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Expression of Programmed Death Ligand 1 (PD-L1) in Breast Cancer patients in India and its Correlation with Prognostic Parameters

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ABSTRACT

Background: The PD-1/PD-L1 pathway represents an adaptive immune resistance mechanism that is exerted by tumor cells. The study was conducted to evaluate the expression of PD-L1 in breast carcinoma in India and to find out its correlation with prognostic parameters. Despite numerous studies, there is a lack of literature for such studies in Indian patients. Moreover, the results obtained from these studies have not been uniform.

Methods: A hospital-based cross-sectional study was conducted on 150 cases of breast carcinoma. The invasive cancer specimens were assessed for routine microscopy and classified into various histopathological subtypes. Bloom Richardson grading was done. Immunohistochemistry for surrogate molecular classification as well as PD-L1 was performed. PD-L1 expression was then compared with several prognostic parameters such as tumor subtype, tumor grade, surrogate molecular classification and pTNM stage. Data analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. A P value of <0.05 was considered statistically significant.

Results: PD-L1 was positive in 14.67% of patients with score 1 in 6% and score 2 in 8.67% of patients. The PD-L1 expression showed a positive correlation with the tumor of higher grades (grade3). It was significantly higher among IBC with medullary features as compared to IBC-NST, IBC with papillary features and the Lobular type. PD-L1 showed a significant association with surrogate molecular classification as well. Its expression was found to be the highest in Triple negative breast cancer subtype as compared to tumors showing ER/PR positivity ($p<0.05$). However, there was no significant association between PD-L1 and TNM staging.

Conclusion: This study revealed a significant association between PD-L1 several prognostic factors such as higher tumor grade (grade3), Triple Negative breast cancer, and IBC with the medullary pattern subtype. The association between PD-L1 and such prognostic parameters signifies its role in tumor mechanism and makes it a potential target for immunotherapy especially in Triple Negative breast cancer cases which lack specific targeted therapies. Moreover, paucity of literature on PD-L1 in breast cancer patients in India and the data showing conflicting results make this study valuable. Our study shall be helpful in further adding to the knowledge of PD-L1 expression in breast cancer especially in Indian women.

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INTRODUCTION

Breast cancer occurs in every country of the world in women at any age after puberty but with increasing rates in later life. In 2020, there were 2.3 million women diagnosed with breast cancer and 685000 deaths globally. Breast cancer is associated more with lost

disability-adjusted life years (DALYs) than any other type of cancer. Improvements in survival began after 1980s due to early detection programmes combined with different modes of treatment available.[1]

Breast cancer is the second leading cause of death. This disease is the primary cause of mortality among women aged 45–55 years, and is the second leading cause of cancer- induced death.[2] There is a large variation in breast cancer survival rates around the world: with an estimated 5-year survival of 80% in high income countries to below 40% for low-income countries. Low- and middle-income countries face resource and infrastructure constraints that challenge the goal of improving breast cancer outcomes by early detection, diagnosis and treatment.[3]

In India, breast cancer has been ranked as number one cancer among females with an age-adjusted rate as high as 25.8 per 100,000 women and mortality of 12.7 per 100,000 women.[4] Among the different breast cancer subtypes, prevalence of triple negative breast cancer (TNBC) has been found to be ranging from 6.7% to 27.9%, with the highest reported percentage in India. The treatment of TNBC is difficult due to lack of targeted therapies. Hence, further research is required for the development of new treatment modalities.[5]

Recently, the role of programmed death ligand 1 (PD-L1) has been explored in relation to breast cancer and its prognosis. PD-1 (programmed cell death-1) is expressed on the surface of activated T cells and PD-L1, its ligand is a T cell inhibitory molecule.[6] Under normal conditions, the immune system performs a series of steps which lead to cancer cell death, known as the cancer immunity cycle. The PD-1/PD-L1 pathway represents an adaptive immune resistance mechanism that is exerted by tumor cells. The PD-L1 expressed on the tumor cells binds to PD-1 receptors on the activated T cells, which leads to the inhibition of cytotoxic T cells. These deactivated T cells remain inhibited in the tumor micro-environment, thus helping in tumor progression.[7]

Discovery of methods to overcome these mechanisms of tumor resistance is a key area of immune oncology research.[8] Over the past few decades, various PD-1/PD-L1 inhibitors have been developed for the treatment of various types of cancer.[9] In a worldwide clinical study, the use of a PD-L1 inhibitor in combination with nab-paclitaxel was shown to significantly prolong the progression-free survival of patients with metastatic triple-negative breast cancer compared to nab-paclitaxel monotherapy.[10]

Since the approval of pembrolizumab for the treatment of advanced melanoma in September 2014, the clinical development of PD-1/PD-L1 inhibitors as anticancer agents has broadened.[11] Recently, the FDA has approved PD-1/PD-L1 inhibitors for the treatment of nine cancer types.[11] This makes research into the regulatory mechanisms of PD-1/PD-L1 expression in cancer cells intriguing.

In the last few years, anti-PD-1/PD-L1 (Programmed death-ligand 1) agents have been evaluated in breast cancer, particularly in the triple negative subtype, with promising results observed when delivered as monotherapy or in combination with conventional treatment.[12]

Several studies have been conducted to evaluate the expression of PD-L1 in breast cancer. Muenst S *et al.* conducted a study on 650 breast cancer cases.[13] PD-L1 was expressed in 152 (23.4 %) of the 650 breast cancer specimens. Expression was significantly associated with several factors like age, tumor size, grade, AJCC primary tumor classification, lymph node status, absence of ER expression, and high Ki-67 expression.

