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IMMUNOHISTOCHEMICAL EXPRESSION AND MOLECULAR PROFILING OF PD-L1 AND KI-67 IN TRIPLE-NEGATIVE BREAST CARCINOMA: CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

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ABSTRACT

Background: Triple-negative breast carcinoma (TNBC) is an aggressive subtype of breast cancer lacking targeted hormonal therapies. Biomarkers such as programmed death-ligand 1 (PD-L1) and Ki-67 have gained importance in understanding tumor behavior, immune evasion, and proliferative activity.

Objective: To evaluate the immunohistochemical expression and molecular profiling of PD-L1 and Ki-67 in TNBC and to assess their correlation with clinicopathological parameters.

Methods: This was a cross-sectional analytical study conducted in the Pathology Department (Histopathology section) of Fauji Foundation Hospital Lahore, from March 2025 to December 2025, including 85 patients diagnosed with triple-negative breast carcinoma (TNBC) to evaluate the immunohistochemical expression and molecular profiling of PD-L1 and Ki-67 and their correlation with clinicopathological parameters.

Results: PD-L1 positivity was observed in 60.0% of patients and was significantly associated with larger tumor size (4.2 ± 1.5 cm vs 3.3 ± 1.2 cm; p=0.008), higher grade tumors (67.9% vs 46.9%; p=0.041), lymph node positivity (69.6% vs 50.0%; p=0.012), and advanced stage disease (72.9% vs 38.2%; p=0.006). High Ki-67 expression was seen in 77.6% of patients and was significantly associated with larger tumor size (4.1 ± 1.4 cm vs 3.0 ± 1.1 cm; p=0.004), higher grade (90.6% vs 56.3%; p=0.009), and lymph node involvement (83.9% vs 47.4%; p=0.047). The combined PD-L1+/high Ki-67 group showed the most aggressive tumor profile, with highest tumor size (4.4 ± 1.5 cm), nodal involvement (81.0%), and advanced stage (73.8%) ($p<0.001$).

Conclusion: PD-L1 and Ki-67 expression are significantly associated with aggressive tumor characteristics in TNBC, and their combined assessment provides enhanced prognostic value for disease stratification and management.

KEYWORDS: Triple-negative breast carcinoma, PD-L1, Ki-67, immunohistochemistry, tumor proliferation, prognostic markers

INTRODUCTION

Triple-negative breast carcinoma (TNBC) is an aggressive subtype of breast carcinoma that is characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression [1]. It comprises between 15 and 20 percent of all breast cancers and is linked to an increased risk of recurrence, early metastasis and worse overall prognosis than other molecular subtypes [3]. The absence of targeted hormonal or HER2-directed therapeutic agents has limited treatment options in TNBC, and it is crucial to identify new biomarkers to advance treatment options [5]. Programmed death-ligand 1 (PD-L1) has emerged as an important immunological marker in various malignancies, including TNBC [2]. The expression of PD-L1 on tumor cells and tumor-infiltrating immune cells is critical in the immune evasion of tumor cells and tumor-infiltrating immune cells [4]. This immune checkpoint pathway has emerged as a major therapeutic target and immune checkpoint inhibitors have shown promising outcomes in PD-L1-positive TNBC patients [6]. Ki-67 is an established proliferation marker that is utilized to determine the growth and aggressiveness of tumors [7]. The high level of Ki-67 expression is linked

with increased cellular proliferation, higher tumor grade, and worse clinical outcomes in breast cancer [9]. High levels of Ki-67 are usually seen in TNBC and can be associated with aggressive tumor biology and rapid disease progression [11].

PD-L1 and Ki-67 immunohistochemical analysis is a valuable source of information about tumor behavior and its possible therapeutic responsiveness [8]. Whereas the expression of PD-L1 might predict response to immunotherapy, Ki-67 is a reflection of tumor proliferative activity and the two may be used to provide complementary prognostic and predictive information [10]. Past studies have shown inconsistent PD-L1 expression in TNBC with some studies demonstrating significant relationships with tumor grade, lymph node involvement, and overall survival [12]. Likewise, the high expression of Ki-67 has consistently been associated with the negative clinicopathological characteristics, such as bigger tumor size and higher histological grade [13]. Nevertheless, the results on their joint prognostic value are not consistent. Molecular profiling of TNBC has also shown its heterogeneity and different subgroups of TNBC have been reported with different biological behaviors and responses to treatment [14]. A combination of immunohistochemical markers and clinicopathological parameters could help to better understand the pattern of diseases and help develop a tailor-made approach to the therapy of various diseases [15]. Although these biomarkers are increasingly becoming of interest, further research is still required to assess the expression of these biomarkers in TNBC as well as their relationship with clinicopathological characteristics especially in the diverse populations [16].

