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Original Article

Pathology Section

Programmed Death-Ligand 1 Immunoexpression in Triple Negative Breast Carcinoma in South Indian Women: A Cross-sectional Study at a Tertiary Care Centre

D DARSHITHA[1], PADMA PRIYA KASUKURTI[2], CLEMENT WILFRED DEVADASS[3]

ABSTRACT

Introduction: Triple Negative Breast Cancer (TNBC) comprises 10-20% of all breast cancer cases and is characterised by a lack of differentiation, subpar response to therapy and a low survival rate. Programmed Death-Ligand 1 (PD-L1) is a type 1 transmembrane protein present in cancer cells. The interaction between PD-1 (present on immune T cells) and PD-L1 facilitates negative immunoregulation, allowing cancer cells to elude the body's immune system, resulting in tumour proliferation. This immune "brake" system translates to the presence of PD-L1 being associated with a poor prognosis and has been linked to low overall survival. Immune checkpoint inhibitors, which are immunomodulatory chemotherapeutic drugs, have the potential to disrupt this interaction and enable Immune Cells (IC) to attack Tumour Cells (TC).

Aim: To analyse the immunohistochemical (IHC) expression of PD-L1 in TNBC patients and examine its association with clinicopathological prognostic variables such as patient age, laterality of the lesion, histopathological type, grade of the tumour, tumour size, lymphovascular invasion, perineural invasion, lymph node involvement, Ki-67 labelling index and tumour stage.

Materials and Methods: This retrospective, cross-sectional study was conducted on 30 cases of modified radical mastectomy specimens from treatment-naïve TNBC patients, received in the Department of Pathology at Ramaiah Medical College, Bengaluru, Karnataka, India from January 2014 to January 2023. Data such as age, sex, tumour size, histological type, histologic grade, lymph node status and hormonal receptor status were collected. Haematoxylin and Eosin (H&E) slides were reviewed and sections containing the highest proportion of viable TCs with surrounding normal breast tissue and IC interface were chosen. IHC detection of PD-L1 was performed on 4-5 µm thick sections of the tumour. The PD-L1 scores of tumour and ICs were determined and the proportions of positive and negative cases were analysed with various clinicopathological variables.

Results: Among the 30 TNBC cases studied for PD-L1 immunoexpression, 70% of the cases (21/30) showed a positive TC score (>1%) and a combined positive score (TC+IC) >1%. IC positivity was observed in 73.33% (22/30) of the cases. A higher histological grade (grade 3) and a patient age older than 50 years were significantly associated with positive TC scores (p-value=0.043), IC scores (p-value=0.049), and combined positive scores in this study.

Conclusion: This study highlights the significant presence of PD-L1 immune expression in TNBC, paving the way for its potential use as a promising marker for identifying patients suitable for targeted immunotherapy in the TNBC phenotype. Future studies are expected to harmonise clone selection and scoring criteria, enabling the development of companion diagnostic kits specifically relevant to TNBC.

Keywords: Immune expression, Immunohistochemistry, Triple negative breast cancer

INTRODUCTION

Breast carcinoma is the leading cause of death among women worldwide. The incidence and fatality rates for breast cancer in India are approximately 14% and 11%, respectively [1]. Breast cancer is molecularly classified to assist with therapeutic decisions. The main intrinsic subtypes include Luminal A, Luminal B, Her-2 neu and triple-negative groups. It is crucial to differentiate between these subtypes based on the genetic or surrogate immunohistochemical expression of Oestrogen Receptor (ER), Progesterone Receptor (PR), HER-2 neu and Ki-67 proliferation indices for effective stratification, prognostication and individualisation of treatment modalities for each subtype [2].