Botti *et al.* attempted to define a standardized protocol suggesting a tumor score for the evaluation of PD-L1 expression in breast cancer cells.[14] In all samples, both qualitative and quantitative parameters were considered. The same immunohistochemistry score has been adopted in our study.

Despite numerous studies, there is still a lack of literature for such studies in Indian patients. Moreover, the results obtained from these studies have not been uniform. Since PD-L1 represents a potential prognostic biomarker in many solid tumors including breast carcinoma, its expression needs to be evaluated in order to understand the role of targeted immunotherapy. Hence, we conducted this study to evaluate the expression of PD-L1 in breast cancer patients in India and find out its correlation with prognostic parameters.

METHODS

Study Design

This study was conducted in the Department of Pathology, Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi.

This is a hospital based cross-sectional study and has received clearance from the hospital's Ethics Committee. Informed written consent was obtained from each patient. Data on each patient was obtained through duly filled clinical forms which were paper-based.

The study was carried out for a duration of 18 months from September 2018 to March 2020. The participants were histopathologically confirmed cases of Invasive Breast Carcinoma (IBC).

Sample Size

As per the study done by Muenst S. *et al.*,[13] 650 breast cancer patients were taken and PD-L1 was

found to be expressed in 152 out of 650 (i.e., 23.4% breast cancer specimens).

Applying the formula to calculate sample size for qualitative variables according to prevalence- $1.96^2xpq/d^2$

Where, p= prevalence (from previous studies)

q=1-p

d= allowable error (10%)

Sample Size n= 3.84x0.23x0.77/0.10x0.10

n= 68

Hence, by using the above formula, a minimum of 68 patients had to be enrolled in this study.

A total of 150 breast carcinoma cases were enrolled in the study, including mastectomy specimens and core needle biopsies. All cases of breast carcinoma received in the Department of Pathology, Safdarjung Hospital. All histo-pathologically proven cases of Invasive Breast Carcinoma were included in this study. Patients who had previously received any form of chemotherapy/radiotherapy for breast cancer and those with the diagnosis of breast sarcomas, metastatic lesions, benign lesions and in situ carcinomas of the breast were all excluded.

Clinical Details

Routine clinical details like file Number, name, age, sex, and clinical diagnosis were taken for every patient.

Collection and Preparation of Materials

Procedure

Core needle biopsies of the breast tissue and mastectomy specimens were obtained in 10% formalin and the representative tissue was grossed and processed as routine. Routine 4 to 5µm sections were cut and sections were stained with hematoxylin and eosin as per the standardised procedure.[15]

Every stained section was evaluated for the following:

Histological grading of the tumor, according to the modified Bloom–Richardson–Elston grading system[16] is given in Table 1.

Pathological Staging pTNM was assessed as per the 8th edition of AJCC Staging System for Breast cancer, wherever possible.[17]

Immunohistochemistry (IHC) for Estrogen Receptor (ER), Progesterone Receptor (PR), Human epidermal growth factor receptor 2 (Her-2neu), Ki 67 and PD-L1 was performed using their respective antibodies.

PD-L1 IHC was done using concentrated and prediluted rabbit monoclonal antibody (Biocare Medical, Catalog number ACI 3171 A, C and API 3171 AA). Clone use was CAL 10.

Table 1. Demographic details and clinical data

Parameter	Frequency
Age (years)	
21-40	39
40-70	106
>70	5
Histopathological subtype	
IBC, NST	141
IBC with medullary pattern	5
Lobular carcinoma	3
Solid invasive papillary carcinoma	1
Grade	
Grade 1	9
Grade 2	89
Grade 3	46
Surrogate Molecular Classification	
Her 2 neu enriched	25
Luminal A	30
Luminal B	51
Triple negative	44
Tumor (T) Stage	
T1	5
T2	43
T3	22
T4	10
Lymph node (N) stage	
N0	18
N1	27
N2	22
N3	10
PD-L1	
Negative	128
Positive	22

STEPS for IHC

Paraffin blocks of relevant sections as revealed by routine diagnosis were cut on poly-L-lysin coated slides which was followed by deparaffinization. Sections were taken through descending concentration of alcohol and ultimately to water. Then, 3% Hydrogen peroxide was added followed by Antigen Retrieval which was carried out using the pressure cooker heating technique. A sniper provided by the same company Biocare was put on each slide to reduce nonspecific background staining. This was followed by the application of Primary Rabbit Monoclonal antibody for 1 hour, and then secondary antibody for 45 minutes. Slides went through several cycles of washes using tris buffer after every significant step. Chromogen-DAB (diamino-benzidine) was used to highlight the antibody expression and Hematoxylin was used as a counter stain. The slides were dehydrated in ascending concentrations of alcohol followed by mounting use of DPX.

Immunohistochemistry for ER, PR, Her-2neu and Ki-67 was used to assess Surrogate Molecular Classification which is as follows:[17]

- Luminal A- ER, PR Positive and Ki 67 <14%
- Luminal B- ER, PR Positive; Her-2neu Negative/Positive and Ki 67 >=14%
- Basal type or Triple Negative- ER, PR, HER-2neu Negative
- Her-2neu Enriched- Her-2neu positive

PD-L1 score was assigned to every case based on the following immunohistochemistry score:[14] Score 0: absence of membranous positivity or mild/moderate cytoplasmic positivity. Score 1+: incomplete but moderate/intense membranous positivity, with/without cytoplasmic positivity, in ≥ 10% of tumor cells. Score 2+: complete and moderate/intense membranous positivity, with/without cytoplasmic positivity, in ≥ 10% of tumor. Score 0 was taken as negative, score 1 as weak positive and score 2 as strong positive.

