

EXPRESSION LEVEL OF PROGRAMMED DEATH LIGAND-1 IN BREAST CARCINOMAS

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Abstract

Background: Programmed death-ligand 1 (PD-L1) is an important immune checkpoint biomarker with established prognostic and therapeutic significance in several malignancies, particularly triple-negative breast cancer. However, data regarding PD-L1 expression in breast carcinoma from Pakistan remain limited. This study evaluated the prevalence of PD-L1 expression and its association with clinicopathological characteristics in breast carcinoma patients from the Hazara region of Khyber Pakhtunkhwa.

Methods: This cross-sectional study was conducted in the Department of Diagnostic Pathology, Ayub Teaching Hospital, Abbottabad, between April 2024 and December 2025. Seventy-one histopathologically confirmed breast carcinoma cases were enrolled using consecutive sampling. Immunohistochemical (IHC) staining for PD-L1 was performed using a rabbit monoclonal anti-human CD274 antibody. Membranous staining in $\geq 1\%$ of tumour cells was considered positive. Staining intensity was categorised as low (1+), moderate (2+) or high (3+). Associations between PD-L1 expression and clinicopathological variables were analysed using the chi-square or Fisher's exact test, while continuous variables were compared using independent-samples t-tests.

Results: PD-L1 expression was detected in 29 of 71 breast carcinomas (40.8%). Among PD-L1-positive tumours, low-intensity staining (1+) was the predominant pattern, observed in 14 (48.3%) cases, followed by high-intensity staining (3+) in 10 (34.5%) and moderate-intensity staining (2+) in 5 (17.2%) cases. A significant association was observed between PD-L1 expression and histological grade ($\chi^2 = 9.264$, $p = 0.010$), with positivity occurring more frequently in intermediate- to high-grade tumours. No significant associations were identified between PD-L1 expression and age category ($p = 0.174$), histological subtype ($p = 0.105$), residence ($p = 0.420$), mean age ($p = 0.251$), or age at menopause ($p = 0.530$).

Conclusions: PD-L1 expression was identified in approximately two-fifths of breast carcinomas and was significantly associated with higher histological grade, supporting its relationship with aggressive tumour biology.

INTRODUCTION

Programmed death-ligand 1 (PD-L1) is a crucial component of the immune system, being expressed on the surface of various cells, including some cancer cells. It is a part of a larger family of transmembrane proteins involved in immune checkpoint pathways¹. It inhibits certain immune responses, inhibits the apoptosis of regulatory T cells and activates the apoptosis of antigen-specific T cells^{1,2}. The interaction between PD-L1 and its receptor, programmed cell death protein 1 (PD-1), helps to regulate immune responses, maintains immune tolerance by preventing autoimmune reactions, and activates proliferative and survival signalling pathways. Therefore, some tumors can exploit the PD-L1/PD-1 pathway to evade detection and destruction by the immune system. When PD-L1 on cancer cells binds to PD-1 on immune cells, it inhibits the immune response against the tumor, allowing the cancer to escape immune surveillance. Its expression has been associated with large tumor size, high grade, high proliferation, estrogen receptor-negative status, and HER2-positive status, and it is inversely correlated with survival in breast cancer^{1,3}. The expression of PD-L1 in various breast carcinomas varies among different molecular subtypes. Studies have reported the prevalence of PD-L1 expression range from 13.3% to 87.3% in different subtypes, with the highest expression rates observed in triple-negative breast cancer (TNBC) subtypes^{4,6}. Immune checkpoint inhibitor therapies have been developed to block the interaction between PD-1 and PD-L1 to prevent the "off" signal from being sent, and allowing the T cells to kill cancer cells. These drugs help the immune system to recognize and attack the cancer cells more effectively, thereby enhancing the body's immune response against cancer cells⁷. Immune checkpoint inhibitors have been approved to treat various cancer types, including breast cancer, bladder cancer, lung cancer, and others^{7,9}. There is a gap in the literature about the PD-L1 expression levels in breast cancer cases in

population of Hazara region of Khyber Pakhtunkhwa. Therefore, we aimed to study the PD-L1 expression levels and its relationship with clinico-pathological parameters such as tumor grade, stage, histological subtype, and socio-demographics in patients of this region. Addition of this information to the literature can indicate PD-L1's potential role as a prognostic biomarker/therapeutic target, aid in refining the current risk stratification models and guide treatment decisions, leading to improved patient outcomes.