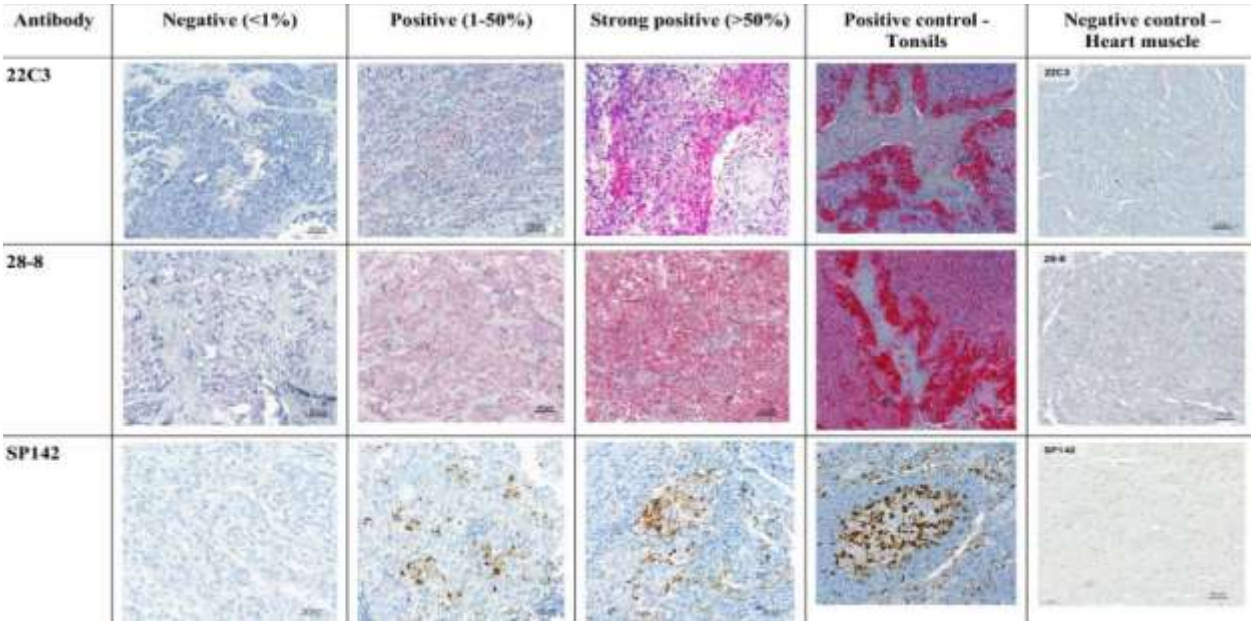


Figure 1: Immunhistochemical staining of tumor tissue and controls with three anti-PD-L1 monoclonal antibodies

Objective

To evaluate the immunohistochemical expression and molecular profiling of PD-L1 and Ki-67 in TNBC and to assess their correlation with clinicopathological parameters.

Methodology

This was a cross-sectional analytical study conducted in the Pathology Department (Histopathology section) of Fauji Foundation Hospital Lahore, from March 2025 to December 2025, ncluding 85 patients.