Statistical Analysis

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If normality was rejected, then non parametric test was used. Qualitative variables were associated using Fisher's Exact test.

Quantitative variables were associated using Mann-Whitney Test (as the data sets were not normally distributed) between two groups and Kruskal Wallis test between three groups. A P value of <0.05 was considered statistically significant.

The data was entered into MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

RESULTS

A hospital-based cross-sectional study was conducted on 150 cases of Invasive Breast Carcinoma in India. Routine clinical details were taken for every patient. ER, PR, Her2 neu and Ki-67 expression were assessed for surrogate molecular classification. PD-L1 expression was assessed according to the immunohistochemistry score.[14] All the relevant demographic and clinical details have been summarised in Table 1.

PD-L1 score was statistically correlated with several parameters such as histological type of the tumor, grade of the tumor, surrogate molecular classification and pathological stage pTNM (wherever possible).[16,17]

The most commonly observed tumor subtype was IBC No Special Type (NST) (94.00%) followed by IBC with medullary pattern (3.33%) and lobular carcinoma (2.00%). Solid invasive papillary carcinoma was seen in only 1 out of 150 patients (0.67%). Also, 61.81% of patients had grade 2 breast cancer followed by grade 3 (31.94%). Grade 1 was the least prevalent grade. The most commonly found surrogate molecular classification was luminal B (34.00%) followed by triple negative (29.33%) and luminal A (20.00%). Her 2 neu enriched breast cancer cases comprised only 25 of 150 patients (16.67%). The study revealed a preponderance (53.75%) of patients with tumor stage T2 followed by T3 (27.50%) and T4 (12.50%). Tumor stage was T1 in only 5 out of 80 patients (6.25%). The most prevalent lymph node stage was N1 (35.06%) followed by N2 (28.57%) and N0 (23.38%). Lymph node stage was N3 in only 10 out of 77 patients (12.99%) (Table 1).

Out of the total 150 breast cancer cases, PD-L1 expression was found to be positive in 22 cases comprising 14.67%. Greater number of cases showed a negative expression of PD-L1 (85.33%).

Immunohistochemistry score assessment was done, the results of which are shown in Table 2 and Figure 1.

Table 2. Immunohistochemistry score of PD-L1 in study subjects

Immunohistochemistry score of PD-L1	Frequency	Percentage
Score 0	128	85.33%
Score 1	9	6.00%
Score 2	13	8.67%
Total	150	100.00%

Most of the cases showed a negative expression of PD-L1(score 0). Among the cases showing positive expression, score 2 was seen in 13 (8.67%) cases and score 1 in 9 (6.00%) cases.

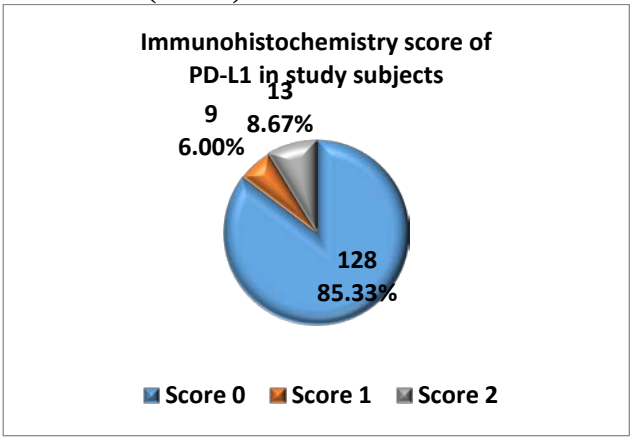

Figure 1. Immunohistochemistry score of PD-L1 in study subjects

The IHC sections of the cases showing strong positivity for PD-L1 (i.e., immunohistochemistry score of 2+) are shown in Figures 2, 3a and 3b. Cases with weak membranous positivity for PD-L1 (i.e., score of 1+) are shown Figure 4a and 4b.