The TNBC account for 12-17% of all breast cancers and represent a physiologically aggressive subtype. Clinically and molecularly, TNBC is heterogeneous and serves as an umbrella term that encompasses various subtypes, particularly the immunomodulatory type. In recent years, there has been a concerted effort to develop specialised therapies tailored to these specific molecular subtypes [3]. PD-L1 expression has been investigated in various malignancies and has been associated with different survival rates in tumours, including squamous cell carcinomas, melanomas, gliomas, lung carcinoma, and colorectal carcinomas. The ligand of PD-1, a type 1 transmembrane protein, is known as PD-L1. When PD-1 binds to PD-L1, it suppresses the expression of proinflammatory and antiapoptotic factors, allowing cancer cells to evade the host immune response and enhance proliferation [4]. PD-L1 plays a crucial role in modulating the immune response against TCs by binding to the immune-inhibitory receptor known as PD-1, a member of the B7-CD28 gene superfamily [5]. PD-L1 overexpression has been linked to increased neoplastic growth, resistance to treatment and cancer recurrence. Studies have also shown that adding anti-PD-L1 medication (targeted therapy) to standard chemotherapy regimens improves survival in metastatic TNBC with positive IHC PD-L1 expression on tumour and ICs [6].

Similar to PD-L1, Ki-67 is also utilised as a biomarker in the TNBC subtype. Since aggressive cancer biology and tumour proliferation are significantly correlated with Ki-67 expression, it is increasingly recognised as a superior prognostic biomarker and its association

with PD-L1 is being studied [7]. As immune checkpoint inhibitors, monoclonal antibodies targeting PD-L1 expression disrupt the aggressive triple-negative molecular pathways, disabling the immune evasion mechanisms. With targeted immunotherapy, this subset of treatment-variable TNBCs—linked to poor overall survival and worse prognosis—shows promising results [8]. The current study aimed to evaluate the proportion of TNBC cases expressing PD-L1 immunohistochemically and to associate this immunoexpression with clinicopathological variables.

MATERIALS AND METHODS

A retrospective, cross-sectional study was conducted over a period of four months, using data available from January 2014 to January 2023 at the Department of Pathology, Ramaiah Medical College, Bengaluru, Karnataka, India.

Inclusion criteria: All consecutive treatment-naïve mastectomy cases diagnosed as triple-negative carcinomas available in the department were included in the study.

Exclusion criteria: Cases diagnosed via core biopsy and mastectomy specimens following chemotherapy were excluded from the study.

Sample size: A minimum sample size of 30 was calculated for statistical probability, and the samples were selected using a convenient sampling method.

Study Procedure

The clinical details and histopathology slides from formalin-fixed paraffin-embedded blocks of all TNBC cases in the study period were retrieved from the archives and reviewed. The tumour blocks with the highest proportion of viable TCs and tumour-infiltrating ICs were selected and submitted for immunohistochemistry.

IHC for PD-L1 was performed using clone CAL 10, following standard protocols. Although SP-142 is FDA-recommended, numerous other clones are being studied for their comparability and effectiveness, with CAL-10 being one of them. Appropriate positive (tonsil) and negative internal controls were used. PD-L1 positivity was separately assessed in TCs, ICs and a combined positive score (CPS=TC+IC) was also derived. Incomplete, partial, or complete membrane staining of any intensity was considered as positive staining. Lymphocytes, plasma cells and macrophages were included in the IC scoring [6].

A semiquantitative estimation of the positive TC percentage was made using 100 viable TCs as the denominator. All TCs and ICs on the entire slide were systematically counted to obtain the mean score. For the combined cell score, TC and IC scores were added. All cases were categorised as positive or negative for PD-L1 immune expression using 1% as the cut-off for TC score, IC score and CPS. Null expression and <1% immunoexpression were considered negative. The proportion of positive and negative cases was determined, and these findings were further correlated with clinicopathological variables like patient age, laterality of the lesion, histopathological type, tumour grade, tumour size, lymphovascular invasion, perineural invasion, lymph node involvement, Ki-67 labelling index and tumour stage. A cut-off of 30% for Ki-67 was used to distinguish between different prognostic subtypes in TNBC [7].

STATISTICAL ANALYSIS

The data were entered into Microsoft Excel, and statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS) version 22.0. The categorical data are expressed in frequencies and percentages. The combined scores and PD-L1 scores of tumour and ICs were analysed with other variables using the Chi-square test. A p-value <0.05 was considered significant in all cases.

RESULTS

The present study observed that out of the 30 patients with TNBC, a majority (56.66%) belonged to the age group under 50 years, with the mean age at presentation being 49 years. Among them, 19 cases (63.33%) presented with left-sided breast carcinoma, and half of the patients had T2 tumour size (17 out of 30). Lymphovascular invasion was seen in 12 (40%) of the patients, whereas perineural invasion was observed in only 10% of the cases (n=3). Eleven out of the 30 patients had nodal involvement (36.66%). Additionally, 56.66% of the TNBC patients in the study belonged to stage 2 cancer [Table/Fig-1].