Inclusion Criteria

- Female patients aged ≥18 years with histopathologically confirmed TNBC (ER-, PR-, HER2-)
- Patients with available formalin-fixed paraffin-embedded (FFPE) tissue blocks
- Patients with complete clinicopathological data
- Patients who had not received neoadjuvant chemotherapy prior to biopsy

Exclusion Criteria

- Patients with non-TNBC breast carcinoma
- Inadequate or poorly preserved tissue samples

- Patients with incomplete clinical or pathological data
- Cases with prior systemic therapy before tissue sampling
- Recurrent breast carcinoma cases

Data Collection

After ethical approval, clinical and pathological data were collected using a structured proforma. Variables included age, tumor size, histological grade, lymph node status, tumor stage, and presence of metastasis. Tissue sections from FFPE blocks were subjected to immunohistochemical staining for PD-L1 and Ki-67 using standardized protocols. PD-L1 expression was evaluated based on membranous staining in tumor cells and/or immune cells and categorized as positive or negative according to established cutoff values. Ki-67 labeling index was calculated as the percentage of positively stained tumor nuclei and categorized into low and high proliferation groups (e.g., <20% vs ≥20%). Molecular profiling was correlated with clinicopathological parameters.

Statistical Analysis

Data were analyzed using SPSS version 26.0. Continuous variables were expressed as mean ± standard deviation, while categorical variables were presented as frequency and percentage. Associations between PD-L1 and Ki-67 expression and clinicopathological variables were assessed using chi-square test or Fisher's exact test. Independent t-test was used for comparison of means where applicable. A p-value ≤0.05 was considered statistically significant.

Results

The study included 85 TNBC patients with a mean age of 49.2 ± 11.6 years, most commonly in the 41–60 year group (49.4%). Mean tumor size was 3.8 ± 1.4 cm, with the majority (54.1%) having tumors between 2–5 cm. High-grade disease predominated, with Grade III tumors in 62.4% of cases. Lymph node positivity was observed in 65.9%, and advanced stage (III–IV) disease in 56.5%, indicating aggressive disease presentation. The mean Ki-67 index was high (38.7 ± 14.2%), and PD-L1 positivity was seen in 60.0% of patients, reflecting high proliferative and immunological activity.

Table 1: Clinicopathological Profile of TNBC Patients (n=85)

Variable	Category	Total n (%) / Mean ± SD
Age (years)	Mean ± SD	49.2 ± 11.6
	≤40 years	24 (28.2%)
	41–60 years	42 (49.4%)
	>60 years	19 (22.4%)
Tumor Size (cm)	Mean ± SD	3.8 ± 1.4
	≤2 cm	18 (21.2%)
	2–5 cm	46 (54.1%)
	>5 cm	21 (24.7%)
Histological Grade	Grade II	32 (37.6%)
	Grade III	53 (62.4%)
Lymph Node Status	Negative	29 (34.1%)
	Positive	56 (65.9%)
Tumor Stage	Stage I–II	37 (43.5%)
	Stage III–IV	48 (56.5%)
Ki-67 Index (%)	Mean ± SD	38.7 ± 14.2
PD-L1 Positivity	Positive	51 (60.0%)

PD-L1 expression showed significant association with aggressive tumor features. It was more frequent in larger tumors (>5 cm: 66.7%) compared to smaller tumors (≤2 cm: 38.9%; p=0.008). Higher expression was also seen in Grade III tumors (67.9% vs 46.9%; p=0.041), lymph node–positive cases (69.6%), and advanced-stage disease (72.9% vs 38.2%; p=0.006). Mean tumor size was higher in PD-L1–positive patients (4.2 ± 1.5 cm vs 3.3 ± 1.2 cm), indicating a correlation with tumor burden, while age showed no significant association.

Table 2: Detailed PD-L1 Expression Stratified with Clinicopathological Variables

Variable	Category	PD-L1 Positive n (%)	PD-L1 Negative n (%)	P-value
Age Group	≤40 years	16 (66.7%)	8 (33.3%)	0.284
	41–60 years	24 (57.1%)	18 (42.9%)	

	>60 years	11 (57.9%)	8 (42.1%)	
Tumor Size	≤2 cm	7 (38.9%)	11 (61.1%)	0.008
	2–5 cm	30 (65.2%)	16 (34.8%)	
	>5 cm	14 (66.7%)	7 (33.3%)	
Histological Grade	Grade II	15 (46.9%)	17 (53.1%)	0.041
	Grade III	36 (67.9%)	17 (32.1%)	
Lymph Node Status	Positive	39 (69.6%)	17 (30.4%)	0.012
Tumor Stage	Stage III–IV	35 (72.9%)	13 (27.1%)	0.006

High Ki-67 expression (≥20%) was observed in 77.6% of patients and was strongly associated with tumor aggressiveness. Patients with high Ki-67 had larger tumors (4.1 ± 1.4 cm vs 3.0 ± 1.1 cm; p=0.004) and a higher proportion of Grade III tumors (90.6% vs 56.3%; p=0.009). Lymph node positivity was also higher (83.9% vs 47.4%; p=0.047). Although high Ki-67 was more frequent in advanced stages (83.3% vs 42.1%).