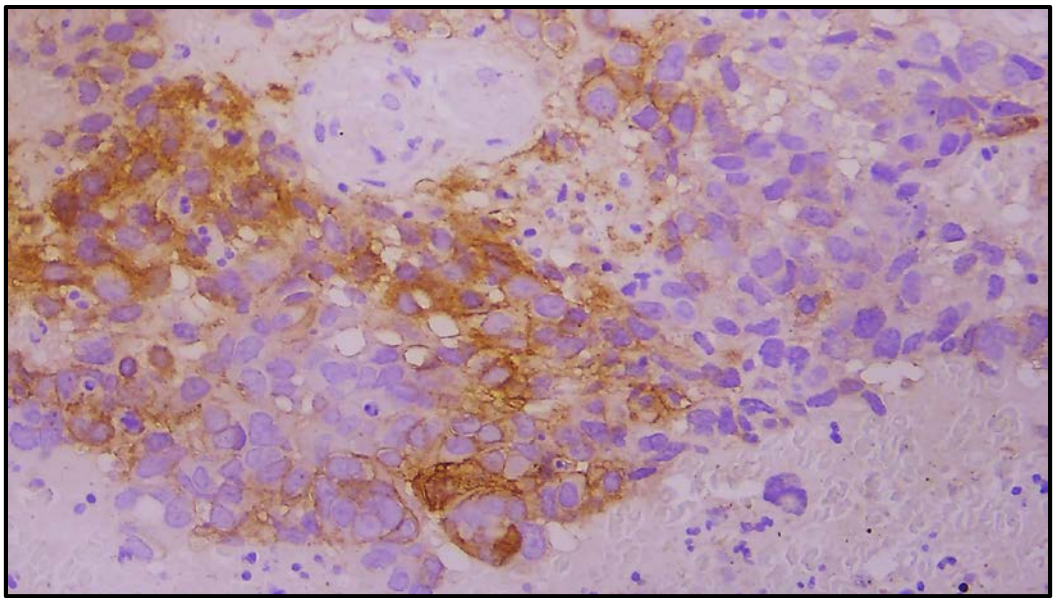

Figure 2. IBC with Medullary features with Immunoreactivity score of 2+. Tumor cells show strong and complete membranous with/without cytoplasmic positivity for PD-L1. Diaminobenzidine chromogen was used to detect PD-L1 positivity with Hematoxylin as counter stain. (40x)

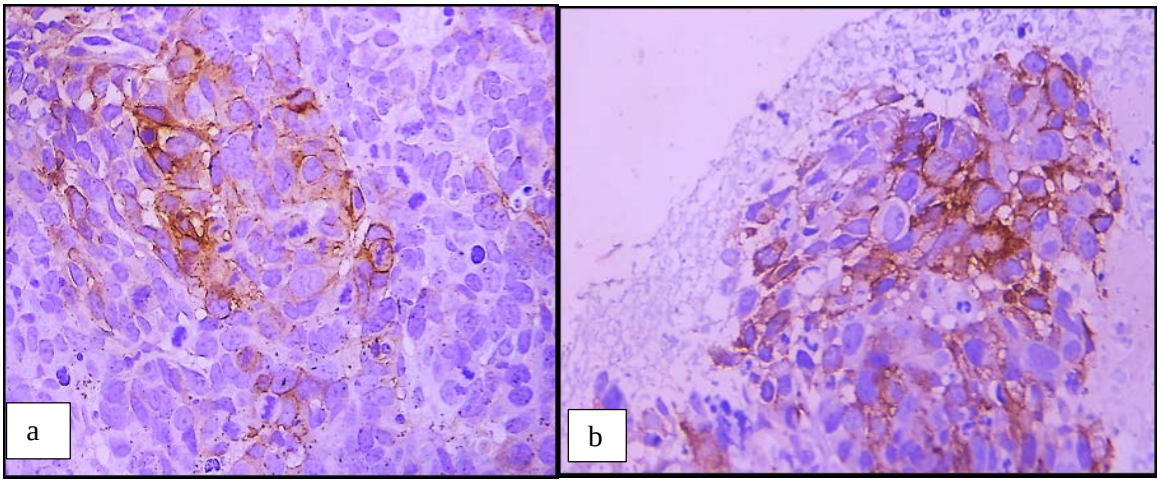

Figure 3. PD-L1 (Score 2+ Strong positive) (a, b) Cells showing strong and complete membranous positivity with/without cytoplasmic positivity for PD-L1. Immunoreactivity score of 2+ was assigned to each of these cases. (40x)

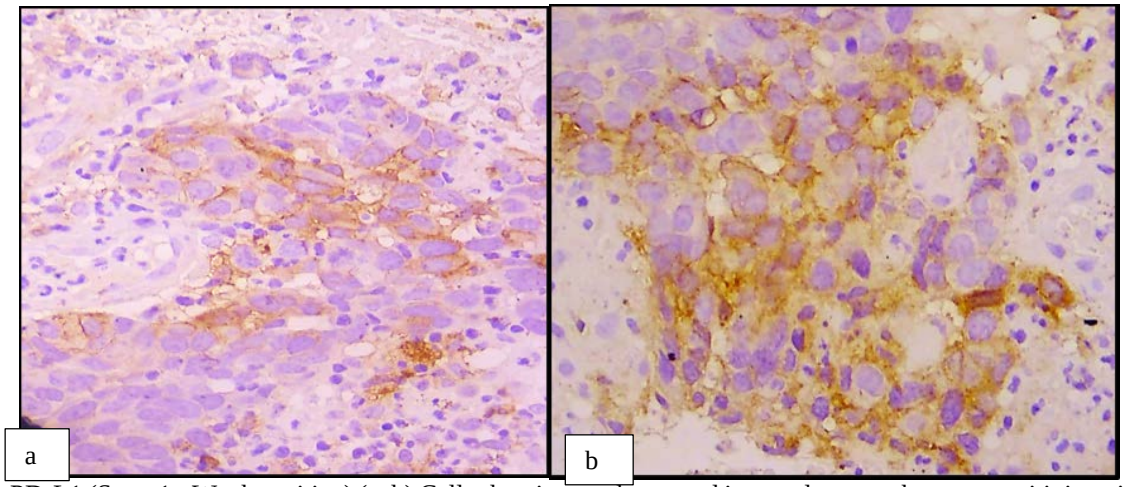

Figure 4. PD-L1 (Score1+ Weak positive) (a, b) Cells showing moderate and incomplete membranous positivity with/without cytoplasmic positivity for PD-L1. Immunoreactivity score of 1+ was assigned to each of these cases. (40x)

Tumor subtype showed a statistically significant association with PD-L1 expression (P value<.05) All of the IBC with medullary pattern cases showed a positive expression of PD-L1 which was significantly

higher as compared to 12.06% of patients with IBC NST. Other subtypes like solid invasive papillary carcinoma or lobular carcinoma cases did not express PD-L1.

