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Original Research Article

# Clinicopathological and Prognostic Value of PD-1/PD-L1 Expression in Patients with Breast Cancer

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## Abstract

Programmed cell death 1 (PD-1), and its ligand programmed cell death-ligand 1 (PD-L1), is important for tumor-immune escape. Expression PD-L1 was observed in various solid tumors, including breast cancer (BC). The aim of this study is to examine the expression of PD-1 in tumor infiltrating lymphocytes (TILs) and PD-L1 in tumor cells (TC) in BC cases, to analyze the association between PD-1 and PD-L1 expression and clinicopathological features, as well as to correlate their expression with overall survival (OS). This is a retrospective study that was conducted on 110 cases of BC. Immunohistochemistry was performed to evaluate PD-1 and PD-L1 expression in TILs and TC respectively. There was no significant association between PD-1expression in TILs and clinicopathological variables. The presence of PD-1+ TIL was positively associated with PDL-1 expression in tumor cells; however, this association was not statistically significant (p = 0.062). On the other hand, PD-L1 expression in TC was significantly associated with lymph node involvement (P <0.0001), advanced stage (P = 0.035), high grade (P <0.0001), high TIL (P = 0.009), and negative ER (P = 0.01). BC cases with PD-L1 expression had a significantly worse OS (HR = 0.102; 95% CI [0.048 - 0.221], p <0.000). PD-L1 expression was an independent prognostic factor in multivariate analysis (HR = 0.195; 95% CI [0.058 - 0.655], p =0.008). In conclusion, PD-L1 expression is associated with advanced tumor stage, aggressive subtypes of BC, lymphatic infiltration, and poor OS in BC.

**Keywords**: Breast Cancer; PD-1/PD-L1 pathway; Immune checkpoint inhibitors; Prognosis.



## INTRODUCTION

Breast cancer (BC) is the most frequently diagnosed cancer and the major cause of death in females, and the second most common cancer around the world [1]. The incidence of BC has steadily increased in recent decades (two million new cases in 2018), but BC mortality appears to decline, perhaps as a result of significant progress in the treatment of BC [2-4]. Five major BC treatments are available, namely radiotherapy, surgery, targeted therapy, chemotherapy, and hormone therapy. Nevertheless, the effectiveness of these therapies in BC patients remains unsatisfactory due to the absence of efficient indicators, which can be used to predict disease pathways and widespread chemical resistance to BC [5, 6]. It is therefore imperative for researchers to identify precise BC biomarkers and potential therapeutic targets for disease treatment to improve survival.

Tumor microenvironment immune responses determine tumor cell biological behavior. By eliminating or inhibiting tumor cells, immune system plays a defensive function. Research has recently revealed an adverse impact on radiotherapy of multiple tumors, as a result of damage to the cell microenvironment [7-10]. Programmed cell death 1 (PD-1), part of a superfamily of B 7-CD28, is a receptor that regulates its activation and apoptosis on the surface of T-, B and NK-cells [11]. Its ligand, programmed cell death-ligand 1 (PD-L1), is produced in certain tumor cells and stimulated B cells and T cells, dendritic nuclei, macrophages and fibroblasts [12]. The activation of PD-1 in combination with PD-L1 is upgraded to prevent cell attacks from cancer cells. Blocking the PD-1/PD-L1 pathway with monoclonal antibodies (against PD-1 or PD-L1) is a promising therapeutic approach that is being investigated in many human cancer studies [13]. Results from these studies suggest that PD-L1, by facilitating PD-1/PD-L1, is important for tumor-immune escape. Expression PD-L1 was observed in various solid tumors, including BC [14]. A recent meta-analysis conducted to evaluate the importance of PD-L1 as a prognostic indicator and determine the correlation between expression of PD-L1

in BC patients and clinicopathological characteristics. It demonstrated that PD-L1 expression is a promising biomarker for the prognosis of BC, and may be helpful to clinicians aiming to select the appropriate immunotherapy for BC [15]. The aim of this study is to examine the expression of PD-1 in tumor infiltrating lymphocytes (TILs) and PD-L1 in tumor cells (TC) in BC cases, to analyze the association between PD-1 and PD-L1 expression and clinicopathological features, as well as to correlate their expression with overall survival.

## MATERIAL AND METHODS

### Study Design

This is a retrospective study that was conducted over the period of four years between March 2014 and March 2018, in Tanta University, Egypt. One hundred and ten cases of BC were included in this study. Data including formalin-fixed and paraffin-embedded tissue blocks were obtained from the archives of the Departments of Pathology, Faculty of Medicine, Tanta University. Clinical and follow-up data of the cases were retrospectively collected from the medical record. This work has been approved by the local institutional ethical committee in accordance with the principles of the Declaration of Helsinki.