MATERIALS AND METHODS

This cross-sectional study was conducted in department of diagnostic pathology of Ayub Medical Teaching Institution (AMTI), Abbottabad. The data collection spanned from April 2024 to December 2025. Seventy-one eligible patients were enrolled into this study using non-probability, consecutive sampling technique. Sample size was calculated using the WHO formula for calculation of sample size for cross-sectional study. The anticipated frequency of PD-L1 expression in breast cancer patients was taken at 7.1% from literature⁶. The absolute precision was taken at 6% and Confidence Interval (CI) at 95% to get a reasonable sample for obtaining statistically significant results.

Biopsy specimen of only those patients was included in this study who were diagnosed to have a breast cancer on histopathology and having adequate tissue sample (Paraffin Block) for IHC analysis. Specimens from patients who had not been diagnosed with breast carcinoma or those currently receiving chemotherapy or radiotherapy were excluded from the study. The data collection was started after receiving approval from the Medical Ethics Committee of Ayub Medical Teaching Institution- Abbottabad (RC-EA-2024/038) and the college of physicians and surgeons- Pakistan. Informed consent was obtained from all participants prior to their inclusion in the study, adhering to ethical standards as per declaration of Helsinki. Biopsies

diagnosed as breast carcinomas were meticulously re-examined to confirm their initial diagnoses, and documentary evidence (biopsy reports) were reviewed to ensure accuracy. These biopsy samples were then processed for examination to quantify the PD-L1 protein expression.

Strict adherence was ensured with recommended protocols for the specimen preparation for histopathological examination to prevent any inadvertent artifacts or contamination¹⁰. Tissue was fixed in 10% neutral buffered formalin for preservation. The tissue containers were marked with patient identification, date, and any clinically relevant information. On gross pathology, the size, color, and any visible abnormalities in the tissue sample were recorded.

The tissue sections were embedded in paraffin wax in labelled cassettes. Sections of appropriate thickness (4 μ m) were cut using a Medite™ Microtome Meditome M530, a semi-automated Microtome manufactured in Germany¹¹. Hematoxylin and Eosin (H&E) stain was applied to the sections to reassure the diagnoses. Slides showing nuclear stratification, hyperchromatic pleomorphic nuclei, atypical mitotic figures, and infiltrating into the surrounding stroma with stromal reaction viewed on 100X and 400X power were considered positive for malignant breast tumors¹².

The IHC (ER, PR, Her 2 neu and PD-L1) was then done in PIMS Islamabad. The steps to retrieve primary antibody for PD-L1 were meticulously followed as stated in guidelines and reported by authors^{10,13}. PD-L1 protein expression was detected using a semi-quantitative method. IHC was done using rabbit monoclonal antibodies produced from the CD274 clone as the anti-human PD-L1 antibody. The IHC positivity was considered if the membrane staining pattern was $\geq 1\%$ as per adopted guidelines^{14,15}. The IHC staining for PD-L1 was scored based on membranous staining intensity, described as follows: Negative: no staining; Low intensity: faint staining in $<10\%$ of cells on high power field (HPF); Moderate intensity: moderate staining (10% to 20% of cells) on HPF; and Strong intensity: strong staining ($>20\%$ of cells). This

scoring method followed previously established protocols^{13,16}.