A significant association was seen in the distribution of grade with PD-L1 negative and positive status (P value<.05). The percentage of patients with positive PD-L1 was 34.78% of grade 3 patients which was significantly higher as compared to 0% of grade 1 and 6.74% of grade 2 patients.

The association observed between the distribution of surrogate molecular classification with PD-L1 expression was also found to be significant (P value<.05). Cases with positive PD-L1 expression was the maximum in triple negative cases (36.36%) which was significantly higher as compared to 20% of HER 2 neu enriched patients. Patients with negative PD-L1 belonged more commonly to Luminal A or Luminal B subtypes (Table 3, Figure 5).

No significant association was seen in the distribution of tumor (T) stage with PD-L1 expression (P value>.05). The percentage of patients with positive PD-L1 was 20% of T1 patients, 32.56% of T2 patients, 9.09% of T3 patients, and 30% of T4 patients with no significant association between them.

The lymph node (N) stage also failed to show any correlation with PD-L1 negative and positive cases (P value>.05). The majority of patients had negative PD-L1; 94.44% of N0, 66.67% of N1, 68.18% of N2 and 70% of N3 and the percentage of patients with positive PD-L1 was 5.56% of N0 patients, 33.33% of N1 patients, 31.82% of N2 patients, and 30% of N3 patients with no significant association observed.

Table 3. Association of surrogate molecular classification with PD-L1 negative and positive expression

Surrogate molecular classification	Negative (n=128)	Positive (n=22)	Total	P value	Test performed
Her 2 neu enriched	20 (80%)	5 (20%)	25 (100%)	<.0001	Fisher's Exact Test
Luminal A	30 (100%)	0 (0%)	30 (100%)		
Luminal B	50 (98.04%)	1 (1.96%)	51 (100%)		
Triple negative	28 (63.64%)	16 (36.36%)	44 (100%)		
Total	128 (85.33%)	22 (14.67%)	150 (100%)		

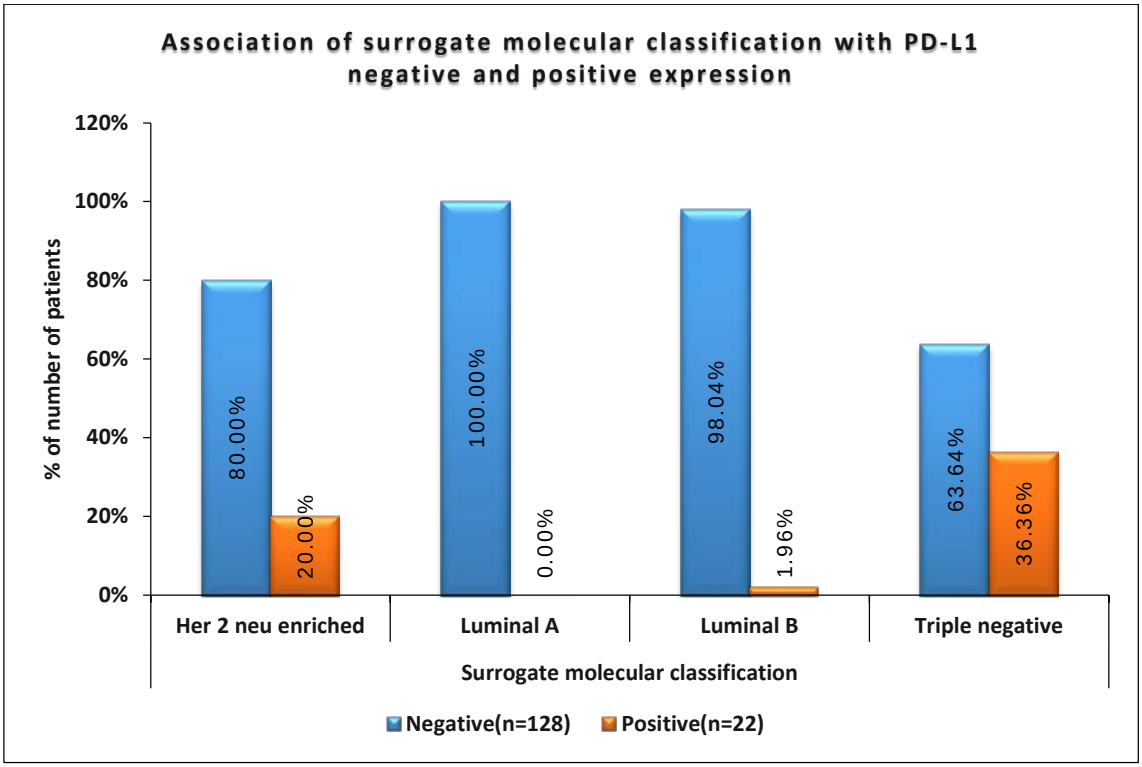

Figure 5. Association of surrogate molecular classification with PD-L1 negative and positive expression

DISCUSSION

Programmed death 1 (PD-1) and its associated receptor, programmed death ligand 1 (PD-L1), appears to be up-regulated in multiple solid malignancies and is typically expressed on the surface of tumor cells.[18] They have attracted attention among various cancers such as lung and breast cancer on account of being novel therapeutic targets. Among the clinical trials that have investigated the use of PD-1/PD-L1 immune checkpoint inhibitors in patients with invasive breast cancer, the use of Atezolizumab (a PD-L1 inhibitor) for treatment of triple-negative breast cancer was found successful in a recent trial in 2019.[19]

In this study, we analyzed the expression of PD-L1 in breast cancer cases in India. The immunohistochemistry expression was then compared with routinely performed prognostic parameters so that it may help further in prognostication of various molecular types of breast cancer.