### Inclusion Criteria

Cases with available data regarding the diagnosis of infiltrating ductal carcinoma (IDC); known hormone receptors and HER-2 immunohistochemistry results; no prior chemotherapy; and sufficient tissue in paraffin blocks to perform IHC tests were included in this study.

### Data Collection

Evaluation of the histopathological features was performed. We assessed the following histological parameters: 1) tumor size (pT); 2) tumor grade (determined according to the modified Bloom and Richardson score) [16]; 3) histological tumor subtype; 4) necrosis; 5) lymph node metastasis (pN); 6) extent of tumor infiltrating lymphocytes (TILs); 7) lympho-vascular; and 8) perineural invasions. The status of estrogen receptor (ER), progesterone receptor (PR), and HER-2 were obtained from the accompanying pathology reports. Breast cancer subtypes were defined as follows: Luminal A (ER and/or PR positive, HER-2 negative), luminal B (ER and/or PR positive, HER-2 positive), HER-2 positive (ER and PR negative, HER-2 positive), triple negative BC (ER negative, PR negative and HER-2 negative) [17]. The level of TILs was evaluated on the basis of the International TILs Working Group recommendations [18].

## METHODS

### Immunohistochemistry

Immunohistochemistry was performed on 4-µm sections that were cut from routine paraffin embedded blocks using an automated stainer (Benchmark Ventana, Tucson, AZ) according to standard protocol and the manufacturer's recommendations for each antibody.

Briefly, paraffin sections were baked at 58 °C overnight and de-paraffinized in xylene. We rehydrated the de-paraffinized sections with graded ethanol and quenched it in 0.3% hydrogen peroxide at 37 ° C for 15 minutes for endogenous peroxidase activities. After that, the sections were processed for high pressure cooking in citrate antigen retrieval solution (pH = 6.0) for about 10 min for PD-L1 and antigen retrieval by EDTA antigen retrieval solution (pH = 8.0) for about 4 min for PD-1. Sections were incubated at 37 °C for 1.5 h with rabbit monoclonal antibodies against PD-1 (1:100, ab137132, Abcam, Cambridge, MA, USA), PD-L1 (1:50, ab174838, Abcam, Cambridge, MA, USA), and ki-67 (monoclonal mouse antibody, DakoCytomation (Dako, Glostrup, Denmark), clone MIB-1, dilution 1 : 100) in a moist chamber. Immunostaining was performed using the EnVision+ System-HRP (AEC) (K4005, Dako, Glostrup, Denmark). Sections were then counterstained with hematoxylin (Sigma-Aldrich, St Louis, MO, USA) and mounted in a non-aqueous mounting medium. All runs included a primary control of the antibody. As external positive controls for PD-1, PDL-1, and ki-67, human tonsil tissue, normal placenta, and skin were used. The use of non-immune normal mouse serum as the primary antibody has obtained negative control.

### Evaluation of TILs level

For statistical analysis, patients were subdivided into two categories low and high (≤60% and >60% respectively) according to the percentage of TIL within the stroma [18].

### Evaluation of PD-1 expression in TILs

PD-1 was considered positive in case of any membranous staining of TILs.

### Evaluation of PD-L1 expression in TCs

Tumors that exhibited>1% staining of tumor cells with staining of any intensity (0 =no expression, 1 = weak, 2 = moderate, 3 = strong) and any distribution (membrane and/or cytoplasm) were considered positive for PD-L1. Both partial and complete cell membrane staining was also considered positive. The staining distribution was scored as follow: 3 ≥ 50%, 2 ≥ 5% to <50%, TC1 ≥ 1% ≤5%, and 0 <1%. Then, the staining intensity is multiplied by the distribution to obtain a final semi-quantitative H score [19].

### Statistical Analysis

Statistical analyses were performed by SPSS software (version 23.0; IBM Corp, Armonk, NY, USA). We used Fisher's exact test for categorical variables and the Student's t-test for continuous variables in order to assess the associations between clinicopathologic factors and PD-L1 expression. We used the Kaplan–

Meier method to estimate the Overall survival (OS) (which was calculated from the date of surgery to the date of death or censoring if patients were alive at the time of last follow-up). Also, the log-rank test was used to perform the non-parametric group comparisons. Univariate and Multivariate analyses were performed using the Cox proportional hazard regression model. The latter analysis was done to study the effects of different variables on OS after adjusting for the possible confounding effects of age, gender, smoking, and laterality and the hazard ratios (HRs) and the associated 95% confidence intervals were reported. All p values are two-sided and $p < 0.05$ was considered to be significant.