The slides were validated at two levels. First, independent review and documentation of the slides by two histopathologists with more than ten years of experience was done and results were compared. We got no incongruence in their results; therefore, third opinion was not needed in any case. Secondly, both positive and negative controls were used simultaneously while reporting the slides for staining intensity of IHC. Negative controls involved oral mucosa samples omitting the primary antibody, while positive controls used samples from tonsils exhibiting mild, moderate, and strong IHC uptake.

Data collection and analysis were conducted using SPSS® v27. Continuous variables such as age were described using means and standard deviations. The distribution of categorical variables, including residence, education, and marital status, was analyzed by calculating the frequency and percentages of each category. The association between the expression of PD-L1 protein and the type of breast carcinoma was evaluated using chi-square/ Fisher's exact tests where appropriate. A significance level of $\leq 5\%$ considered statistically significant.

RESULTS

A total of 71 histopathology confirmed breast cancer cases were included in this study. The mean age of the patients was 55.87 ± 15.81 years, and the mean age at menopause was 48.34 ± 2.29 years. PD-L1 expression was observed in 29 (40.8%) cases. The majority of patients belonged to the older age group 30 (42.3%). Histologically, Grade II/III (intermediate to high grade) tumors constituted the predominant category 56 (78.9%), while IDC/NST was the most common histological subtype 61(85.9%). (Table I)

Among PD-L1-positive tumors, low-intensity staining (1+) was the most frequent pattern, observed in 14 (48.3%) cases, followed by high-intensity staining (3+) in 10 (34.5%) cases. (Table II)

A statistically significant association was observed between histological grade and PD-L1 expression ($\chi^2 = 9.264$, $p = 0.010$) as shown in table III. No

significant associations were found between PD-L1 expression and age category ($p = 0.174$), histological subtype ($p = 0.105$), or residence ($p = 0.420$). (Table III)

Independent-samples t-tests were performed to compare age and age at menopause between PD-L1-positive and PD-L1-negative patients. Patients with PD-L1-positive tumors were slightly older than those with PD-L1-negative tumors (58.48 vs. 54.07 years), but the difference was not statistically significant ($p = 0.251$). Similarly, no significant difference was observed in age at menopause between PD-L1-positive and PD-L1-negative patients ($p = 0.530$). (Table IV)

Of the 71 breast carcinoma cases, 29 (40.8%) showed PD-L1 positivity, while 42 (59.2%) were PD-L1 negative. Among PD-L1-positive cases, 6

were ER/PR positive, 6 were HER2 positive, and 17 were triple-negative breast cancers. Among PD-L1-negative cases, 39 were ER/PR positive and 3 were HER2 positive. (Table V).

The Hematoxylin and Eosin slide from our study is shown in figure I.

As shown in the figure 1, the negative control shows complete absence of brown chromogenic staining, confirming assay specificity. Our study case demonstrates diffuse, strong membranous PD-L1 expression involving the tumour cells at both low-power (100X) and high-power (400X) magnifications. The staining pattern and intensity are comparable to those of the positive control, validating robust PD-L1 expression in the study specimen. (Fig II).

Table I. Demographic and Clinicopathological Characteristics of Breast Cancer Patients (n = 71)

Variable		n (%)
Age Category	Young Adult (20–39 years)	14 (19.7)
	Middle Aged (40–59 years)	27 (38.0)
	Older Age (≥ 60 years)	30 (42.3)
Residence	Abbottabad	58 (81.7)
	Mansehra	12 (16.9)
	Peshawar	1 (1.4)
Histological Grade	Grade I	12 (16.9)
	Grade II/III	56 (78.9)
	Grade III	3 (4.2)
Histological Subtype	IDC	61 (85.9)
	Medullary carcinoma	3 (4.2)
	Mucinous carcinoma	7 (9.9)
PD-L1 Expression	Positive	29 (40.8)
	Negative	42 (59.2)
Variable	Mean $\pm$ SD	Median (IQR)
Age (years) (n=71)	55.87 $\pm$ 15.81	53 (44–70)
Age at Menopause (years) (n=44)	48.34 $\pm$ 2.29	50 (45–50)