In our study on 150 patients with breast cancer, PD-L1 was expressed in only 22 (14.67%) cases with score 1 in 9 (6%) and score 2 in 13 (8.67%) of patients.

As reported by Lou J *et al.*,[20] PD-L1 was found to be over-expressed in 37.5% of breast invasive ductal carcinoma samples, with only 1 out of 22 (4.5%) tumor adjacent normal breast tissue samples.

The difference in PD-L1 expression could be due to the use of different antibody clones and different scoring systems. Some studies also include cells in tumor microenvironment such as macrophages and TILs in PD-L1 scoring.

Tumor subtypes

In our study, the predominant subtype was IBC NST (94.00%) followed by IBC with the medullary pattern (3.33%). A significant association was seen in the distribution of tumor subtype with PD-L1 expression (P value<.05). Every case of IBC with the medullary pattern showed a strong, positive PD-L1 expression. Also, a significant association was seen in the distribution of tumor subtype with immunohistochemistry score of PD-L1 (p value<.05). The score 1 of PD-L1 positivity was seen in only IBC NST subtype (6.38%), whereas IBC with medullary pattern cases showed a stronger PD-L1 expression (score 2+).

A similar association was also reported by Doğukan et al., [(21)] where tumor PD-L1 positivity rate was relatively low in patients with invasive carcinoma, NST (23.8%) and high in breast carcinomas with medullary features (83.3%) and metaplastic carcinoma (66.6%).

Histological Grade

In our study, grade 1 was seen in 6.25% of patients, grade 2 in 61.81% and grade 3 in 31.94%. All of the patients with Grade 1 were PD-L1 negative. The percentage of patients with positive PD-L1 was 34.78% among grade 3 patients which was significantly higher as compared to 0% of grade 1 patients and 6.74% of grade 2 patients. The association of PD-L1 expression with higher tumor grade was statistically significant (P-value<0.05).

A significant association was seen in the distribution of grade with the immunohistochemistry score of PD-L1 (P<0.05). The percentage of patients with score 2 was 23.91% among grade 3 patients which was significantly higher as compared to 0% of grade 1 patients and 2.25% of grade 2 patients. We also found that with an increase in the grade of the tumor, the intensity of the PD-L1 expression was higher.

Our findings were in line with the study by Li F et al., who found that PD-L1 positive expression was associated with the histopathological grade (P<0.05).[22] They showed that the association of PD-L1 with histopathology grade indicates that the higher score of PD-L1 expression in breast cancer specimens is associated with larger and more aggressive tumors. In contrast, a few studies have found no association of grade and PD-L1 scoring.

In a study by Yuan et al., all patients' histology grades were grade 2 (45%) or grade 3 (55%). In primary tumors, there was no significant association between the positivity of PD-L1 and grading of breast cancer (P=0.515).[23]

Among studies which found no association between PD-L1 expression and grade, the reason could be the smaller number of low-grade tumors, and different antibody clones used in the study.

Surrogate molecular classification

In our study, in the majority (34.00%) of patients, surrogate molecular classification was luminal B followed by triple negative (29.33%). A significant association was seen with a positive expression of PD-L1 and Triple negative breast cancer (P-value<0.05). Also, 36.36% of triple negative patients showed positivity which was significantly higher compared to 20% of HER 2 neu enriched patients, 0% of luminal A patients and 1.96% of luminal B patients.

In addition, a significant association was seen in the distribution of surrogate molecular classification with the immunohistochemistry score of PD-L1 (P-value<0.05). The percentage of patients with a stronger expression of PD-L1 (Score 2+) was higher in triple negative breast cancer (25%). This was significantly higher compared to 8% of HER 2 neu enriched patients and none in Luminal subtypes.

Similarly, in a study by Zhou *et al.*, the expression of PD-L1 in tumor cells was evaluated with the molecular tumor type, showing that in TNBC, the expression rate of PD-L1 in tumor cells was 47.8% and that of PD-1 in para tumor-infiltrating immune cells was 43.5%, which was higher than the expression rates in other subtypes; these differences were statistically significant.[24]

Similar to our findings, in a previous study by Muenst S *et al.*, the expression of PD-L1 was found to be the highest in the basal-like/TNBC subtype, and lowest in the Luminal A subtype (P<0.0001).[13]

Kim *et al.*, also observed a higher PD-L1 expression in the HER2 neu positive and TNBC subtypes than in the ER+/PR+ subtypes.[25] Similarly, Gatalica *et al.*, demonstrated that PD-1 and PD-L1 expression was more common in the TNBC subtype than in the Luminal-like subtypes.[26]

A meta-analysis was conducted to evaluate the prognostic significance of TILs and PD-L1 expression as well as their association with various clinicopathological characteristics in triple negative breast cancer (TNBC).[27] Similar to the level of TILs, the study found no significant associations between PD-L1 expression and tumor size, tumor stage, histological grade, and Ki67 index (All P-values≥0.05). However, the study revealed that tumoral PD-L1 expression was strongly associated with high levels of TILs in TNBC patients (OR = 8.34, 95% CI: 2.68 to 25.95, P-value<0.001).