Table 3: Ki-67 Proliferation Index Correlation with Tumor Biology

Variable	Category	High Ki-67 n (%)	Low Ki-67 n (%)	P-value
Age Group	≤40 years	20 (83.3%)	4 (16.7%)	0.312
	41–60 years	32 (76.2%)	10 (23.8%)	
	>60 years	14 (73.7%)	5 (26.3%)	
Tumor Size	≤2 cm	10 (55.6%)	8 (44.4%)	0.004
	2–5 cm	38 (82.6%)	8 (17.4%)	
	>5 cm	18 (85.7%)	3 (14.3%)	
Histological Grade	Grade II	18 (56.3%)	14 (43.7%)	0.009
	Grade III	48 (90.6%)	5 (9.4%)	
Lymph Node Status	Positive	47 (83.9%)	9 (16.1%)	0.047
Tumor Stage	Stage III–IV	40 (83.3%)	8 (16.7%)	0.163

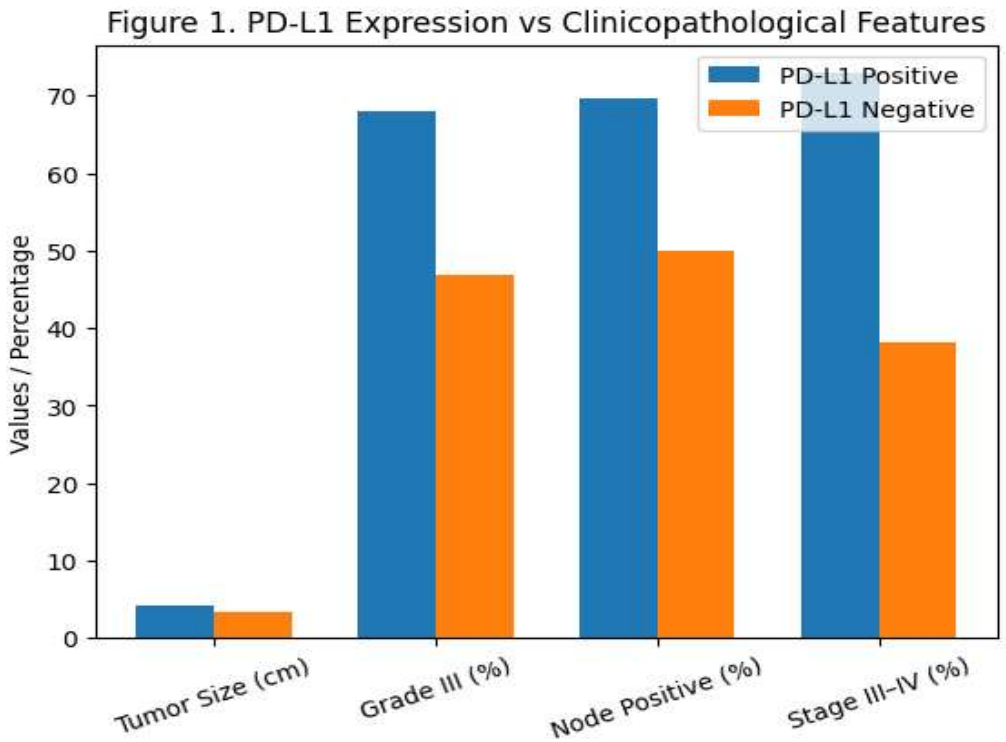


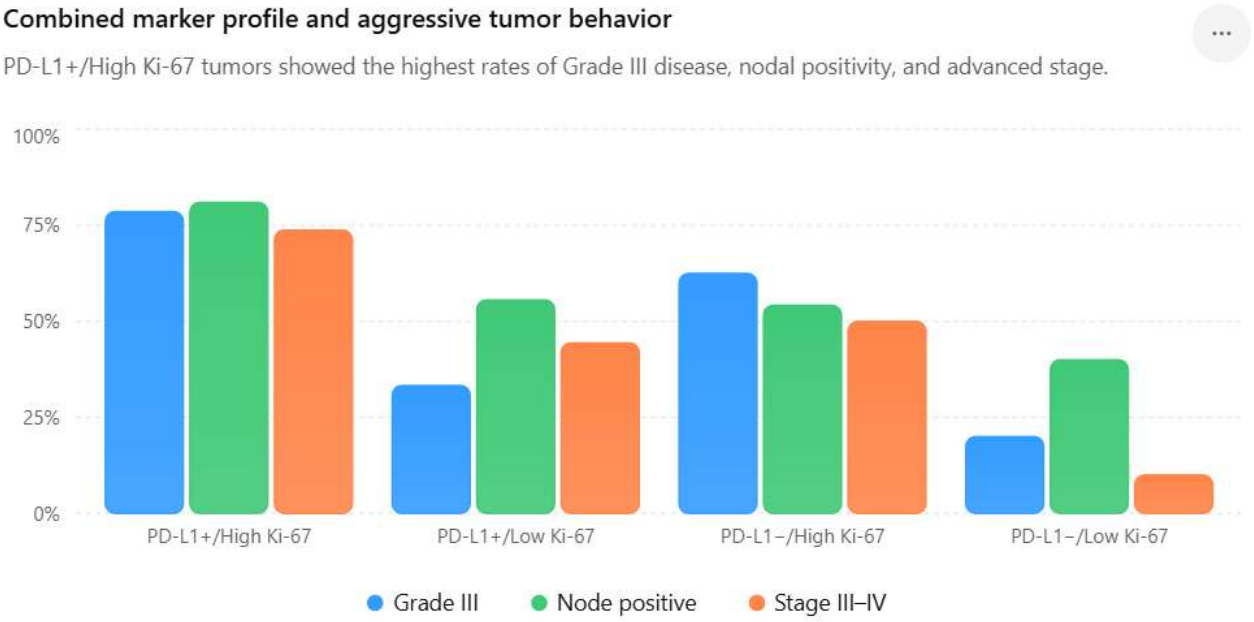
Figure 2. Comparative Analysis of Clinicopathological Features According to PD-L1 Expression in Triple-Negative Breast Carcinoma