Table II. Distribution of PD-L1 immunohistochemical staining intensity among PD-L1-positive breast carcinomas (n = 29)

PD-L1 Intensity	n	%
Low (1+)	14	48.3
Moderate (2+)	5	17.2
High (3+)	10	34.5

Table III. Association Between Age Category and PD-L1 Expression

Parameters		Positive n (%)	Negative n (%)	p-value
Age Category	Young Adult (20–39 years)	5 (35.7)	9 (64.3)	0.174
	Middle Aged (40–59 years)	8 (29.6)	19 (70.4)	
	Older Age (≥60 years)	16 (53.3)	14 (46.7)	
Histopathological Grade	Grade II	1 (8.3)	11 (91.7)	0.010*
	Grade II/III	28 (50.0)	28 (50.0)	
	Grade III	0 (0.0)	3 (100.0)	
Histopathological Subtype	IDC/NST	22 (36.1)	39 (63.9)	0.105
	Medullary carcinoma	3 (100.0)	0 (0.0)	
	Mucinous carcinoma	4 (57.1)	3 (42.9)	

Table IV. Comparison of Age and Age at Menopause According to PD-L1 Expression

Variable	PD-L1 Positive (n=29) Mean ± SD	PD-L1 Negative (n=42) Mean ± SD	Mean Difference (95% CI)	p-value
Age (years)	58.48 ± 15.61	54.07 ± 15.88	4.41 (-3.19 to 12.01)	0.251
Age at Menopause (years)	48.57 ± 2.32	48.13 ± 2.30	0.44 (-0.97 to 1.85)	0.530

Table V. Distribution of ER/PR-positive, HER2-positive and triple-negative breast carcinoma cases according to PD-L1 expression

PD-L1 status	No. of cases	ER/PR positive	HER2 positive	TNBC
PD-L1 negative	42	39	3	—
PD-L1 positive	29	6	6	17

TNBC: triple-negative breast cancer, ER/PR: estrogen receptor/progesterone receptor, HER2: human epidermal growth factor receptor 2.

