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Expression of Programmed Death Ligand 1 in Breast Cancer in Mexican Women

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Abstract

Background: Breast cancer is one of the most common malignant forms of neoplasia worldwide; programmed death protein 1 (PD-1), an inhibitory receptor of T lymphocytes, and its ligand programmed death ligand 1 (PD-L1), play an important role in the ability of tumor cells to evade the host's immune system.

Methods: We conducted a descriptive, observational study using retrospective data and an open evaluation using immunohistochemistry to determine the general prevalence of PD-L1 expression in 63 women with breast cancer who underwent a modified radical mastectomy, or quadrantectomy, with axillary lymph node removal.

Results: The prevalence of PD-L1 expression was 32% in patients with breast cancer treated with radical mastectomy. PD-L1 expression was higher in patients with large tumor size (19% for pT1, 37% for pT2, 50% for pT3, and 100% for pT4), metastasis in regional lymph nodes (25% for N0, 38% for N1, 75% for pN2, and 38% for pN3), and higher histological grade carcinoma (0% for grade 1, 23% for grade 2, and 50% for grade 3).

Conclusions: These findings suggest that PD-L1 expression is heterogeneous in breast cancer tumors and that its expression varies highly in tumor regions over time. The evaluation of PD-L1 expression is significant, because of the therapeutical implications that could improve the outcomes and prognosis of these patients.

Keywords: Breast cancer; Neoplasm; Obesity; Pathogenesis

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Introduction

Breast cancer is one of the most common malignant forms of neoplasia worldwide. According to the available evidence, breast cancer is the second most frequent type of cancer in the world and the first in women. Annually, about 2.1 million new cases are detected and 627,000 deaths are caused by this disease [1]. The treatment of malignant neoplasia has evolved in recent decades with the development of new chemotherapeutic agents and immunotherapy approaches.

In Mexico, breast cancer is the leading cause of death by malignant tumors in women; according to a Mexican retrospective study, which evaluated 379 patients, the molecular subclassification showed a luminal A subtype in 36.67%, luminal B in 37.73%, human epidermal growth factor receptor 2 (HER2) positive in 8.44% and triple-negative carcinomas in 17.15% of cases, thus demonstrating that breast carcinoma occurs at an earlier age in Mexican women compared to women in the USA (54.63 years versus 61 years) [2].

Programmed death ligand 1 (PD-L1) has become the focus of recent immune-oncology research. PD-L1 is expressed in tumor cells and participates in suppressing the local immune response through its binding to the programmed death protein 1 (PD-1) receptor on T lymphocytes [3]. PD-1 is expressed on the surface of T, B, and natural killer (NK) lymphocytes, activated monocytes, and dendritic cells, whereas PD-L1 is expressed in virtually all cell types.

The PD-1/PD-L1 pathway plays a critical role in the ability of tumor cells to evade the host's immune system [3]. Despite its name, PD-1 does not induce cell death directly but rather reduces growth factors and survival signals [4]. PD-1 has two ligands: PD-L1 (B7-H1) and PD-L2 (B7-CD). Binding of PD-1 to its ligand PD-L1 induces downregulation of the activity of tumor-reactive T lymphocytes, increases apoptosis, and reduces their immunogenicity [4].

In solid tumors, the inhibitory PD-1/PD-L1 pathway can be used to silence the immune system through overexpression of PD-L1 on the surface of tumor cells, which inhibits the function of T lymphocytes. PD-L1 can be overexpressed in multiple types of cancer, such as melanoma and lung, kidney, and gastric cancer. Monoclonal antibodies against PD-L1 are used as part of the current treatment of these tumors. PD-L1 has been shown to be overexpressed in breast cancer, particu-

larly in patients with the triple-negative immunophenotype, and its expression is associated with poor prognostic factors, such as large tumor size and high mitotic index [5].

Anti-PD-L1 treatment has been conceived as a way to block this ligand and to "release the brakes" on T lymphocytes to allow them to exert their cytotoxic effects leading to the death of tumor cells [4]. The expression of PD-L1 in breast carcinomas has been evaluated [3, 6]. A meta-analysis by Zhang et al [4] included five studies with 2,546 cases and found that PD-L1 expression is associated with lymph node metastases, high histological grade, estrogen receptor negativity, and the triple-negative immunophenotype. Other studies also support a strong relationship between PD-L1 expression and basal-type tumors.