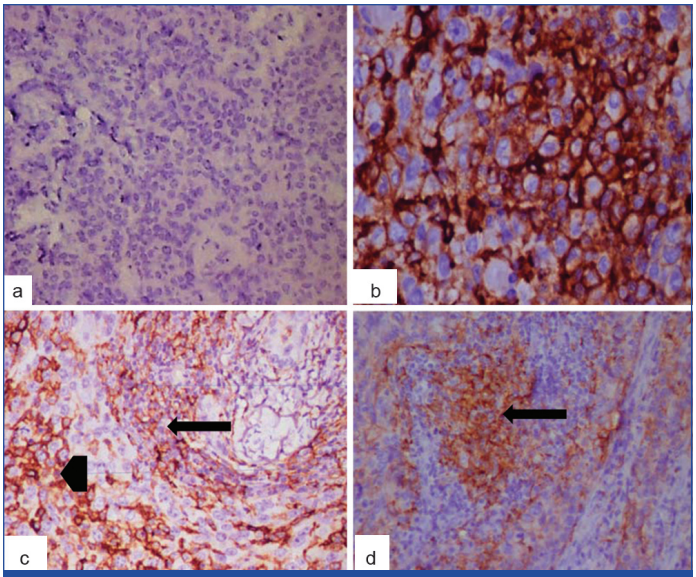
Parameters	n (%)
Laterality	
Right	11 (36.67)
Left	19 (63.33)
Histopathological type	
IDC-NOS	27 (90)
Medullary	3 (10)
Grade of tumour	
1	1 (3.33)
2	13 (43.33)
3	16 (53.33)
Tumour size	
T1	5 (16.67)
T2	17 (56.66)
T3	8 (26.67)
Lymphovascular invasion	
Present	12 (40)
Absent	18 (60)
Perineural invasion	
Present	3 (10)
Absent	27 (90)
Ki-67 (done in 17 cases)	
<30%	3 (17.65)
>30%	14 (82.35)
Tumour stage	
1	5 (16.67)
2	17 (56.66)
3	8 (26.67)

[Table/Fig-1]: Baseline variables for the study subjects (n=30).

PD-L1 combined positive scores and TC positive scores of >1% were seen in 21 out of 30 cases (70%), while IC scores of >1% were found in 73.33% of the cases (n=22) [Table/Fig-2]. PD-L1 scores were significantly associated with the age of the patients, with 11 subjects over 50 years of age showing higher scores with positivity of >1% (p-value of 0.043). PD-L1 IC scores were also higher in older age groups, with a significant p-value of 0.049 [Table/Fig-3]. The histological grade of the tumour was significantly associated with PD-L1 combined scores and IC scores, with higher-grade tumours displaying scores of >1%. Ki-67 immunoexpression was found not to be associated with PD-L1 scores [Table/Fig-3].

DISCUSSION

PD-L1, also known as CD 279, was originally described by Ishida et al., in mouse T cell tumours. PD-1, a transmembrane protein of the CTLA-4 superfamily, is widely expressed on ICs like activated T cells, B cells and monocytes and negatively regulates immune responses by binding with its ligand PD-L1, which belongs to the B7 family of T cell co-inhibitory molecules. PD-1 consists of an extracellular IgV-like domain and its cytoplasmic region has an Immunoreceptor



[Table/Fig-2]: a) Tumour Cells (TC) negative for PD-L1 immunostaining(10x); b) Tumour Cells (TC) positive for PD-L1 immunostaining (complete, intense, membranous staining in >1% of TCs,40x); c) Tumour Cells (TC) (arrow head) and Immune Cells (IC) (arrow) positive in the same case (>1% cells); d) Only Immune Cells (IC) positive at the interface (>1% cells, arrow).