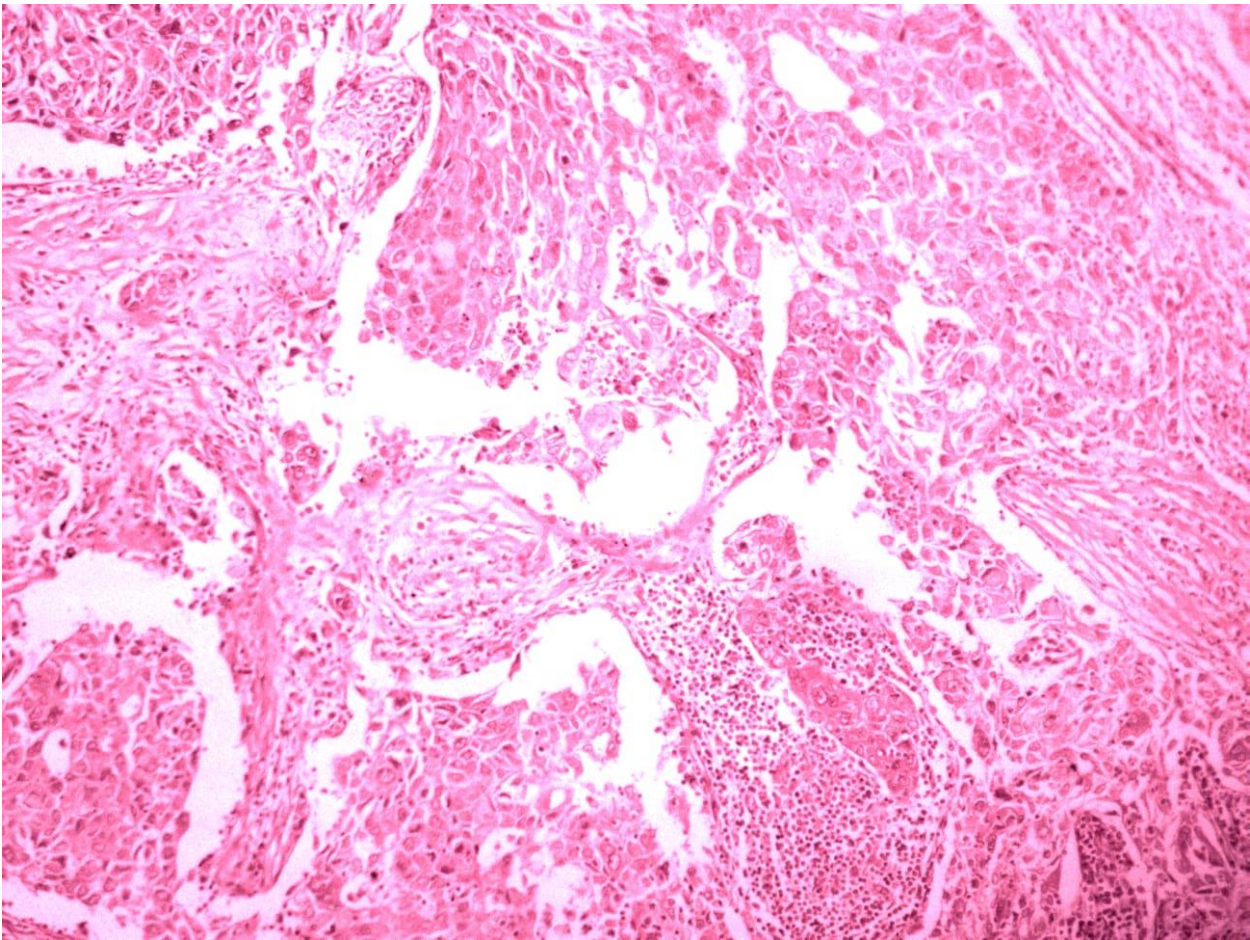


Figure 1: Hematoxylin and Eosin slide from our study

	100X	400X
Negative Control		

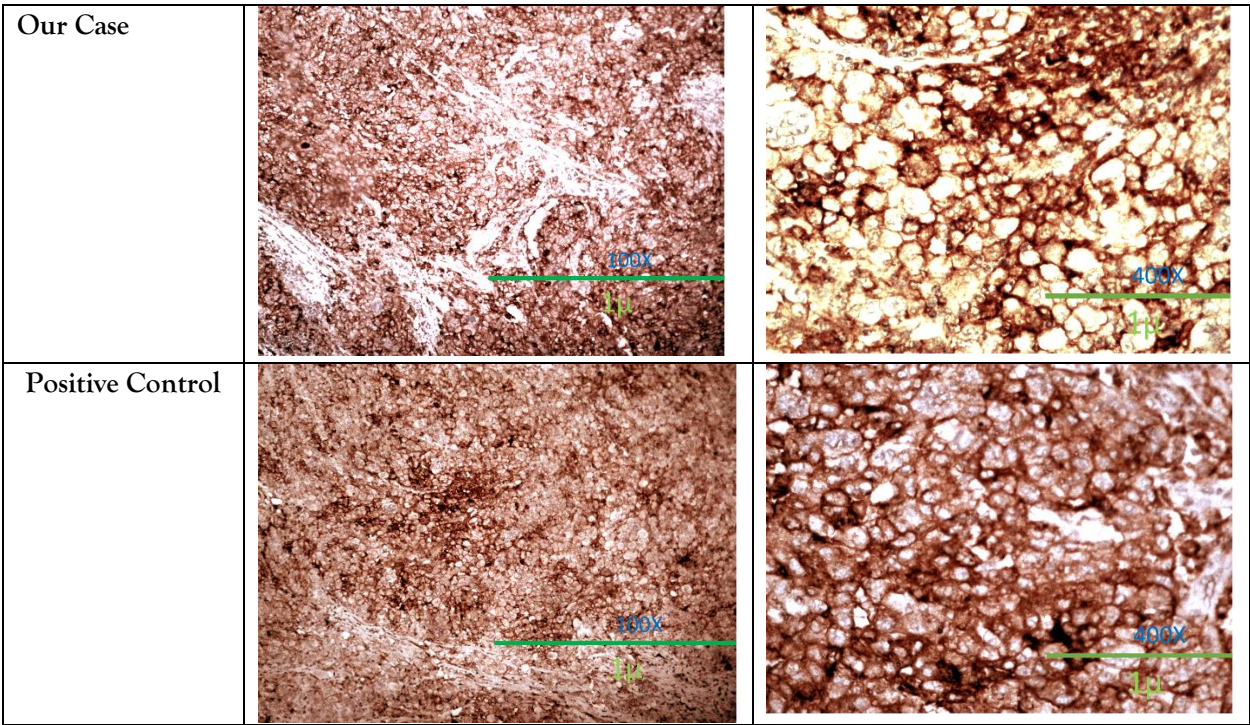


Figure 2: Representative IHC stained slides for PD-L1

DISCUSSION:

This study evaluated the expression of PD-L1 in breast carcinomas and its association with clinicopathological characteristics. PD-L1 expression was identified in 29 (40.8%) of breast carcinoma cases, with low-intensity staining representing the most common pattern among positive tumours. A statistically significant association was observed between PD-L1 expression and histological grade, whereas age, age at menopause, residence, and histological subtype were not significantly associated with PD-L1 status.

A study found that the expression rates of PD-L1 and PD-1 in TNBC were 47.8% and 43.5%, respectively, which were significantly higher than the expression prevalence in other subtypes ⁴. Another study found that PD-L1 was expressed in 23.4% of the 650 breast cancer specimens ². In contrast, a study reported the expression of PD-L1 in 7.1% of breast cancers ¹⁷. Similarly, a large cohort study involving 821 breast cancer cases found PD-L1 expression in tumour cells in only 6.5% of tumours ¹⁶. Such variation across studies may be attributable to differences in antibody

clones, immunohistochemical protocols, scoring criteria, cut-off values for positivity, tumour heterogeneity, and patient demographics. Nevertheless, the prevalence observed in the present study is comparable to more recent reports from Asian