## RESULTS

### Patient Characteristics

One hundred and ten BC cases were included in this study. The clinicopathological characteristics of the patients were detailed in Table 1. Briefly, the median age was 52 years (29-87 years). Forty-four cases (40%) were luminal A, 39 cases were luminal B (35.5%), while 7 (6.4%) and 20 cases (18.2%) were HER positive and basal-like (triple negative BC), respectively. Nearly, half of the patients had stage II disease (52.7%) and negative lymph nodes (44.5%).

### Correlation of PD-1 Expression in TIL with Clinicopathological Variables

PD-1 expression in TIL was seen in 32/110 (29.1 %) of BC cases (Fig 1A). The Expression of PD-1 in TIL was significantly associated with high TIL count ($p<0.0001$), Table 2. There was no significant association between PD-1expression in tumor cells and the remaining clinicopathological variables.

The presence of PD-1+ TIL was positively associated with PDL-1 expression in tumor cells; however, this association was not statistically significant ($p = 0.062$).

Interestingly, it was noted that PD-1 expression was more common in TNBCs than the other subtypes; 9/20 cases (45%) of TNBCs were positive for PD-1. However, no significant association has been found between PD-1 expression and the intrinsic subtypes ($p = 0.149$) (Fig. 3 A, Table 3).

### Correlation of PD-L1 Expression in Tumor Cells with Clinicopathological Variables

Correlation of PD-L1 Expression with different clinicopathological Variables was summarized in Table 2.

PD-L1 was expressed on the cell membranes of tumor cells in 34 cases (30.9%) (Fig.1B). PD-L1 expression was significantly associated with lymph node involvement ($P <0.0001$), advanced stage ($P = 0.035$), high grade ($P <0.0001$), high TIL ($P = 0.009$), and negative ER ($P = 0.01$).

Notably, the expression of PDL-1 was significantly different among the intrinsic subtypes of BC. The prevalence of PDL-1+ TC was the highest in TNBC subtype (60%) (Fig 2A-D) and the lowest in the luminal B subtype (23.1 %) ($p = 0.021$) (Fig-3B, Table-3).

### Prognostic Significance of PD-1 and PD-L1 Expressions

The mean follow-up time was 53.5±11.2 months (16–60 months). Univariate and multivariate analyses of overall survival were summarized in Table-4.

In univariate survival analysis, no association has been detected between PD-1 expression in TILs and OS (HR =0.742; 95% CI: 0.369-1.492; p= 0.403; Fig-4A). Similarly, in subset analyses by intrinsic subtype, PD-1 expression in TIL was not associated with OS in all subtypes (Table-5). In multivariate analysis, the presence of PD-1+ TIL was not found to be an independent prognostic factor for OS (HR 1.599; 95% CI [0.557 - 4.591], $p = 0.384$).

On the other hand, BC cases with PD-L1 expression had a significantly worse overall survival; (HR = 0.102; 95% CI [0.048 - 0.221], $p <0.0001$), (Table 4 and Fig. 4B). PD-L1 expression was an independent prognostic factor in multivariate analysis (HR = 0.195; 95% CI [0.058 - 0.655], $p =0.008$).

In multivariate analysis; lymph node status, tumor grade, LVI, and the expression of PD-L1 have been proved to be independent negative prognostic factors for OS ($p = 0.002$, $p = 0.045$, $p = 0.026$, and $p = 0.008$, respectively) (Table-4).

In subset analyses by intrinsic subtype, the expression of PD-L1 was associated with decreased OS in the luminal A ($p<0.0001$), the luminal B ($p <0.0001$), the HER2 ($p = 0.008$), and the triple negative subtype ($p = 0.014$) (Table-5 and Fig-4 C-F).