The PD-L1+/High Ki-67 group had the highest mean tumor size (4.4 ± 1.5 cm), highest proportion of Grade III tumors (78.6%), lymph node positivity (81.0%), and advanced-stage disease (73.8%) ($p<0.001$). In contrast, the PD-L1−/Low Ki-67 group showed the least aggressive profile, with smaller tumors (2.8 ± 1.0 cm), lower Grade III frequency

(20.0%), lower nodal involvement (40.0%), and minimal advanced-stage disease (10.0%). This highlights the combined prognostic significance of these markers.

Table 4: Combined PD-L1 and Ki-67 Expression with Aggressive Tumor Behavior

Profile	n (%)	Grade III n (%)	Node Positive n (%)	Stage III–IV n (%)	Mean Tumor Size ± SD	P-value
PD-L1+ / High Ki-67	42 (49.4%)	33 (78.6%)	34 (81.0%)	31 (73.8%)	4.4 ± 1.5	<0.001
PD-L1+ / Low Ki-67	9 (10.6%)	3 (33.3%)	5 (55.6%)	4 (44.4%)	3.5 ± 1.2	
PD-L1− / High Ki-67	24 (28.2%)	15 (62.5%)	13 (54.2%)	12 (50.0%)	3.7 ± 1.3	
PD-L1− / Low Ki-67	10 (11.8%)	2 (20.0%)	4 (40.0%)	1 (10.0%)	2.8 ± 1.0	