Parameters	Combined score and PD-L1 Tumour Cell (TC) score		PD-L1 Immune Cell (IC) score	
	<1% (n=09)	>1% (n=21)	<1% (n=08)	>1% (n=22)
Age (years)				
<50	7 (77.78%)	10 (47.62%)	6 (75%)	11 (50%)
>50	2 (22.22%)	11 (52.38%)	2 (25%)	11(50%)
p-value	0.043*		0.049*	
Laterality				
Right	3 (33.33%)	8 (38.10%)	3 (37.5%)	8 (36.36%)
Left	6 (66.67%)	13 (61.90%)	5 (62.5%)	14 (63.64%)
p-value	0.571		0.637	
Histopathological type				
IDC-NOS	9 (100%)	18 (85.71%)	8 (100%)	19 (86.36%)
Medullary	0	3 (14.29%)	0	3 (13.64%)
p-value	0.328		0.379	
Grade of tumour				
1	0	1 (4.76%)	0	1 (4.55%)
2	7 (77.78%)	6 (28.57%)	6 (75%)	5 (22.73%)
3	2 (22.22%)	14 (66.67%)	2 (25%)	16 (72.73%)
p-value	0.0432*		0.0307*	
Tumour size				
T1	3 (33.33%)	3 (14.29%)	3 (37.5%)	3 (13.63%)
T2	4 (44.45%)	11 (52.38%)	3 (37.5%)	12 (54.55%)
T3	2 (22.22%)	7 (33.33%)	2 (25%)	7 (31.81%)
p-value	0.477		0.350	
Lymphovascular invasion				
Present	5 (55.56%)	7 (33.33%)	5 (62.5%)	7 (31.82%)
Absent	4 (44.44%)	14 (66.67%)	3 (37.5%)	15 (68.18%)
p-value	0.231		0.137	
Perineural invasion				
Present	1 (11.11%)	2 (9.52%)	1 (12.5%)	2 (9.09%)
Absent	8 (88.89%)	19 (90.48%)	7 (87.5%)	20 (90.91%)
p-value	0.672		0.621	
Lymph node involvement				
Present	4 (44.44%)	7 (33.33%)	4 (50%)	7 (31.82%)
Absent	5 (55.56%)	14 (66.67%)	4 (50%)	15 (68.18%)
p-value	0.479		0.371	
Ki-67 (done in 17 cases)				
<30%	0	2 (14.29%)	0	2 (13.33%)
>30%	3 (100%)	12 (85.71%)	2 (100%)	13 (86.67%)
p-value	0.187		0.526	
Tumour stage				
1	2 (22.22%)	3 (14.29%)	2 (25%)	3 (13.63%)
2	5 (55.56%)	12 (57.14%)	4 (50%)	13 (59.09%)
3	2 (22.22%)	6 (28.57%)	2 (25%)	6 (27.27%)
p-value	0.845		0.759	

[Table/Fig-3]: PD-L1 scores in relation to different variables in the study.

Tyrosine-based Inhibitory Motif (ITIM) and an Immunoreceptor Tyrosine-based Switch Motif (ITSM). The interaction between PD-1 and PD-L1 is primarily responsible for preventing autoimmunity and regulating immune tolerance. In cancer, this same mechanism allows PD-L1 expressed by TCs to inhibit T cell activation, facilitating cancer immune escape [9].

PD-L1 is widely studied in various cancers, including breast cancer, glioma, melanoma and lung cancer, due to its significant high expression and role in maintaining the immunosuppressive microenvironment in cancer. In breast cancer, PD-L1 expression is found to be highest in TCs and ICs in triple-negative carcinoma, particularly in basal-like types and serves as a promising prognostic marker. While gene expression data on PD-L1 is more reproducible, surrogate immunohistochemical analysis has proven to be heterogeneous, with inconsistencies depending on the tumour type, the antibody used, cut-off values for immunopositivity, the type of cell studied and the study population. However, triple-negative tumours are aggressive and often unresponsive to standard chemotherapeutic regimens. In this context, cancer immunotherapy is continuously explored and the use of anti-PD-L1 checkpoint inhibitors is evaluated for their prognostic and predictive roles. Although the routine implementation of PD-L1 testing has faced numerous technical challenges, several research studies have demonstrated better overall survival in triple-negative patients with PD-L1 positivity when treated with targeted therapy like atezolizumab prior to chemotherapy [10,11].

