

ORIGINAL ARTICLE

Programmed Death-Ligand 1 (PD-L1) expression in triple-negative breast cancer: A cross-sectional study from a Tertiary Diagnostic Laboratory in Pakistan.

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ABSTRACT... Objective: To evaluate the frequency of PD-L1 expression by combined positive score (CPS) and analyze the clinic-pathological characteristics in TNBC patients presenting at Chughtai Lab, Lahore. **Study Design:** Cross Sectional Analytical study. **Setting:** Chughtai Lab, Lahore. **Period:** Sep 2024 to Feb 2025. **Methods:** Newly diagnosed TNBC patients. Based on diagnosis, 85 patients were diagnosed with TNBC. All of the tumors were classified as invasive ductal carcinomas. Immunohistochemistry was performed via DAKO 22C3 pharm-Dx assays. PD-L1 expression was divided into three categories: CPS 0, CPS <10, and CPS ≥10. The data regarding pathological characteristics and demography like patient age, tumor grade, and Ki-67 index was collected. The SPSS version 20 was used for data analysis. **Results:** Out of 85 TNBC patients, 75 (88.2%) were classified as tumors with grade 3, with 10 (11.8%) classified as grade 2. Regarding the distribution of PD-L1 expression, we found that 7 (8.2%) patients had CPS 0, 20 (23.5%) had CPS <10, and 58 (68.2%) had CPS ≥10. Ki-67 values were greater than 14% for all tumours. PD-L1-positive expression (CPS ≥10) was observed more frequently in grade 3 tumors, and the association was statistically significant ($p < 0.001$). **Conclusion:** The study demonstrates the high prevalence of PD-L1 positivity in TNBC patients, indicating that there may be a valuable role for immunotherapy for TNBC patients. This study contributes to the growing body of evidence for the use of PD-L1 testing in the routine diagnostic process for TNBC patients in Pakistan.

Key words: Breast Carcinoma, Estrogen Receptors, Programmed Death Ligand 1, Triple Negative Breast Cancer.

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INTRODUCTION

Triple-negative breast cancer (TNBC) is one of the most aggressive types of breast cancer, accounting for approximately 10-20% of all breast cancers worldwide.¹ TNBC lacks expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), and is therefore associated with aggressive disease behaviour and limited treatment options.¹ TNBC is most prevalent among younger women worldwide.²

In recent years, immunotherapy has emerged as a promising new treatment for a variety of solid tumors, including TNBC.^{3,4} The PD-L1 positivity has become an important predictor of a patient's likelihood of responding to ICI treatment.⁵ The Combined Positive Score (CPS) is a widely used method for assessing PD-L1 expression in breast cancer.^{6,7,8} and the DAKO 22C3 pharmDx is the

most widely used assays approved by FDA.

PD-L1 testing is increasingly integral to treatment decision-making worldwide; however, patterns of PD-L1 expression vary across geographic regions, tumor subtype,s the assay employed, scoring methodology, and patient characteristics.⁹⁻¹² Within Pakistan and throughout Asia breast cancer has high morbidity due to delayed diagnosis, limited access to targeted therapies, and poor overall outcomes.¹³ Accordingly, understanding local patterns of PD-L1 expression is essential for the appropriate integration of PD-1/PD-L1-based combination therapies into existing treatment protocols.¹⁴

This study focused on PD-L1 expression using the validated DAKO 22C3 pharmDx Assay in the TNBC patient population, diagnosed at Chughtai Lab in Lahore, Pakistan.

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The findings of this study will provide critical, locally relevant data supporting the global understanding of TNBC immunobiology and the potential utility of immunotherapy as an adjunct to improve.

