Immunohistochemical Evaluation of PD-L1 Expression in Triple-Negative Breast Cancer: An Analysis of 83 Cases

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Abstract

Background: Triple negative breast cancer (TNBC) is an aggressive disease associated with a poorer outcome when compared with other subtypes of breast cancer. Programmed death-ligand 1 (PD-L1) status in TNBC patients has been associated with prognostic value, high PD-L1 expression has reported to predict worse outcomes. PD-1/PD-L1 inhibitors has been approved in the treatment of TNBC in recent times. The aim of this study was to evaluate the expression of PD-L1 in TNBC.

Method: This was a cross-sectional study conducted among 83 cases of TNBC at the Department of Pathology, Bangladesh Medical University, Dhaka at the period of 2021-2023. Demographic and pathological parameters (histologic type, Nottingham histologic grade, tumor stage etc.) were assessed. Immunohistochemical expression of PD-L1 in tumor cells was observed. Collected data were placed in a data sheet. Results were collected and tabulated. Statistical analysis was performed on the tabulated data by chi-square test using SPSS 25.0.

Result: PD-L1 expression was positive in 19.3% (16/83) of the cases. Among these 2.4% (2/83) cases were strongly positive. No expression was seen in 80.7% (67/83) of cases.

Conclusion: PD-L1 expression was observed in about one-fifth of the study samples. Therefore, immunotherapy with anti-PD-1/PD-L1 agent can be considered as treatment option in selected TNBC patients.

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Introduction

In women, breast cancer is the most commonly diagnosed cancer and a leading cause of cancer death. With an estimated 2.3 million new cases annually, or around 24% of all cancers in women, female breast cancer has now overtaken lung cancer as the primary cause of cancer incidence worldwide.[1]

Triple-negative breast cancer (TNBC) refers to cancer with negative expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2).[2] TNBCs comprise 15-20% of all breast cancers and more commonly affect women of Indian, African American, and Hispanic descent, than the white population.[3,4] The prevalence of TNBC in the Indian subcontinent is estimated to be much higher compared to the western population. Two meta-analyses from India regarding the prevalence of TNBC, showed the pooled prevalence ranges from 25% to 31%.[5,6]

PD-1 (programmed cell death protein 1) is an immune checkpoint receptor expressed in T cells. It can bind its ligands PD-L1 and PD-L2 expressed on antigen-presenting cells such as B cells or macrophages. A major functional role of this signaling pathway is the inhibition of self-reactive T cells, to protect against autoimmune disease.[7] Tumor cells can also express PD-L1 and suppress the T-cell functions, which leads to apoptosis of T cells and potentiates tumor progression.[8,9]

Immune checkpoint inhibitors targeting PD-1/PD-L1 have impacted the treatment of many cancers such as non-small cell lung carcinoma, cervical squamous cell carcinoma, urothelial carcinoma, head and neck squamous cell carcinoma, gastric adenocarcinoma and melanoma.[4]

The KEYNOTE-522 trial showed the benefits of PD-L1 inhibitor in combination with chemotherapy over chemotherapy only in TNBC patients. Patients who received the combination therapy showed improved pathological complete response rate and event-free survival compared to who didn't.[10] Based on this trial, the Food and Drug Administration (FDA) of the United States of America granted approval for PD-1 inhibitor pembrolizumab for patients with locally advanced or metastatic TNBC.[11]

Therefore, given the therapeutic implication of PD-L1, this study was designed to assess PD-L1 expression in TNBCs.

Methods

This was a cross-sectional study conducted among 83 cases of TNBC during 2021-2023 at the Department of Pathology, Bangladesh Medical University, Dhaka. Demographic data was collected from the departmental records. Paraffin blocks, Hematoxylin and Eosin-stained (H&E) slides and ER, PR, Her-2/ Neu immunohistochemistry (IHC) slides of diagnosed cases of TNBCs were collected from Pathology department, BMU. H&E-stained sections and IHC slides of each case were re-evaluated to confirm the diagnosis. Core biopsy specimens, tumor with extensive areas of necrosis, paraffin blocks without adequate tumor tissue and patients who received neoadjuvant therapies were excluded. Sections from paraffin-embedded block were stained for IHC using pre-diluted, ready to use PD-L1 antibody following standard protocol compatible with DAKO REALTM EnVision system. The presence of PD-L1 staining in syncytiotrophoblast of term placenta served as a positive control.