Today, the usefulness of immunotherapy in breast cancer has been reported in some studies: KEYNOTE-086 examined pembrolizumab in metastatic, triple-negative breast cancer, where an objective response rate of 21.4% and 5.7% was found in treatment-naive and previously treated PD-L1-positive patients [7]. Recently, in KEYNOTE-355, it was demonstrated that the combination of pembrolizumab and chemotherapy had meaningful improvement in progression-free survival and overall survival versus placebo-chemotherapy among patients with metastatic triple-negative breast cancer with Combined Positive Score (CPS) of 10 or more (9.7 months versus 5.6 months; 23 months and 16.1 months, respectively) [8].

PD-L1 expression studies have been performed using various techniques such as immunohistochemistry, measuring messenger ribonucleic acid (mRNA) levels, and flow cytometry [9, 10]. The evaluation of PD-L1 expression is significant, because of the therapeutical implications that could improve the outcomes and prognosis of these patients. We used immunohistochemistry to investigate the general prevalence of PD-L1 expression in patients with breast cancer.

Materials and Methods

Patients and specimens

We conducted a descriptive, observational study using retrospective data. Patients treated by Medica Sur Hospital over a 5-year period, from January 2013 to December 2017, were included. The patients were eligible if they had a diagnosis of breast cancer confirmed by histopathology and had undergone a modified radical mastectomy or quadrantectomy with lymph node dissection. The case number, age, histological type, tumor size, positive lymph nodes, and tumor, node, metastases (TNM) pathology stage were recorded on a registration sheet created for this purpose. This study was approved by our scientific and bioethical committee (CONBIOETICA). As it was a retrospective analysis and data were de-identified, we did not contact patients nor asked for consents. Given the retrospective nature of this analysis, no intervention was made in the study population.

Immunohistochemistry

The most representative paraffin block for each patient (i.e.,

the block containing material with the highest quantity and quality of tumor tissue, absence of necrosis, etc.) was selected for the PD-L1 immunohistochemical study. For all samples, the immunohistochemical process was performed manually using PD-L1 (CD274) Clone BSR from Bio-SD. Placenta tissue was included as a positive control.

Evaluation of immunohistochemistry

There is no specific protocol for PD-L1 immunohistochemical assessment in breast cancer. Following previous studies, positivity was defined as a PD-L1-positive cell membrane in ≥ 1% cells. An intensity score of 1+ was considered as weak positivity or incomplete immunoreactivity of the membrane, 2+ as moderate or intense positivity and incomplete membrane immunoreactivity, and 3+ as intense positivity and complete immunoreactivity of the membrane. The relationships between PD-L1 expression and variables such as age, histological type, histological grade, tumor size, lymph node metastasis, and immunophenotype (luminal A, luminal B, HER2, and triple negative) were determined (Table 1). These relationships are expressed as numerical values and percentages.

Results

We collected data for 77 patients who met the inclusion criteria during the 5-year period established for the study (January 2013 to December 2017). Fourteen patients were excluded because of a lack of complete histological material within the institution (exclusion criterion), giving a total number of 63 patients.

The 63 patients selected were Mexican Hispanic-non-black patients and had an established diagnosis of breast cancer, and their clinical and immune profile information and the histological material were complete within the pathology service of the Medica Sur Hospital. The samples from all patients underwent PD-L1 immunohistochemical analysis with a positive external control (the placenta). PD-L1 staining was positive in samples from 20 (32%) patients. The average age of patients with PD-L1 expression was 60.35 years (range: 33 - 86 years). We found a non-significant tendency of PD-L1 positivity in women aged 54 or less (P = 0.839).

We found a direct relationship between the expression of PD-L1 and tumor size; the percentages of patients whose samples were positive for PD-L1 were 19% (n = 5) for T1, 37% (n = 11) for T2, 50% (n = 3) for T3, and 100% (1%) for T4. We examined the relationship between PD-L1 expression and the presence of lymph node metastasis. PD-L1 expression increased with the number of metastases for N0-N2. The percentages of patients positive for PD-L1 were 25% (n = 9) for N0, 38% (n = 5) for N1, and 75% (n = 3) for N2 (Table 1). The percentage for N3 was 38% (n = 3).