METHODS

This cross-sectional analytical study was conducted in Lahore at Chughtai Lab with Institutional Review Board approval (CIP/IRB/1355A) dated: 12-12-2025, having extensive immuno-histochemical (IHC) testing facilities. The total number of cases included in this study was 85 newly diagnosed cases of triple-negative breast cancer (TNBC), which had been received at Chughtai Lab during the study period. As all cases were confirmed as invasive ductal carcinoma by histopathological examination and all hormone receptors (estrogen receptor [ER], progesterone receptor [PR], and human epidermal growth factor receptor 2 [HER2]) were confirmed by IHC, it was ensured that all enrolled Tumors were ER-negative, PR-negative, and HER2-negative. To be included in the study, patients needed to have a newly diagnosed TNBC, had adequate amounts of Formalin Fixed Paraffin Embedded (FFPE) tissue for IHC analysis, and given written consent for diagnostic testing along with permission to utilize de-identified information from the diagnostic tests for research purposes. Patients were excluded from the study if they had previously received neoadjuvant chemotherapy, targeted therapy, or immunotherapy due to the potential impact of treatment exposure on PD-L1 expression. Additionally, patients whose initial presentation of breast cancer was recurrent/metastatic and where there was insufficient tumor tissue to assess PD-L1 expression were excluded as well. All tumor tissues were processed in the Chughtai Lab histopathology unit where fresh tumor tissues are routinely fixed in formalin and paraffin embed. Tumortissue sections were cut at 3-4 microns thick and subjected to IHC for PD-L1 expression utilizing the DAKO 22C3 pharmDx IHC assay. All assays were performed in accordance with the product's standardized protocol as specified by the manufacturer which included antigen retrieval, PD-L1 antibody incubation, EnVision FLEX visualization, and counterstaining. To evaluate PD-L1 expression, Combined Positive Score (CPS) was utilized which is the sum of the number of PD-L1 positive tumor cells, lymphocytes, and macrophages divided by

the total viable tumor cells, and multiplied by 100. Tumors were classified into three CPS categories based on CPS value; CPS 0, CPS <10, and CPS \geq 10. A Tumor was classified as PD-L1 positive if CPS \geq 10. The stained slides were evaluated independently by two experienced pathologists; when the pathologists had differences in scoring, a consensus score was made by means of a joint review through a multiheaded microscope.

All clinical and pathological data were entered into SPSS version 20, and data analysis was performed using this software. Patient age (quantitative variable) was reported as mean \pm standard deviation, while histological grade and PD-L1 expression category (categorical variables) were reported as frequencies and percentages. The association between PD-L1 expression and tumor grade was assessed using the chi-square test, with a p-value \leq 0.05 considered statistically significant.

RESULTS

The study consisted of an overall total of 85 TNBC (Triple-negative breast cancer) patients, who were all confirmed to have Invasive Ductal Carcinoma (IDC). The average age was 54.5 years (\pm 9.02), and the average size of the tumors was 3.7 cm (\pm 1.41). The majority of patients (75 patients; 88.2%) had High Grade tumors (Grade III) and there were 10 patients (11.8%) with intermediate grade (Grade II) tumors. All patients had negative estrogen receptor (ER), progesterone receptor (PR), and HER2 status, confirming their TNBC status. All patients exhibited a high proliferation index as measured by Ki-67 (>14%). Regarding tumor staging, T2 was the most common stage (60% of patients) followed by T3 (24.7% of patients) and T1 (15.3% of patients). Nodal involvement was noted in 58 patients. The N1 stage was noted in 34 patients (40%), N2 was noted in 24 patients (28.2%), and N0 was noted in 27 patients (31.8%) (Table-I).

PD-L1 expression, assessed by the Combined Positive Score (CPS), showed that 58 tumors (68.2%) were PD-L1 positive (CPS \geq 10), 20 tumors (23.5%) had CPS <10, and 7 tumors (8.2%) showed no PD-L1 expression (CPS 0) (Table-II). On histopathology, Grade 3, invasive ductal breast carcinoma showing Estrogen, progesterone, and Her2 negative expression, but PD-L1

positive expression (Figure-1).