PD-L1 Score

Selected cases had at least 100 evaluable or viable tumor cells; damaged or necrotic cells were excluded. The percentage of PD-L1 positive tumor cells to the total number of tumor cells were determined. Staining of anti-PD-L1 antibodies was evaluated in the tumor cell membrane.

Membranous staining was recorded as positive when viable tumor cells exhibited complete circumferential or partial linear plasma membrane staining at any intensity.

- Negative: 0 to < 1% of tumor cells
- Positive: 1–50% tumor cells
- Strong positive: > 50% tumor cells.[12]

The statistical analysis was carried out using the Statistical Package for Social Sciences version 25.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics (frequencies and percentages) were used to summarize the patients' demographic and histological characteristics and were presented in tables, figures and charts. The frequencies of different entities were expressed as percentage.

Results

Demographic and histopathological variables i.e. age, histological type, histologic grade and tumor stage were assessed and immunohistochemical expression of PD-L1 was observed. Age variation ranged in between 22 years to 70 years. Most of the cases (38.5%) were found in the 5th decade. Mean age of patients was 44.46 (± 10.3) years. When the laterality of occurrence was considered, it was observed that in 39 (47%) cases the tumor existed in right breast while in 44 (53%) cases tumor occurred in left breast. The vast majority of the cases were infiltrating ductal carcinoma (73 cases, 88%), followed by 5 (6%) invasive breast carcinoma NST (IBC-NST) with medullary features and 5 (6%) metaplastic carcinomas. Among the total 83 cases, PD-L1 expression was positive (> 1% tumor cells) in 19.3% (16/83) of the cases, while no expression was seen in 80.7%. (Figure 1 & 2). Among the positive cases 2.4% (2/83) cases were strongly positive cases (Figure 3).

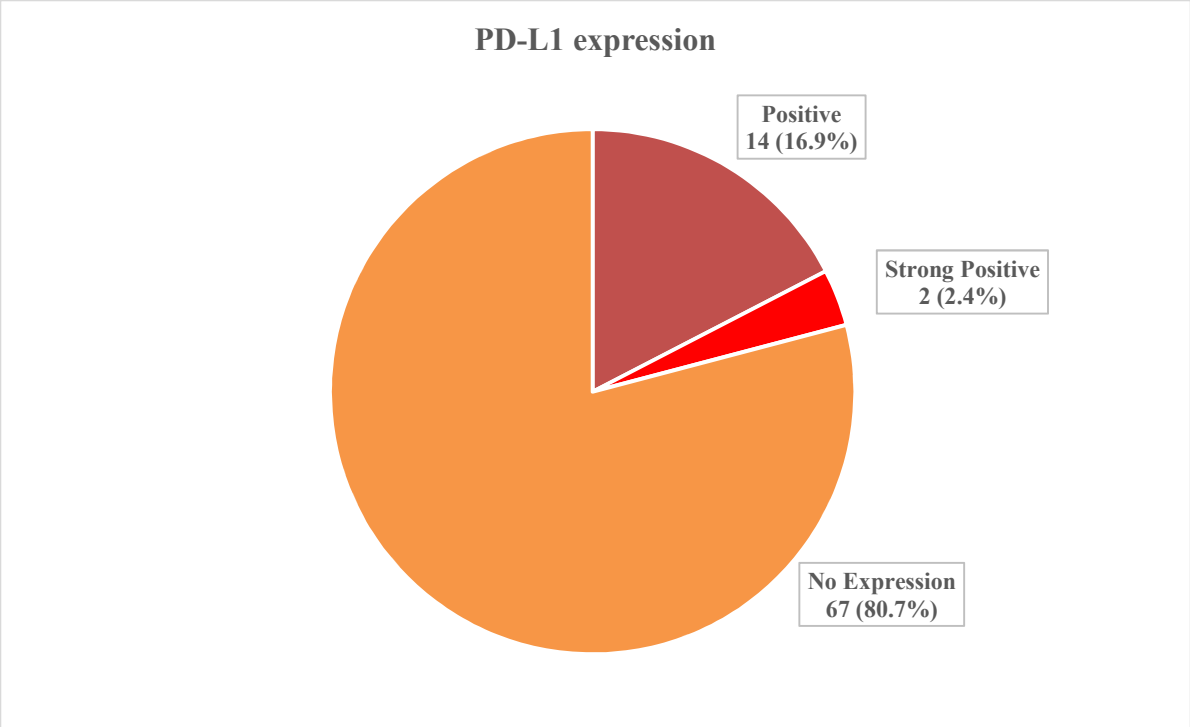

Figure 1. Distribution of PD-L1 expression (n=83).