Such results allow for future research based on the expression of PD-L1 with addition of other markers such as p53, EGFR, in the molecular classification. This in turn will further determine the association among various markers. Positive expression of PD-L1, particularly in triple negative breast cancer cases may serve as new targets for immunotherapy in the coming years.

pTNM classification

In our study, the majority (53.75%) of patients had tumor stage T2 followed by T3 (27.50%), T4 (12.50%), and T1 in only 6.25% of the patients. Also, 35.06% of patients had lymph node stage N1 followed by N2 (28.57%), N0 (23.38%), and N3 in only 12.99% of the patients. No significant association was seen in the distribution of tumor(N) and lymph node (N) stage with PD-L1 expression (P-value>0.05).

Similar to our study, Doğukan *et al.*,[21] reported that there was no statistically significant relationship between tumoral or microenvironmental PD-L1 expression status and main clinicopathological and survival parameters such as tumor stage (P=0.545), node and metastasis (0.716).

Muenst *et al.*[13] found that PD-L1 expression in breast cancer specimens was significantly associated with positive lymph node status. The finding indicates that activation of the PD- 1/PD-L1 pathway may help these tumors evade antitumor immune responses and consequently proliferate and spread more rapidly. However, this mechanism needs to be further explored to arrive at a definite conclusion.

Constantinou C *et al*. conducted a study on 84 TNBC cases to explore the relationship between p53, p63, c-kit, Ki67, cMet, claudin7, CK5/6, CK17, AR, PTEN, EGFR, ALK, PDL-1 and c-MYC expression with the clinicopathological features in TNBC patients.[28] PD-L1 expression was positive in 10.4% of the patients. The study failed to show any statistically significant relationship between PD-L1 and tumor grade as well as the number of the positive lymph nodes.

PD-L1 expression in breast cancer has shown conflicting results across various studies and nations worldwide.

Our study aimed to provide additional data on the expression of PD-L1 in breast cancer patients, especially in Indian population. The study revealed the association of PD-L1 with various routinely assessed prognostic factors such as tumor subtype, tumor grade and surrogate molecular classification. We also found a significant relationship between PD-L1 expression and Triple Negative Breast cancer, thus making it a potential target for immunotherapy in such cases, which lack specific therapies.

CONCLUSION

This study was conducted to evaluate the expression of PD-L1 in breast cancer patients in India. The expression of PD-L1 was seen in 14.67% of the breast cancer cases with immunohistochemistry score of 1 in 6% and 2 in 8.67%. A significant association was seen between PD-L1 expression and several prognostic parameters such as tumor grade, tumor subtype and surrogate molecular classification. PD-L1 expression was associated with a higher tumor grade. All the cases of IBC with the medullary pattern showed a positive and stronger expression for PD-L1, which was found to be the highest in Triple negative breast cancer subtype (36.36%) compared to tumors showing ER/PR positivity. However, no relationship was found between PD-L1 expression and TNM staging.

Since breast cancer is highly heterogeneous, PD-L1 expression may vary among different countries as well as with several other factors such as molecular subtypes or different grades. Available data on PD-L1 expression has shown conflicting results. Moreover, there is paucity of research on expression of PD-L1 in breast cancer patients especially in the Indian subcontinent. Thus, our study will be helpful in

further adding to the knowledge of PD-L1 expression in breast cancer particularly in Indian women. The study will also add to the already existing literature on relationship of PD-L1 expression in breast cancer with various routinely used prognostic parameters.

In recent era of personalized treatments, more attention is being given to the development of new targeted therapies, particularly for triple negative breast cancer subtype. Immunohistochemistry is a relatively cheaper and easier procedure to evaluate the expression of new emerging markers including PD-L1. Thus, PD-L1 expression is a novel marker that may be considered as a potential target for immune therapy of breast cancer patients who have IBC with medullary pattern or triple negative breast cancer subtype (TNBC).

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CONFLICT OF INTEREST
None.