When stratified by histological grade, PD-L1 positivity (CPS ≥ 10) was predominantly observed in grade 3 tumors, with 58 of 75 grade 3 tumors (77.3%) demonstrating CPS ≥ 10 compared to none of the grade 2 tumors. Conversely, all seven CPS 0 cases were grade 2 tumors, while CPS < 10 was observed in three grade 2 and 17 grade 3 tumors. Pearson's chi-square test indicated a statistically significant association between PD-L1 expression and tumor grade ($\chi^2 = 60.435$, $df = 2$, $p < .001$). These findings suggest that PD-L1 positivity is more frequent in higher-grade TNBC tumors (Table-III).

TABLE-I

Clinicopathological characteristics of TNBC Patients (N = 85)

Variable	Category / Value	n (%) / Mean \pm SD
Age (years)	Mean \pm SD	54.5 \pm 9.02
Tumor size (cm)	Mean \pm SD	3.7 \pm 1.41
Histological Grade	Grade 2	10 (11.8%)
	Grade 3	75 (88.2%)
ER/PR/HER2	All negative	85 (100%)
Ki-67	>14%	85 (100%)
T Stage	T1	13 (15.3%)
	T2	51 (60.0%)
	T3	21 (24.7%)
N Stage	N0	27 (31.8%)
	N1	34 (40.0%)
	N2	24 (28.2%)

TABLE-II

PD-L1 Expression by CPS

CPS Category	n (%)
CPS 0	7 (8.2%)
CPS < 10	20 (23.5%)
CPS ≥ 10	58 (68.2%)

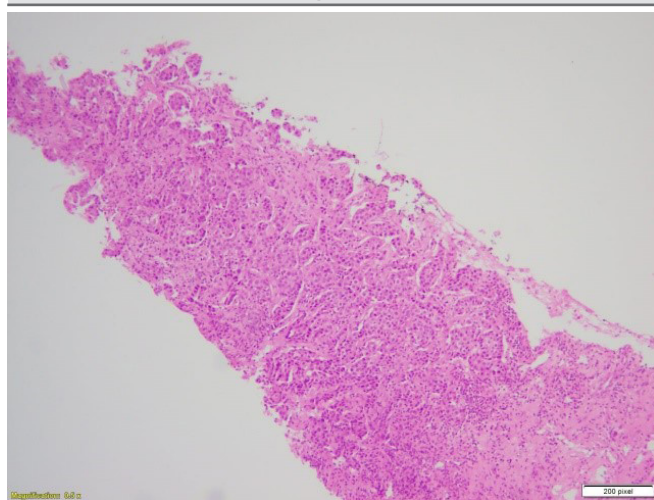
TABLE-III

Association between tumor grade and PD-L1 expression

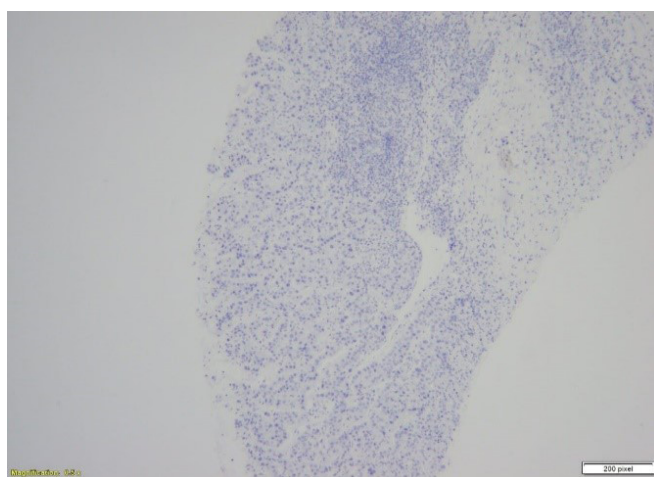
CPS Category	Grade 2	Grade 3	Total	P
CPS 0	7	0	7	$<.001$
CPS < 10	3	17	20	
CPS ≥ 10	0	58	58	
Total	10	75	85	