In a meta-analysis of 38 reports, the overall pooled expression rate of PD-L1 in breast tumour tissue was found to be 24% in TCs, 33% in ICs, and 25% in both TCs and ICs [10]. Doğukan R et al., studied 61 TNBC cases and established PD-L1 positivity of 37.7% in TCs and 47.5% in the tumour microenvironment. The authors did not find a significant association with any clinicopathological variables except Ki-67 [11]. Shi S et al., studied 89 cases of TNBC and 62 cases of normal breast tissue, detecting PD-L1 expression in 29.2% of TNBC cases and concluding that its expression was significantly higher compared to normal breast tissue. In this study, PD-L1 was also positively correlated with tumour size, Ki-67 proliferative index and Tumor, Nodes, Metastasis (TNM) staging [12].

Guo H et al., evaluated PD-L1 using a 1% cut-off and concluded that PD-L1 was positive in 35% of TNBC cases by combined TC score and IC score, 31% by IC score, and 16% by TC score. The authors concluded that PD-L1 by immune score is associated with worse clinical outcomes [13]. Oner G et al., used the SP 263 clone and considered PD-L1 greater than 1% as positive, finding that 50% of TNBC cases were PD-L1 positive in TCs and 46% in ICs. These authors also suggested that patients with a high PD-L1 expression in TCs were likely to have a better outcome [14].

Srivastava V et al., studied 30 patients with locally advanced breast cancer, demonstrating that the majority were in the age group of 41 to 50 years. The authors categorised PD-L1 immunoexpression using the modified H-score and, importantly, studied its expression before and after neoadjuvant chemotherapy. They found that 36.7% of cases were positive for PD-L1, indicating a statistically significant

change in PD-L1 expression from positive to negative after the administration of neoadjuvant chemotherapy (p-value=0.036). This is a significant observation, as changes in PD-L1 expression in the same patient before and after chemotherapy may serve as a predictor of neoadjuvant chemotherapy response [15].

Huang X et al., studied the concordance between three approved PD-L1 assays, the DAKO 28-8, DAKO 22C3, and Ventana SP 142, and employed similar scoring methods and cut-offs as the present study. The authors concluded that these three clones did not agree with each other, exhibiting different rates of positivity. The present study's high rate of PD-L1 positivity could similarly be explained [16].

The current study attempted to evaluate PD-L1 immunoexpression in treatment-naive TNBC cases using a 1% cut-off in TCs, ICs and a combined positive score (CPS/TCIC) and demonstrated positivity in a high proportion of cases. CPS and TCs were positive in 70% of cases, while ICs were positive in 73% of cases. Only higher histological tumour grade and an age over 50 years in patients were significant variables associated with positive PD-L1 immune expression.

Limitation(s)

One limitation of the present study was that the sample size concerning treatment-naive triple-negative breast carcinomas is small, as the incidence of TNBC was low. Furthermore, PD-L1 immunoexpression is heterogeneous and depends on numerous variables, including the population studied, the cell type examined, the antibody clones used, the established cut-off, the histology and hormone receptor status of the tumour and the tumour microenvironment, which could have influenced the results obtained in this study.

CONCLUSION(S)

The present study reported high PD-L1 expression in TCs and ICs in the stromal microenvironment of TNBC. This finding has promising therapeutic implications for the use of targeted immune checkpoint inhibitors. However, PD-L1 expression is highly heterogeneous, with variable expression in TCs and ICs in different parts of the tumour and across different clones. It is suggested that a more uniform and internationally standardised protocol is urgently needed to explore the full potential of PD-L1 as an impactful biomarker in breast cancer. Therefore, further research is warranted to understand the extensive role of PD-L1 in determining treatment for TNBC.

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PARTICULARS OF CONTRIBUTORS:
1. Resident, Department of Pathology, Ramaiah Medical College, Bengaluru, Karnataka, India.
2. Assistant Professor, Department of Pathology, Ramaiah Medical College, Bengaluru, Karnataka, India.
3. Professor, Department of Pathology, Ramaiah Medical College, Bengaluru, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Padma Priya Kasukurti,
Assistant Professor, Department of Pathology, Ramaiah Medical College,
M S Ramaiah Road, Gokula, Bengaluru, Karnataka, India.
E-mail: padmakasukurti@gmail.com

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