The percentage of patients with positive PD-L1 expression was highest in those with the triple-negative immunophenotype (58%; n = 7), followed by HER2 expression (40%; n = 2) and luminal B HER2-negative expression (36%; n = 8). PD-L1 was expressed in only 15% (n = 3) of patients with

Table 1. Characteristics of the Patients

PD-L1 expression in breast cancer tumors	Total number	Positive cases	% Positive cases	Negative cases
Tumoral size (TS)				
T1 (< 20 mm)	26	5	19%	21
T2 (> 20 mm - < 50 mm)	30	11	37%	19
T3 (> 50 mm)	6	3	50%	3
T4 (thoracic wall invasion or tumoral ulceration)	1	1	100%	0
Total	63	20		43
Node metastases (PN)				
N0 (no metastases)	36	9	25%	27
N1 (micrometastases or metastases in 1 - 3 lymph nodes)	13	5	38%	8
N2 (metastases in 4 - 9 axillary lymph nodes	4	3	75%	1
N3 (metastases in 10 or more lymph nodes)	10	3	30%	7
Total	63	20		43
Immunochemistry				
Luminal A	20	3	15%	17
Luminal B HER2 positive	4	0	0	4
Luminal B HER2 negative	22	8	36%	14
HER2	5	2	40%	3
Triple negative	12	7	58%	5
Total	63	20		43
Histological grade				
Grade 1	6	0	0	6
Grade 2	31	7	23%	24
Grade 3	26	13	50%	13
Total	63	20		43

Luminal A breast cancer is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive at least ≥ 1% of tumor cells), HER2-negative (≤ 10% tumor cells membrane) and has low levels of the protein Ki-67 in tumor cells (≤ 14%). Luminal B breast cancer is hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive at least ≥ 1% in tumor cells), and either HER2-positive (at least >10% of tumor cells complete membrane) or HER2-negative with high levels of Ki-67 (> 14%). Triple-negative/basal-like breast cancer is hormone-receptor negative (estrogen-receptor and progesterone-receptor negative; < 1% of tumor cells) and HER2-negative (≤ 10% tumor cells membrane). PD-L1: programmed death ligand 1; TNM: tumor, node, metastases.

luminal A cancer and in none of the patients with the luminal B HER-positive immunophenotype (Table 1).

The histological diagnoses of the patients whose samples expressed PD-L1 were as follows: 50% (n = 10) had infiltrating ductal carcinoma without specific pattern, 20% (n = 4) lobular, 15% (n = 3) micropapillary carcinoma, 5% (n = 1) ductal carcinoma with metaplastic component, and 5% (n = 1) mucinous carcinoma and mixed carcinoma (ductal and lobular).

The intensity of PD-L1 immunostaining in neoplastic cells was examined. In most of the 20 patients whose samples were positive for PD-L1 (70%, n = 14), fewer than 5% of cells were positive for PD-L1: 1% of cells were positive in eight patients (40%), 2% in two patients (10%), and 5% in four patients (20%). About 30% of cells were stained in two patients (10%), and 20%, 40%, 70%, and 95% in one patient each (5%). The intensity of staining was variable; the staining intensity was rated as 1+ or 2+ for eight patients and 3+ for six (30%).

PD-L1 expression was observed in tumor lymphocytes from nine patients, stromal expression was observed in five patients (8%), cytoplasmic expression was observed in 10 patients, and nuclear expression was observed in two patients. These values were independent of the PD-L1 positivity assessed exclusively as membrane immunostaining and were therefore not included as statistical data.

Discussion

In our study, the prevalence of PD-L1 expression was 32% in patients with breast cancer who underwent radical mastectomy at the Pathological Anatomy Service, Medica Sur Hospital, during the 5-year period from 2013 to 2017. We identified a higher expression of PD-L1 in patients with large tumor size (19 for pT1, 37% for pT2, 50% for pT3, and 100% for pT4),

metastasis in regional lymph nodes (75% for pN2 and 38% for pN3), and in high histological grade carcinomas (23% for grade 2 and 50% for grade 3).

PD-L1 expression was higher in the samples with the more aggressive immunophenotypes of triple negative (58%) and HER2 expression (40%). Little or no PE-L1 expression was detected in samples of the luminal B HER2-negative (36%), luminal A (15%), and luminal B HER2-positive (0%) phenotypes. These features highlight the heterogeneity of PD-L1 expression in tumor cells. We emphasize that this study was performed with complete tumor cuts, not with small biopsies or microarrays.

Studies have confirmed the relationships between PD-L1 expression and worse survival; for example, a retrospective study in Indonesia demonstrated that the overexpression of PD-L1 in triple-negative breast cancer patients is associated with worse prognosis, independent of other established risk factors [11]. In our study, we identified a direct relationship between PD-L1 expression and tumor size. The percentages of tumors whose samples expressed PD-L1 were 19% for T1, 37% for T2, 50% for T3, and 100% for T4. In the analysis of the relationship with lymph nodes with metastases, PD-L1 expression increased with metastases from N0 to N2 (75%), although the percentage (38%) was lower for N3. We also found a direct relationship between PD-L1 expression and the histological grade: 23% of grade 2 and 50% of grade 3 carcinoma samples expressed PD-L1, but no grade 1 samples were positive for PD-L1.