FIGURE-1

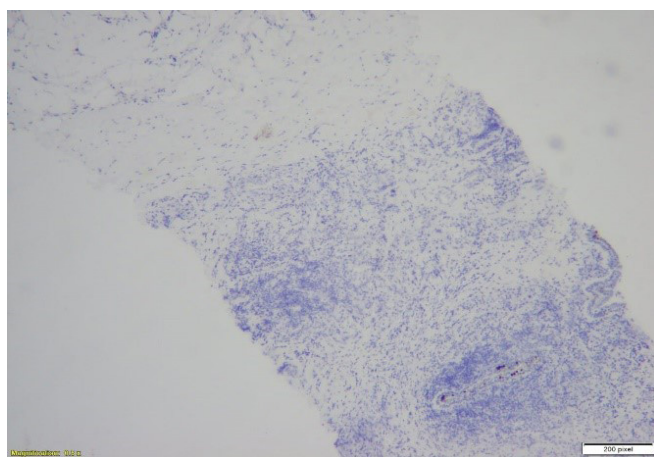
Histopathological evaluation of Invasive ductal carcinoma, ER, PR, Ki67, and PD-L1 expressions



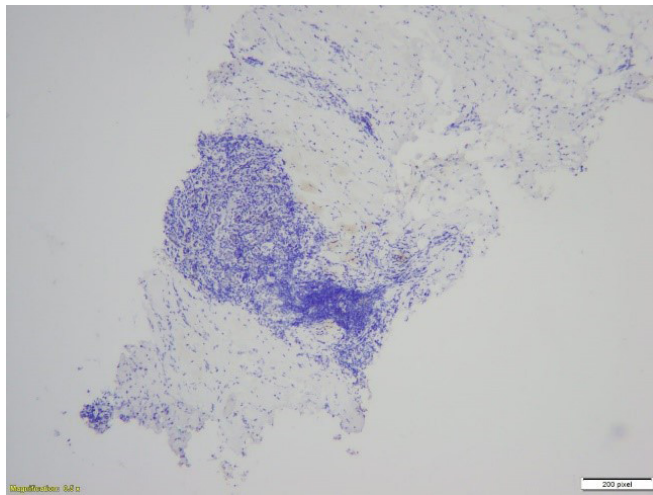
Invasive ductal carcinoma grade 3



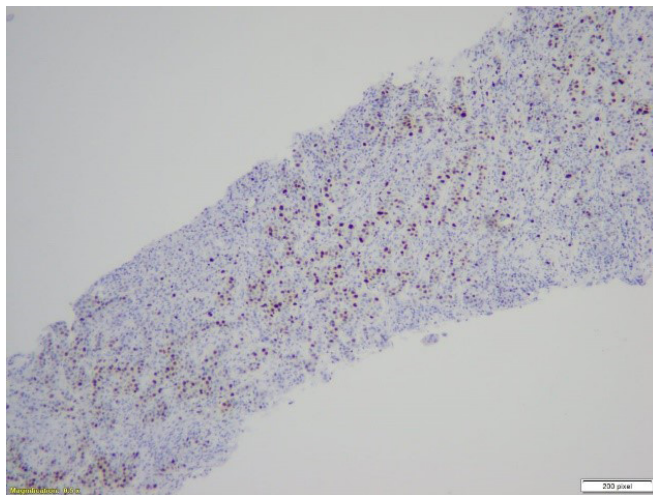
Negative Estrogen Expression



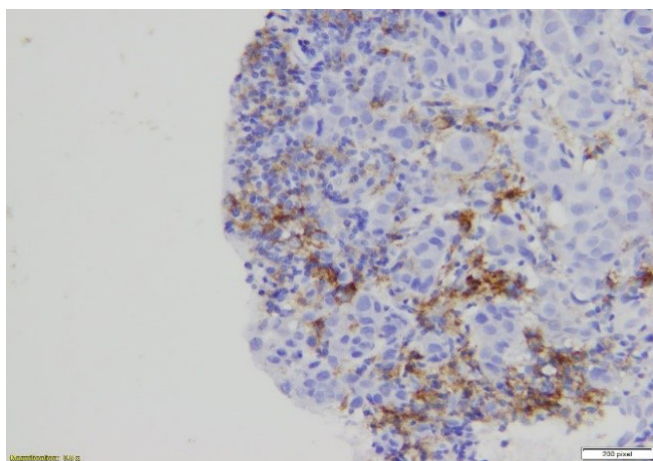
Negative Progesterone Expression



Negative HER2 Expression



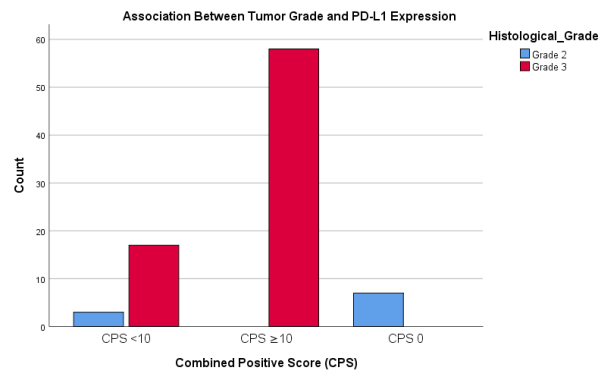
Ki67 was >14%



PD-L1

FIGURE-2

PD-L1 positivity (CPS ≥ 10) was more common in grade 3 tumors.



DISCUSSION

Triple-negative breast cancer is recognized as a highly aggressive breast cancer subtype with limited treatment options and elevated mortality rates. In recent years, there has been a growing body of research focused on increasing the accuracy of assessing PD-L1 in TNBC. Cha et al. (2021) compared PD-L1 expression between 22C3, SP142 and SP263 antibody clones as part of their study of TNBC specimens, they concluded that 22C3 assay provides much greater prognostic information than either SP142 or SP263 assay; particularly regarding resected breast carcinomas.¹ Huang et al. (2021) directly compared the three PD-L1 immunohistochemistry assays approved by the FDA showing large differences in the rates of positivity for all three assays². A multicenter cohort study published in 2023 found PD-L1 positivity in $\approx 60\%$ of TNBC samples utilizing a cutoff value of CPS ≥ 10 ^{15,16} and it was further validated in other studies.¹⁷

The current findings matches the therapeutic indications for administering PD-1 inhibitors for the treatment of TNBC.¹⁸ Cortés et al. (2022) reported that in patients with metastatic TNBC with PD-L1 CPS ≥ 10 , pembrolizumab added to chemotherapy improved treatment outcomes.