populations. Rajadurai et al. (2024), for example, reported a PD-L1 positivity rate of 37.7% (95% CI: 29.6%–46.3%) among patients with advanced triple-negative breast cancer (TNBC) in Malaysia ¹⁸. The relatively high prevalence observed in our cohort suggests that a substantial proportion of breast cancer patients in the local population may potentially benefit from immunotherapeutic approaches targeting the PD-1/PD-L1 pathway.

An important finding of this study was the significant association between PD-L1 expression and histological grade (p=0.010). PD-L1 positivity was considerably more frequent in intermediate- to high-grade tumours than in lower-grade lesions. This observation is consistent with findings from previous studies demonstrating that PD-L1 expression is associated with aggressive tumour characteristics such as high histological grade, and increased tumour proliferation ^{3, 16}. The

association between PD-L1 expression and higher tumour grade may reflect the biological interplay between tumour aggressiveness and immune evasion mechanisms¹⁹.

High-grade tumours typically possess greater genomic instability and a higher mutational burden, resulting in increased neoantigen production and enhanced immune recognition²⁰. In response to immune activation, tumour cells may upregulate PD-L1 expression as an adaptive mechanism to suppress cytotoxic T-cell activity and evade immune-mediated destruction^{1, 21}. Consequently, increased PD-L1 expression may act as an indicator of both tumour aggressiveness and active tumour-immune interaction.