REFERENCES

1. Breast cancer- WHO. Available from: https://www.who.int/news-room/fact-sheets/detail/breast-cancer#:~:text=In%202020%2C%20there%20were%202.3,the%20world%27s%20most%20prevalent%20cancer.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009;59:225-49.
3. Najafi S, Sadeghi M, Abasvandi F, Shajari MR, Mohebi K, Ghandchi H. Prognostic factors influencing prognosis in early breast cancer patients. Prz Menopauzalny 2019;18(2):82-8.
4. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women. *Asia Pac J Clin Oncol* 2017;13:289-95. doi: 10.1111/ajco.12661.
5. Thakur KK, Bordoloi D, Kunnumakkara AB. Alarming burden of triple-negative breast cancer in India. *Clin Breast Cancer* 2018;18:393-9. doi: 10.1016/j.clbc.2017.07.013.
6. Cancer immunotherapy and the PD-1/PD-L1 checkpoint pathway. Available from: https://www.abcam.com/cancer/cancer-immunotherapy-and-the-pd1pdl1-pathway.
7. Shi Y. Regulatory mechanisms of PD-L1 expression in cancer cells. *Cancer Immunol Immunother* 2018;262:1-9. doi: 10.1007/s00262-018-2226-9.
8. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat. Rev. Cancer*. 2016;16:275–287. doi: 10.1038/nrc.2016.36.
9. Shen X, Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. *BMJ*. 2018;362:k3529. doi: 10.1136/bmj.k3529.
10. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N. Engl. J. Med.* 2018;379:2108–2121. doi: 10.1056/NEJMoa1809615.
11. Gong J, Chehrazi-Raffle A, Reddi S, Salgia R. Development of PD-1 and PD-L1 inhibitors as a form of cancer immunotherapy: a comprehensive review of registration trials and future considerations. *J Immunother Cancer* 2018;6(1):8. doi: 10.1186/s40425-018-0316-z.
12. Planes-Laine G, Rochigneux P, Bertucci F, Chretein AS, Viens P, Sabatier R, et al. PD- 1/PD-L1 Targeting in breast cancer: The first clinical evidences are emerging. A literature review. *Cancers* (Basel) 2019;11(7):1033. doi: 10.3390/cancers11071033.
13. Muenst S, Schaerli AR, Gao F, Däster S, Trella E, Droeser RA, et al. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. *Breast cancer Res Treat* 2014;146:15-24. doi: 10.1007/s10549-014-2988-5.
14. Botti G, Collina F, Scognamiglio G, Di Bonito M, Liguori G, Cantile M. Programmed death ligand 1 (PDL-1) expression in triple negative breast cancer cells: new proposal for a tumor score definition. *Int J Clin Exp Pathol* 2016;9:11693-96. Available from:- https://www.scopus.com/record/display.uri?eid=2-s2.0-85006141718&origin=inward&txGid=4b6d22bd14ce62bc9ec5d6fe19f5d192
15. Layton C, Bancroft JD. The Hematoxylin and Eosin. In: Suvarna SK, Layton C, Bancroft JD (Eds). Theory and Practice of Histological Techniques, 7th edition. Philadelphia: Elsevier; 2013. pp. 179-220.
16. Collins LC. Breast. Rosai and Ackerman Surgical Pathology, 11th edition. Philadelphia: Elsevier; 2017. pp. 1434-527.
17. Hortobagyi GN, Connolly JL, D'Orsi CJet al. Breast. In: Amin MB, Edge S, Greene F et al, eds; American Joint Committee on Cancer. AJCC cancer staging manual. 8th ed. New York, NY: Springer, 2017; 589–636.
18. Uhercik M, Sanders AJ, Owen S, Davies E, Sharma AK, Jiang WJ, et al. Clinical Significance of PD1 and PDL1 in Human Breast Cancer. *Anticancer Res* 2017;37(8):4249- 54. doi: 10.21873/anticanres.11817
19. Heimes AS, Schmidt M. Atezolizumab for the treatment of triple-negative breast cancer. *Expert Opin Investig Drugs.* 2019 Jan;28(1):1-5. doi: 10.1080/13543784.2019.1552255.
20. Lou J, Zhou Y, Huang J, Qian X. Relationship between PD-L1 expression and clinical characteristics in patients with breast invasive ductal carcinoma. *Open Med (Wars)* 2017;12:288-92. doi: 10.1515/med-2017-0042.
21. Doğukan R, Uçak R, Doğukan FM, Tanık C, Çitgez B, Kabukcuoğlu F. Correlation between the expression of pd-l1 and clinicopathological

parameters in triple negative breast cancer patients. *Eur J Breast Health* 2019;15(4):235-41. doi: 10.5152/ejbh.2019.4912.
22. Li F, Ren Y, Wang Z. Programmed death 1 Ligand 1 expression in breast cancer and its association with patients' clinical parameters. *J Cancer Res Ther*. 2018 Jan;14(1):150-154. doi: 10.4103/jcrt.JCRT_602_17.
23. Yuan C, Liu Z, Yu Q, Wang X, Bian M, Yu Z, Yu J. Expression of PD-1/PD-L1 in primary breast tumours and metastatic axillary lymph nodes and its correlation with clinicopathological parameters. *Sci Rep*. 2019 Oct 7;9(1):14356. doi: 10.1038/s41598-019-50898-3.
24. Zhou T, Xu D, Tang B, Ren Y, Han Y, Liang G, et al. Expression of programmed death ligand-1 and programmed death-1 in samples of invasive ductal carcinoma of the breast and its correlation with prognosis. *Anticancer Drugs* 2018;29(9):904-10. doi: 10.1097/CAD.0000000000000683.
25. Kim HM, Lee J, Koo JS. Clinicopathological and prognostic significance of programmed death ligand-1 expression in breast cancer: a meta-analysis. BMC Cancer. 2017 Oct 17;17(1):690. doi: 10.1186/s12885-017-3670-1.
26. Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao, et al. Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. *Cancer Epidemiol Biomarkers Prevention* 2014;23:2965-70. doi: 10.1158/1055-9965.EPI-14-0654.
27. Lotfinejad P, Asghari Jafarabadi M, Abdoli Shadbad M, Kazemi T, Pashazadeh F, Sandoghchian Shotorbani S, et al. Prognostic Role and Clinical Significance of Tumor-Infiltrating Lymphocyte (TIL) and Programmed Death Ligand 1 (PD-L1) Expression in Triple-Negative Breast Cancer (TNBC): A Systematic Review and Meta-Analysis Study. *Diagnostics* (Basel). 2020 Sep 17;10(9):704. doi: 10.3390/diagnostics10090704.
28. Constantinou C, Papadopoulos S, Karyda E, Alexopoulos A, Agnanti N, Batistatou A et al.Expression and clinical significance of Claudin-7, PDL-1, PTEN, c-Kit, c-Met,c-Myc, ALK, CK5/6, CK17, p53, EGFR, Ki67, p63 in triple-negative breast cancer–a single centre prospective observational study. *In Vivo* 2018;32:303-11. doi: 10.21873/invivo.11238.

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