³ Schmid et al. (2022) also found similar results.⁴ All of the tumors studied here had a high Ki-67 index (>14%), consistent with the known high-proliferative phenotype of TNBC.¹⁹ Similarly, higher-grade tumors (69.4%) had greater association with PD-L1 positivity than lower-grade tumors (17.6%).²⁰

The observed positivity rates suggest that an interesting proportion of Pakistani TNBC patients may potentially be candidates for pembrolizumab-based regimens. Strengths of this investigation lie primarily in the fact that it was carried out on a clinically homogenous cohort of TNBC and utilized the therapeutically pertinent 22C3 assay. Notwithstanding, there are some apparent limitations, such as the cross-sectional nature of the survey and the lack of information about survival or granular tumour-related metrics which may be addressed in future studies.

CONCLUSION

This study reveals high prevalence of PD-11 positivity in Pakistani triple negative breast cancer (TNBC) patients. This tumor cohort predominantly included high-grade highly proliferative lesions, highlighting the aggressive biological nature of TNBC. These data suggest that PD-1L1 testing may help to identify patients who are likely to benefit from immune checkpoint inhibition. While the integration of routine PD-1L1 assessment into personalized therapeutic approaches needs to be considered, further multicenter, outcome-based studies are needed to determine the efficacy of immunotherapy in this population and provide evidence-based guidelines.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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2	Zonaira Rathore: Data collection.
3	Fatima Khalid: Data analysis.
4	Saira Rathore: Manuscript writing.
5	Omar Chughtai: Data entry.
6	A S Chughtai: Critical analysis.