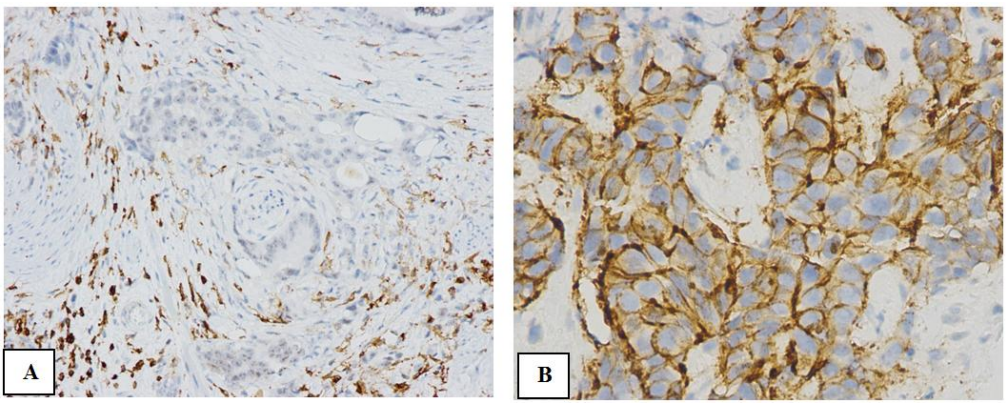


**Fig-1: PD-1 Expression in TIL (A), PD-L1 expression in tumor cells (B)**

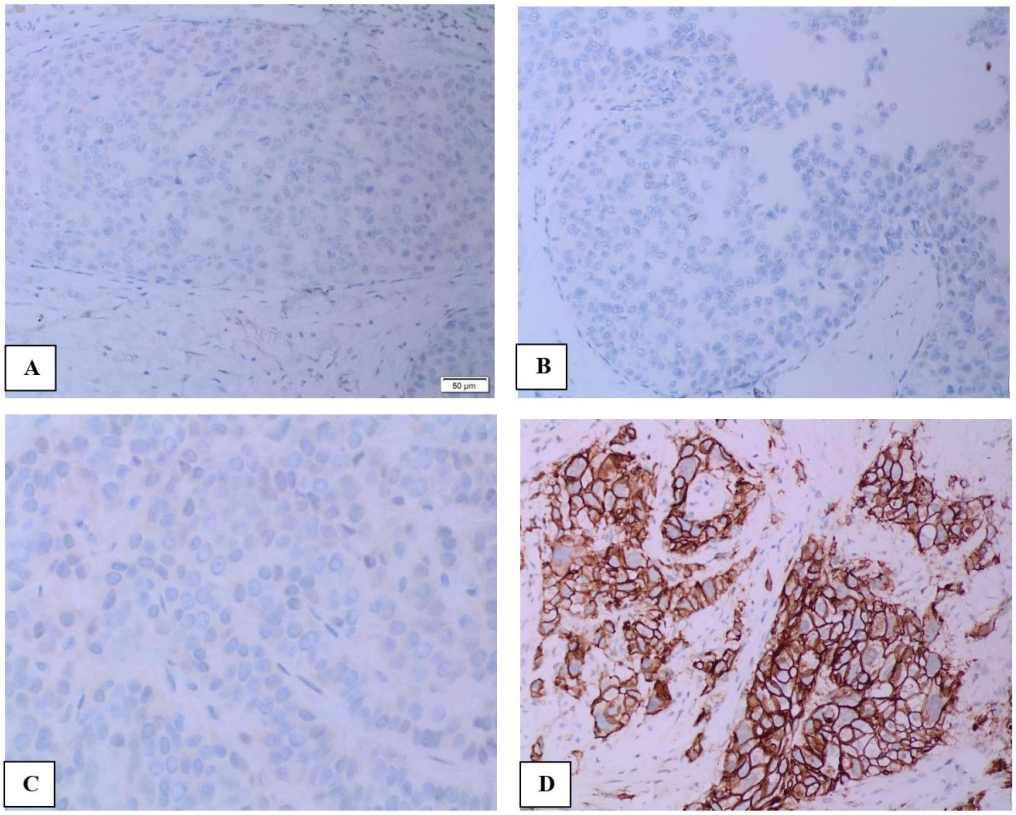


**Fig-2: TNBC, ER (A), PR (B), HER2 (C), strong PD-L1 expression in tumor cells (D)**

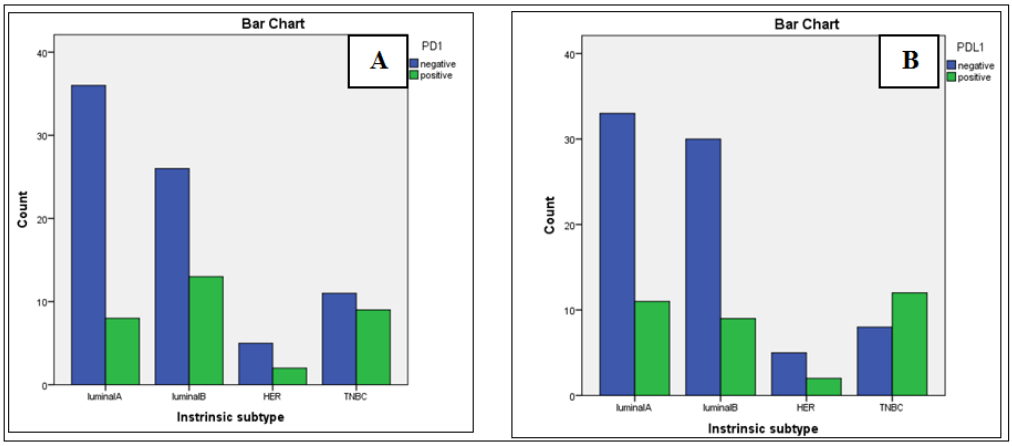


**Fig-3: Correlation of PD-1 (A) and PD-L1 (B) expression with the intrinsic