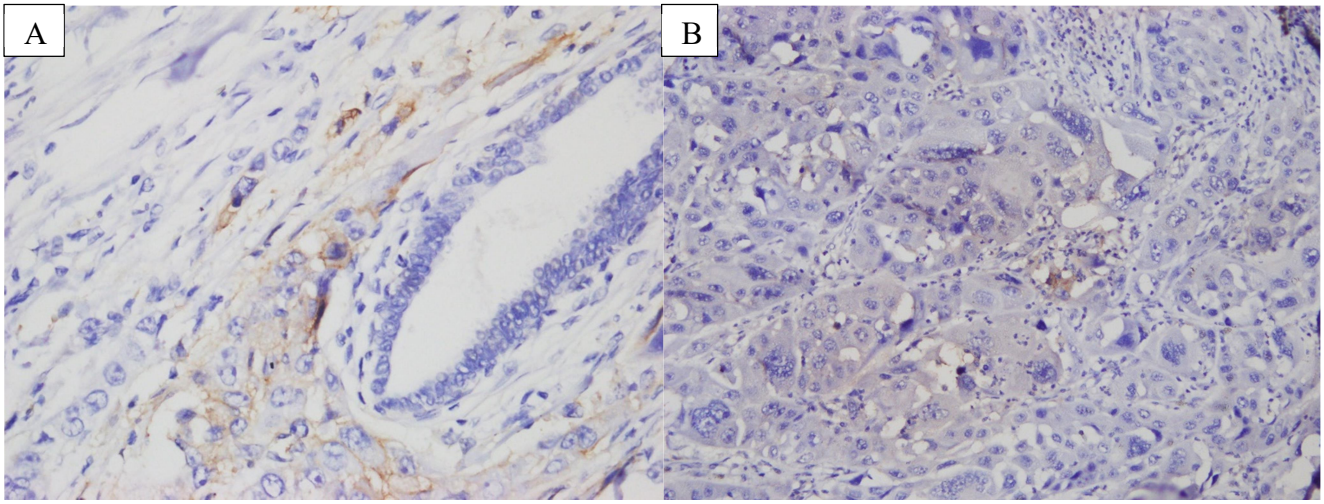

Figure 2. (A) Photomicrograph showing positive PD-L1 staining (40x); (B) Photomicrograph showing negative PD-L1 staining (40x)

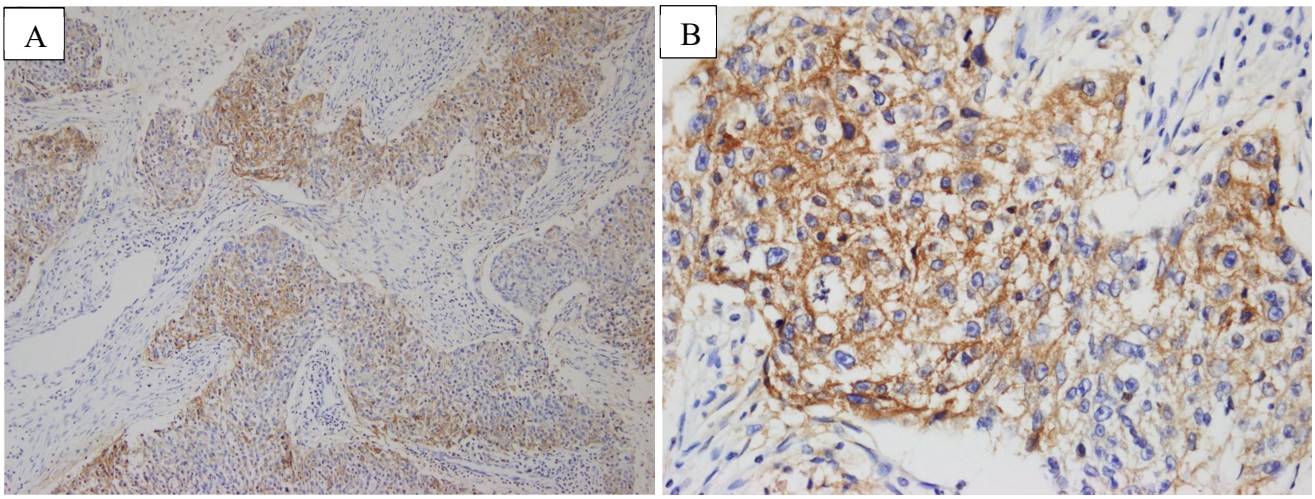

Figure 3. (A) Photomicrograph showing strong positive PD-L1 staining (4x); (B) Strong positive PD-L1 staining (40x)

Discussion

Chemotherapy is the standard treatment in TNBC. In the metastatic setting, TNBC, has a relatively shorter median survival period. Adding the programmed death-1 (PD-1)-targeting agents atezolizumab or pembrolizumab to chemotherapy was recently shown to improve outcomes in both early-stage and metastatic TNBC.