Muenst et al [12] reported a higher expression of PD-L1 in samples from patients with tumor size of pT3 (42.9%), followed by pT4 (35.7%), those with lymph node metastasis of N2 (50.0%), and those with histological grade of 3 (31.4%). Similar findings were reported by Ali et al [10], who reported higher PD-L1 expression in patients with T2 or higher grade (70.1%), N1 or higher (81.3%), and histological grade 3 (84.5%). Consistent with these findings, Soliman et al [13] found a higher expression in grade 3 tumors (30.5%).

Other studies have also examined the relationship between PD-L1 expression and the HER2-expressing and triple-negative immunophenotypes, and the inverse relationship with the luminal A and B phenotypes. Our results showed increased expression of PD-L1 in patients with breast carcinoma of the triple-negative immunophenotype (58%), followed by the HER2-expressing (40%) and luminal B HER2-negative (36%) phenotypes. We also found much lower expression in patients with the luminal A (15%) or luminal B HER2-positive (0%) immunophenotype. Muenst et al [12] also reported a stronger relationship with the HER2 (33.9%) followed by the triple-negative (30.7%) phenotypes. Ali et al [10] also found the highest positivity in the HER2-expressing (50%) and triple-negative (31.6%) phenotypes. Another study focused specifically on PD-L1 expression in breast carcinoma involving the triple-negative phenotype and its association with clinical and pathological outcomes [14]. In that study of 238 triple-negative carcinomas, PD-L1 expression was positively associated with histological grade 3 (88.5% of cases), tumor sizes pT1 (46%) and pT2 (45%), and lymph node metastases (81.5%).

In a study using flow cytometry, Soliman et al [13] found higher expression of the PD-L1 protein in the basal subtype (triple negative) and in cases involving lymph node metastases. Similarly, in a study of the mRNA from 5,454 mammary carcinoma samples, Sabatier et al [9] found that PD-L1 was upregulated in 20% of all carcinomas and in 38% of triple-negative cases.

Using RNA sequencing, Mittendorf et al [15] reported higher expression of PD-L1 in triple-negative breast cancer cases. The samples were evaluated using immunohistochemistry, and overexpression was identified in 19%. The researchers also identified an association between a higher number of CD8 T lymphocytes and the loss of expression of *PTEN*, which was attributed to the *PI3K* pathway [14]. Also, Oshi et al [16] demonstrated that a high abundance of regulatory CD4⁺ T cells had significantly elevated gene expression of PD-L1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) in triple-negative breast cancer. On the other side, a high activity of the E2F pathway is associated with cancer aggressiveness (triple-negative breast cancer, higher pathological stage, histological grade, and expression of Ki67), metastasis, PD-L1 expression and treatment response, because of cell proliferation-related gene sets such as G2M signaling [17].

Finally, we highlight that PD-L1 expression was heterogeneous and highly variable, in both the percentage of positive cells and staining intensity. Most of the samples stained weakly (< 5% positivity in 70% of positive samples), and the highest staining intensity (95%) was obtained in only one patient, who had the triple-negative phenotype. The staining intensity was 1+ or 2+ for eight patients and 3+ for six. PD-L1 expression in tumors appears to be heterogeneous and to vary highly in tumor regions over time. This heterogeneity can lead to a false-negative interpretation of the immunohistochemistry results from small biopsies [6]. We therefore recommend that PD-L1 expression should not be examined with immunohistochemistry in small biopsy samples.

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Conflict of Interest

JMRM has provided advisory and speaker services to Ipsen, Bristol-Myers Squibb, Merck Sharp & Dohme and Novartis and received sponsorship for travel and expenses from Bayer. RDH has provided advisory services Merck Sharp & Dohme and received sponsorship for travel and expenses from Roche. The rest of authors declare no conflict of interests.

Informed Consent

As it was a retrospective analysis and data were de-identified, we did not contact patients nor asked for consents.

Author Contributions

Original idea: PJEJ and RDH. Data recollection and literature analysis: PJEJ, EAF, LFAM, CDIT, and EAMC. Data analysis and interpretation: RDH and JMRM. Writing manuscript first draft: all authors. Writing manuscript final draft: MGMG, RDH and JMRM. Oversight and leadership responsibility for the research activity, planning and execution, including mentorship to the core of the team: RDH and JMRM.

Data Availability

The authors declare that data supporting the findings of this study are available within the article.

Abbreviations

mRNA: messenger RNA; NK: natural killer; PD-1: programmed death protein 1; PD-L1: programmed death ligand 1; RNA: ribonucleic acid

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