subtypes of breast cancer cases**

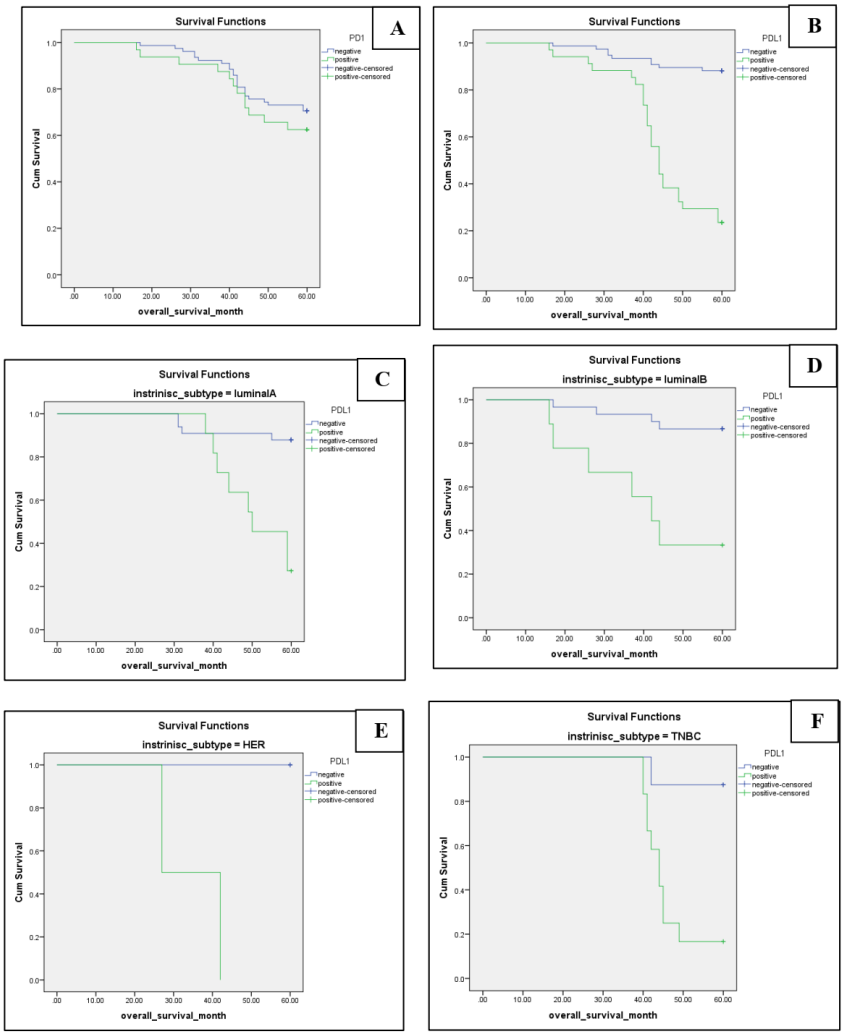


**Fig-4: Kaplan–Meier survival curves for overall survival according to the expression of PD-1 and PD-L1. (A) PD-1expression in TILs, (B) PD-L1 expression in TC, (C–F) Survival curves for overall survival depending on the PD-L1 expression in TC for the indicated breast cancer intrinsic subtypes**