In this study 83 cases were included. The mean age of patients were 44.46 ± 10.3 years ranging from 22 to 70 years. The majority (38.5%) of the cases were in the 5th decade. However, two studies from Europe showed that the median age at the time of diagnosis was 55 and 54 years [13,14] TNBC is diagnosed at an earlier age in patients in the subcontinent. In the study of Ishitha et al., conducted in the southern India, the mean age of TNBC patients was 46±12 years [15]. A study from Pakistan showed that, 71% of the TNBC cases were in women < 50 years [16]. A previous study from Bangladesh by Mostafa et al. showed that patients mean age at diagnosis was 45.6 years.[17] Therefore, this study is similar in respect to age at the time of diagnosis, compared with other studies in the region.

The current study found that the vast majority of the cases were infiltrating ductal type (n = 73, 88%), followed by Invasive breast carcinoma with medullary features (n = 5, 6%) and metaplastic carcinoma (n = 5, 6%). Schmidt et al.(2022) and Vangangelt et al.(2020) also showed similar findings, as their study showed majority of the patients (90% and 65% respectively) had invasive ductal carcinoma [12,14]. Similarly 96% of cases were classified as invasive ductal carcinoma, not otherwise specified; in the study of Ishitha et al. [15]. Thus, the finding of this study, that majority of TNBCs are of infiltrating duct type are similar with previous studies.

The majority (49/83, 59%) of the tumors were in Nottingham histological grade 3 in this study. Moorman et al. also found higher frequency (77%) of grade 3 tumors in their study.[18] The study by Schmidt et al. found 66% had grade 3 cancers.[12] Similarly in an Indian study by Akhtar et al.,(2015) observed that majority of TNBCs were in grade 3 as compared to other grades.[19] Hence, the finding of this study, that grade 3 tumors is the majority of TNBC is similar to other studies.

In this study, PD-L1 staining was done with 28-8 clone. Positive expression was seen in 19.3% (16/83) of the cases. Among positive cases, 2.4% (2/83) were strongly positive. No expression was seen in 80.7% (67/83) of cases. Among the 248 TNBCs studied by Mori et al., PD-L1 (E1L3N clone) expression was detected in 103 (41.5%).[20] In the study of Bharadwaj et al., conducted on Indian women with TNBC, showed that 12.9% of cases were PD-L1 positive.[21] In the study of Gajaria et al. PD- L1 (SP263 clone) positivity of tumor cells was seen in 14.7% of the case [22]. The variation in PD-L1 expression is due to use of different clones of PD-L1 and cut off values in different studies as shown by Schmidt et al. and Huang et al. The study of Schmidt et al. (2022) compared three PD-L1 antibodies (22C3, 28-8 and, SP142). They showed 22% positivity including one strong positive case for 28-8 clone which is similar to the current study. The 22C3 and 28-8 clones showed identical results in terms of positivity [12]. Another comparative study (28-8, 22C3, and SP142 clone) by Huang et al. showed PD-L1 positivity in 16% of tumor cell with 28-8 clone. They also showed 28-8 and 22C3 assays demonstrated 93% concordant cases with substantial agreement (kappa co-efficient 0.72). There was less agreement between SP142 and the other assays.[23] Currently, the 22C3 clone is the only FDA-approved companion diagnostic test (FDA, 2023). In the present study, 28-8 clone was used, due to the unavailability of 22C3 from the suppliers. However, previous studies have demonstrated a high concordance rate between 28-8 and 22C3 assays across a range of tumors.[24]

Conclusion

The expression of PD-L1 in a subgroup of triple-negative breast tumors in Bangladeshi women was shown in this study, confirming its significance as a biomarker in this condition. Evaluation of PD-L1 expression has important clinical implications since it can help identify pateints who might benefit from immunotherapy and help develop more personalized treatment plans.

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