DISCUSSION

This paper compared and contrasted the immunohistochemical expression of the PD-L1 and Ki-67 in triple-negative breast cancer and demonstrated significant correlations with adverse clinicopathological features, which suggests that they may serve as prognostic biomarkers.The results indicate that both the markers, either separately or jointly, can represent the aggressiveness of the tumors and can have implications in the risk stratification and therapeutic decision making processes [17].One significant finding was that PD-L1 positivity (60.0%), showed a significant association with a larger tumor size, higher histological grade, involvement of lymph node, and advanced stage disease.Mean tumor size was greater in PD-L1–positive patients (4.2 ± 1.5 cm vs 3.3 ± 1.2 cm; p=0.008), and PD-L1 expression was more frequent in Grade III tumors (67.9% vs 46.9%; p=0.041) and advanced-stage cases (72.9% vs 38.2%; p=0.006).These results indicate that the expression of PD-L1 is associated with the more aggressive tumor biology.Earlier studies have also suggested a positive relationship between the higher PD-L1 expression in tumours with higher stage, grade, and nodal tumours, and its utility as a predictor of tumor aggressiveness and elicitation of immunotherapy [18].The Ki-67 expression was also significantly increased and 77.6% of the patients in the study exhibited high proliferative index.High Ki-67 was significantly associated with larger tumors (4.1 ± 1.4 cm vs 3.0 ± 1.1 cm; p=0.004), higher grade tumors (90.6% vs 56.3%; p=0.009), and increased lymph node positivity (83.9% vs 47.4%; p=0.047).These findings suggest that the proliferative activity is intimately connected to the tumor progression and metastatic possibilities.The past studies have all indicated that high degree of Ki-67 expression is associated with poor prognostic characteristics, such as rapid tumor growth and increased risk of tumor metastasis in TNBC [19].Notably, the joint analysis of PD-L1 and Ki-67 presented more information about the behavior of tumors.The

most aggressive profile was that of the PD-L1+/High Ki-67 group with the largest mean tumor size (4.4 ± 1.5 cm), highest proportion of Grade III tumors (78.6%), lymph node involvement (81.0%), and advanced stage disease (73.8%) ($p<0.001$).Contrastingly, PD-L1-/low Ki-67 group had the least aggressive characteristics with smaller tumors (2.8 ± 1.0 cm) and minimal advanced-stage disease (10.0%).This implies that there is a synergistic action between immune evasion and high rate of proliferation in promoting tumor aggressiveness.Other past studies have equally emphasized that prognostic analysis that combines immunological and proliferative markers is more successful than single-marker analysis in prognosticating the outcome [20].The absence of such a strong correlation between age and the expression of both PD-L1 and Ki-67, indicates that the tumor biology of TNBC could be more of a factor of intrinsic molecular factors than patient demographics.Past studies have also shown that the TNBC behaviour is more influenced by molecular heterogeneity and not just by age [21].The high correlation of the two markers with positivity of the lymph nodes further reinforces the values of the two markers in predicting the metastatic potential.PD-L1-positive and high Ki-67 tumors exhibited increased nodal involvement, thus, increased possibility of spreading the disease.Past studies have also shown that not only the immune checkpoint expression, but also high indices of proliferation are correlated with increased metastatic behavior [22].These results have significant clinical implications.The expression of PD-L1 can be used to identify patients who might respond to the immune checkpoint inhibitor therapy, with Ki-67 potentially being utilized to help assess the aggressiveness of the tumor and that the intensity of treatment should be determined.When combined these markers can perhaps enable more personalized approaches to therapy in TNBC.The use of a combination of biomarkers to enhance treatment stratification has also been supported by previous studies.On the whole, this paper shows that PD-L1 and Ki-67 are useful markers in TNBC, and both are significantly correlated with adverse clinicopathological markers.Their joint expression gives them better prognostic value to justify their use in tumor characterization and possibly in clinical use.

CONCLUSION

It is concluded that PD-L1 and Ki-67 expression are significantly associated with aggressive clinicopathological features in triple-negative breast carcinoma. PD-L1 positivity correlated with larger tumor size, higher histological grade, lymph node involvement, and advanced stage, while high Ki-67 expression reflected increased proliferative activity and tumor aggressiveness. The combined PD-L1+/high Ki-67 profile demonstrated the most aggressive tumor behavior, whereas PD-L1−/low Ki-67 tumors showed more favorable characteristics. These findings suggest that both markers, particularly in combination, serve as valuable prognostic indicators and may aid in risk stratification and personalized therapeutic decision-making in TNBC.

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