**Table-1: Clinicopathological features of breast cancer patients included in this study**

| Characteristic | | N | % |
|---|---|---|---|
| Age | Median (range) 52 (29-87) | | |
| | <50 | 47 | 42.7% |
| | ≥50 | 63 | 57.3% |
| Tumor size | <2cm | 24 | 21.8% |
| | 2-5cm | 62 | 56.4% |
| | > 5cm | 24 | 21.8% |
| Lymph node status | Negative | 49 | 44.5% |
| | Positive < 3 | 37 | 33.6% |
| | Positive > 3 | 24 | 21.8% |
| Stage | 1 | 12 | 10.9% |
| | 2 | 58 | 52.7% |
| | 3 | 31 | 28.2% |
| | 4 | 9 | 8.2% |
| Grade | G 1 | 29 | 26.4% |
| | G 2 | 41 | 37.3% |
| | G 3 | 40 | 36.4% |
| LVI | Absent | 72 | 65.5% |
| | Present | 38 | 34.5% |
| Necrosis | Absent | 63 | 57.3% |
| | Present | 47 | 42.7% |

| | | | |
|---|---|---|---|
| TIL | Low | 39 | 35.5% |
| | Moderate | 52 | 47.3% |
| | High | 19 | 17.3% |
| Ki-67 | Low | 62 | 56.4% |
| | High | 48 | 43.6% |
| ER | Negative | 39 | 35.5% |
| | Positive | 71 | 64.5% |
| PR | Negative | 65 | 59.1% |
| | Positive | 45 | 40.9% |
| HER | Negative | 64 | 58.2% |
| | Positive | 46 | 41.8% |
| Intrinsic subtype | Luminal A | 44 | 40.0% |
| | Luminal B | 39 | 35.5% |
| | HER | 7 | 6.4% |
| | TNBC | 20 | 18.2% |
| PD-1 | Negative | 78 | 70.9% |
| | Positive | 32 | 29.1% |
| PDL-1 | Negative | 76 | 69.1% |
| | Positive | 34 | 30.9% |

**Table-2: Association between PD-1 and PD-L1 expressions and clinicopathological features in breast cancer cases**

| **Clinicopathologic feature** | | **PD-1** | | | | **P-value** | **PDL-1** | | | | **P-value** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Negative** | | **Positive** | | | **negative** | | **positive** | | |
| | | **N** | | **N** | | | **N** | | **N** | | |
| Age | <50 | 36 | 44.9% | 11 | 40.6% | 0.257 | 32 | 44.7% | 15 | 41.2% | 0.844 |
| | >50 | 42 | 55.1% | 21 | 59.4% | | 44 | 55.3% | 19 | 58.8% | |
| Tumor size | <2cm | 18 | 23.1% | 6 | 18.8% | 0.2 | 17 | 22.4% | 7 | 20.6% | 0.95 |
| | 2-5cm | 40 | 51.3% | 22 | 68.8% | | 43 | 56.6% | 19 | 55.9% | |
| | > 5cm | 20 | 25.6% | 4 | 12.5% | | 16 | 21.1% | 8 | 23.5% | |
| Lymph node status | Negative | 35 | 44.9% | 14 | 43.8% | 0.864 | 43 | 56.6% | 6 | 17.6% | **<0.0001** |
| | Positive < 3 | 27 | 34.6% | 10 | 31.3% | | 24 | 31.6% | 13 | 38.2% | |
| | Positive > 3 | 16 | 20.5% | 8 | 25.0% | | 9 | 11.8% | 15 | 44.1% | |
| Stage | 1 | 9 | 11.5% | 3 | 9.4% | 0.755 | 10 | 13.2% | 2 | 5.9% | **0.035** |
| | 2 | 39 | 50.0% | 19 | 59.4% | | 45 | 59.2% | 13 | 38.2% | |
| | 3 | 24 | 30.8% | 7 | 21.9% | | 17 | 22.4% | 14 | 41.2% | |
| | 4 | 6 | 7.7% | 3 | 9.4% | | 4 | 5.3% | 5 | 14.7% | |
| Grade | G 1 | 23 | 29.5% | 6 | 18.8% | 0.064 | 24 | 31.6% | 5 | 14.7% | **<0.0001** |
| | G 2 | 32 | 41.0% | 9 | 28.1% | | 35 | 46.1% | 6 | 17.6% | |
| | G 3 | 23 | 29.5% | 17 | 53.1% | | 17 | 22.4% | 23 | 67.6% | |
| LVI | Absent | 53 | 67.9% | 19 | 59.4% | 0.93 | 54 | 71.1% | 18 | 52.9% | 0.65 |
| | Present | 25 | 32.1% | 13 | 40.6% | | 22 | 28.9% | 16 | 47.1% | |
| Necrosis | Absent | 46 | 59.0% | 17 | 53.1% | 0.573 | 40 | 52.6% | 23 | 67.6% | 0.141 |
| | Present | 32 | 41.0% | 15 | 46.9% | | 36 | 47.4% | 11 | 32.4% | |
| TIL | Low | 35 | 44.9% | 4 | 12.5% | **<0.0001** | 34 | 44.7% | 5 | 14.7% | **0.009** |
| | Moderate | 36 | 46.2% | 16 | 50% | | 30 | 39.5% | 22 | 64.7% | |
| | High | 7 | 9.0% | 12 | 37.5% | | 12 | 15.8% | 7 | 20.6% | |
| Ki-67 | Low | 45 | 57.7% | 17 | 53.1% | 0.661 | 45 | 59.2% | 17 | 47.1% | .3680 |
| | High | 33 | 42.3% | 15 | 56.9% | | 31 | 40.8% | 17 | 50% | |
| ER | Negative | 25 | 32.1% | 14 | 43.8% | 0.244 | 21 | 27.6% | 18 | 50% | **.010** |
| | Positive | 53 | 67.9% | 18 | 56.3% | | 55 | 72.4% | 16 | 47.1% | |
| PR | Negative | 46 | 59.0% | 19 | 59.4% | 0.969 | 42 | 55.3% | 23 | 67.6% | 0.22 |
| | Positive | 32 | 41.0% | 13 | 40.6% | | 34 | 44.7% | 11 | 32.4% | |
| HER | Negative | 47 | 60.3% | 17 | 53.1% | 0.491 | 41 | 53.9% | 23 | 67.6% | 0.178 |
| | Positive | 31 | 39.7% | 15 | 46.9% | | 35 | 46.1% | 11 | 32.4% | |
| PD1 | negative | - | - | - | - | - | 58 | 76.3% | 20 | 58.8% | 0.062 |
| | Positive | - | - | - | - | | 18 | 23.7% | 14 | 41.2% | |
| PDL1 | negative | 58 | 74.4% | 18 | 56.3% | 0.062 | - | - | - | - | - |
| | positive | 20 | 25.6% | 14 | 43.8% | | - | - | - | - | |

