Umer, Naveed Sharif, and Abdul Salam. Study methodology was developed by Umer and Abdul Salam. Data collection and data cleaning were carried out by Mehwish Nowshad, Umer, Aiman Ajmeer, and Sidra Mashal. Statistical analysis was performed by Umer and Abdal Ahmad. The manuscript was drafted by Umer, Naveed Sharif,

Mehwish Nowshad, Aiman Ajmeer, Sidra Mashal, and Abdal Ahmad. Critical revision of the manuscript for important intellectual content was carried out by Naveed Sharif and Abdul Salam. The study was supervised by Abdul Salam and Naveed Sharif. All authors critically reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Acknowledgments
The authors acknowledge the Head of the Institute of Pathology and Diagnostic Medicine, Khyber Medical University, Peshawar, Pakistan, for granting permission to use archival tissue samples and related clinicopathological data. The authors also thank the faculty and technical staff of IPDM for their assistance with tissue processing and immunohistochemical staining.

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