The findings of the present study support the growing body of evidence suggesting that PD-L1 may serve as an important prognostic and predictive biomarker in breast cancer. Recent studies have highlighted the close association between PD-L1 expression and tumour-infiltrating lymphocytes (TILs), particularly CD8+ T cells. Vučinić et al. (2023) reported a strong positive correlation between PD-L1 positivity and stromal lymphocytic infiltration²², while Wang et al. (2024) demonstrated that PD-L1 positive tumour associated macrophages were associated with enhanced anti-tumour immune responses and favourable clinical outcomes²³. These observations indicate that PD-L1 expression reflects a dynamic tumour microenvironment characterised by ongoing immune activity.

No significant association was observed between age category and PD-L1 expression in the present study. Although PD-L1 positivity appeared numerically higher among patients aged 60 years or above, the difference did not achieve statistical significance. Similarly, the mean age of PD-L1 positive patients was slightly higher than that of PD-L1-negative patients, but this difference was not statistically significant. These findings are in agreement with several studies that have reported no consistent relationship between patient age and PD-L1 expression^{4, 17}. The lack of association suggests that PD-L1 expression is predominantly determined by tumour biology rather than host demographic factors.

Likewise, age at menopause was not significantly associated with PD-L1 status in the present study. The mean age at menopause was comparable between PD-L1-positive and PD-L1-negative groups. Although hormonal factors are well-established contributors to breast carcinogenesis, current evidence suggests that immune checkpoint expression is more closely linked to tumour-specific molecular and immunological characteristics than to reproductive history. This may explain the absence of a significant relationship between menopausal age and PD-L1 expression in the present cohort.

The present study also demonstrated no statistically significant association between histological subtype and PD-L1 expression. However, noteworthy differences in positivity rates were observed among subtypes. All medullary carcinomas expressed PD-L1, while positivity rates were 57.1% for mucinous carcinomas and 36.1% for invasive ductal carcinoma of no special type (IDC/NST). Although these differences did not reach statistical significance, likely due to the small number of cases in certain subgroups, they are biologically plausible. Previous studies have similarly demonstrated increased PD-L1 expression in medullary and basal-like breast cancers, which are characterised by prominent lymphocytic infiltration and enhanced immune activation^{3, 16}.

Other findings of our study were the heterogeneous nature of PD-L1 expression in breast cancer. From a clinical perspective, high-intensity PD-L1 expression may be particularly relevant because several studies have demonstrated improved responses to immune checkpoint inhibitors among patients with strongly PD-L1 positive tumours^{24, 25}. The emergence of immunotherapeutic agents such as pembrolizumab and atezolizumab has significantly altered the treatment landscape of breast cancer, particularly in TNBC, where PD-L1 positivity is now routinely used to guide treatment selection²⁶. The observed prevalence of PD-L1 positivity in the present study has important implications for clinical practice in Pakistan. Access to immunotherapy remains limited in many developing countries; however, the identification

of a substantial proportion of PD-L1-positive tumours suggests that biomarker-guided immunotherapeutic strategies may become increasingly relevant in the future. Recent clinical trials have demonstrated improved progression-free and overall survival among patients with PD-L1-positive breast cancers receiving immune checkpoint inhibitors in combination with chemotherapy^{26, 27}. Consequently, routine assessment of PD-L1 expression may facilitate more personalised treatment approaches and improve outcomes in selected patient populations. Our limitations include a single institution study and a relatively modest sample size. This has limited our findings as no significant associations for less common tumour subtypes could be addressed. However, our study adds valuable local data to the limited literature on this topic from our region. This study highlights the role of PD-L1 as prognostic biomarker and a therapeutic target for breast carcinoma. Future research should focus on multicentre data with large sample size, incorporating molecular subtyping, survival analysis, and treatment outcomes. This will clarify the role of PD-L1 in breast cancer management within the local population.

CONCLUSION:

PD-L1 expression was detected in approximately two-fifths of breast carcinomas and was significantly associated with higher histological grade. These findings suggest that PD-L1 may serve as a valuable prognostic biomarker and a therapeutic target for breast cancer patients. PD-L1 levels may help in identifying patients who could benefit from immune checkpoint inhibitor therapy.

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