Statistically significant P-values (P <0.05) are highlighted in bold.

**Table-3: Association between PD-1 and PD-L1 expressions and intrinsic subtype of breast cancer**

| Instrinsic subtype | PD-1 | | | | p value | PD-L1 | | | | p value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Negative | | Positive | | | Negative | | Positive | | |
| | N | % | N | % | | N | % | N | % | |
| Luminal A | 36 | 81.8% | 8 | 18.2% | 0.149 | 33 | 75.0% | 11 | 25.0% | **0.021** |
| Luminal B | 26 | 66.7% | 13 | 33.3% | | 30 | 76.9% | 9 | 23.1% | |
| HER | 5 | 71.4% | 2 | 28.6% | | 5 | 71.4% | 2 | 28.6% | |
| TNBC | 11 | 55.0% | 9 | 45.0% | | 8 | 40% | 12 | 60% | |

Statistically significant P-values (P <0.05) are highlighted in bold.

**Table-4: Univariate and multivariate analyses of overall survival according to clinical, histopathological, PD-1 and PD-L1 expressions**

| Features | Univariate | | Multivariate | |
|---|---|---|---|---|
| | (HR) (95% CI) | P-value (Cox regression) | (HR) (95% CI) | P-value (Cox regression) |
| Age | .912 (0.469-1.774) | 0.787 | 1.488 (0.439-5.046) | 0.524 |
| Tumor size | 1.171 (0.439-3.121) | 0.753 | 3.268 (0.792-3.496) | 0.102 |
| Lymph node status | 0.090 (0.033-0.246) | **<0.0001** | 0.109 (0.026-0.457) | **0.002** |
| Stage | 0.136 (0.015-1.216) | 0.074 | 0.678 (0.04-11.613) | 0.788 |
| Grade | 0.176 (.061-0.51) | **0.001** | 0.216 (0.048-0.964) | **0.045** |
| LVI | 0.365 (.187-.711) | **0.003** | 0.315 (0.114-0.870) | **0.026** |
| Necrosis | 1.135(0.775-2.233) | 0.713 | 0.689 (0.281-1.693) | 0.417 |
| TIL | 0.480 (0.161-1.428) | 0.187 | 0.438 (0.11-1.753) | 0.243 |
| Ki-67 | 0.554 (0.285 -1.077) | 0.082 | 0.676 (0.219-2.087) | 0. 496 |
| ER | 1.99(1.024-3.864) | **0.042** | 1.21 (0.208-7.048) | 0.832 |
| PR | 1.502(0.736 -3.068) | 0.264 | 0.993 (0.262-3.759) | 0.991 |
| HER | 1.329 (0.661-2.672) | 0.424 | 0.230 (0.024-2.161) | 0.199 |
| Instrinsic subtype | 0.497 (0.110-2.247) | 0.06 | 1.361 (0.133-13.893 | 0.795 |
| PD-1 | 0.742 (0.369-1.492) | 0.403 | 1.599 (0.557-4.591) | 0.384 |
| PDL-1 | 0. 102 (0.048- 0.221) | **<0.0001** | 0.195 (0.058-0.655) | **0.008** |

HR: Hazard ratio, CI: Confidence interval. Statistically significant P-values (P<0.05) are highlighted in bold.

**Table-5: Univariate analyses for the effect of PD-L1 and PD-1 expressions by intrinsic subtype on overall survival**

| PD-1 expression, by intrinsic subtype | P-value | PD-L1 expression, by intrinsic subtype | P-value |
|---|---|---|---|
| Luminal A | 0.298 | Luminal A | **<0.0001** |
| Luminal B | 0.188 | Luminal B | **<0.0001** |
| HER | 0.350 | HER | **0.008** |
| TNBC | 0.918 | TNBC | **0.004** |

Statistically significant P-values (P <0.05) are highlighted in bold.

## DISCUSSION

Since its discovery in early 1990s by the by Japanese scholar Ishida [20], the role of the checkpoint inhibitor PD-1, and its ligand PD-L1, in inhibiting anti-tumor immune cells has been well-established [21]. Recent reports have also suggested clinicopathological and prognostic values of PD-1/PD-L1 pathway in common types of cancer such as colorectal cancer and lung cancer [22, 23]. In the present retrospective study, we demonstrated that the expression of PD-L1 in BC cells was associated with higher stage and grade of the tumors, as well as more aggressive lymphatic invasion. Patients with PD-L1 were more likely to have Her2-positie and triple-negative subtypes of BC. The regression analysis also showed that positive expression of PD-L1 was associated with poor survival. In contrary, the expression of PD-1 within the TILs was neither associated with advanced BC nor poor prognosis.

As mentioned before, the expression of PD-1, and its ligand PD-L1, in tumor microenvironment and TILs is a major contributor to the immune evasion mechanism exhibited by many cancers [24]. Thus, immunotherapy targeting PD-1/PD-L1 pathway has emerged as one of the most effective, and safe, treatment regimens for many solid tumors [25]. With such advancement in our understanding of the pivotal role of PD-1/PD-L1 pathway in cancer cell immunogenicity and the effective anti-tumor effects of PD-1/PD-L1 inhibitors, it was recently hypothesized that the PD-L1/PD-1 pathway correlates with tumor invasion, metastasis, and prognosis [26]. However, in the setting of BC, previous studies showed inconsistent results regarding the clinicopathological and prognostic values of PD-1/PD-L1 pathway. Results from this study showed that the expression of PD-L1 within BC cells was associated with advanced cancer, more aggressive subtypes, and lymphatic invasion. The prognostic value of PD-L1 was clear in this work in which PD-L1 expression was an independent predictor of worse OS. In concordance with these findings, previous immunohistochemistry study demonstrated significant association between PD-L1 expression and advanced

tumor stage, positive lymph node, negative hormonal receptors, and worse OS [27]. Other reports by Cimino-Mathews and colleagues [28], Muenst and colleagues [29], and Tsang and colleagues [30] showed similar findings. In their recent meta-analysis study on five retrospective studies, Zhang and colleagues [31] showed that positive expression of PD-L1 was associated with higher grades, stages, lymphatic infiltration, and poor OS of BC. In contrast to findings in this study, a recent report by and Uhercik and colleagues [32] demonstrated favorable outcomes with positive PD-L1 expression in BC. The source of such high heterogeneity regarding the prognostic values of PD-L1 within the published literature is unclear and further studies are still needed.

On the other hand, no statistically significant association between PD-1 expression in TILs and clinicopathological features of BC has been detected in this study. Moreover, the PD-1 expression was not an independent predictor of mortality. Such findings was similar to Uhercik and colleagues' report [32] In contrary, Muenst and colleagues [33] reported that higher tumor grade, positive lymph node, and worse prognosis was associated with PD-1$^+$ TIL.

## CONCLUSION

In conclusion, this study demonstrated that PD-L1 expression was significantly associated with lymph node involvement, advanced tumor stage, high grade, aggressive subtypes of breast cancer, and poor OS. Furthermore, this work confirmed that PD-L1 is an independent negative predictor of survival in patients with breast cancer. Further studies with larger sample size are needed before wider investigations of anti-PD-L1 antibodies in clinical trials.

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