Original Article

Effect of neoadjuvant chemotherapy (NAC) on programmed cell death ligand (PD-L1) in patients of carcinoma breast: A prospective study in Indian tertiary care setting

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Abstract

Context: Several studies have reported that PD-L1 has shown therapeutic activity in various tumor types. However, its expression changes in a person on administration of NAC which is reported by very few studies. **Aims:** To find out the difference in the expression of PD-L1 by tumor cells after the administration of NAC. **Settings and Design:** This prospective study was conducted on 30 patients who were diagnosed with locally advanced breast carcinoma (LABC) between 2017 and 2019 and those who received NAC followed by surgery. **Methods and Material:** Breast cancer specimens were collected using core needle biopsy prior to administration of NAC and IHC was performed. Frequency and staining intensity of PD-L1 by tumor cells were analyzed. PD-L1 expression was dichotomized into two groups according to the frequency distributions of the H-scores. **Statistical Analysis Used:** The differences in expression of PD-L1 along with various parameters were analyzed using Chi-square test and Student's t test. **Results:** The mean age of the patients in our study was 51.37 ± 11.37 years. The response of NAC according to the RECIST criteria showed that most of patients (83.3%) showed complete response. Of the 30 cases, 11 (36.7%) patients were PD-L1 positive before the administration of NAC. We found a significant change in expression from positive to negative status, i.e., seven patients changed from positive to negative ($p = 0.036$). Upon comparing the PD-L1 expression before NAC, significant association was observed between the primary tumor (T) and tumor stage with high PD-L1 expression ($p = 0.020$ and $P = 0.034$). After NAC, 18 (69.2%) patients who were ER positive and 18 (69.2%) patients who were PR positive showed negative PD-L1 expression while none of them were positive in PD-L1 positive patients ($p = 0.018$ and $P = 0.018$). **Conclusion:** PD-L1 expression in a same person changes upon administration of NAC which may indirectly be used as a predictor of response to NAC.

Keywords: Breast cancer, neoadjuvant chemotherapy, Programmed cell death ligand

Introduction

Breast cancer is the most common cancer among women, affecting 2.1 million women each year and causing the largest number of women with cancer-related deaths. In 2018, breast cancer deaths were reported at 627,000, i.e., 15% of all women cancer-related deaths.[1] While the breast cancer rates among

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women in developed countries are higher, their rates are rising globally in almost every region.[2] In the Indian scenario, breast cancer is the most common cancer among women in metropolitan cities.[3] Despite the remarkable advances in therapies for breast cancer, there are still women who die of this disease at the end. In Western population, the incidence of advanced stage disease has decreased due to routine screening protocols. However, we face an increase in the number of locally advanced and metastatic cases in the Indian setting even at a younger age at the time of diagnosis. In such cases, NAC remains the cornerstone of treatment.

Immunotherapy is a newly emerging tool in breast cancer management and can be safely applied as an adjunct or alternative to chemotherapy in the treatment of breast cancer. Cancer cells frequently express tumor antigens that, in principle, can be recognized by the patient's immune system; however, the resultant immune responses are ineffective and often do not parallel clinical tumor regression.[4]

Adaptive and innate immune responses play an important role in tumor immune monitoring and may restrict neoplasm development and growth. Tumors develop in a complex network of epithelial cells, blood vessels, lymph channels, cytokines and chemokines, and invade immune cells known as tumor microenvironments.[5] It has been shown that adaptive immune system cells conduct monitoring and can remove nascent tumors via various immune modulatory pathways.

There is a complex interaction between the T-cells and antigen-presenting-cells (APC) which involves T-cell receptor along with multiple co-stimulatory receptors. This can either activate or inhibit T-cell function. Programmed cell death-1 (PD-1) is an example of such co-stimulatory receptor that belongs to CD28/CTLA-4 family. It conveys an inhibitory signal to T-cell, thus impeding immune response; a protective mechanism designed for self-antigens.[6] Tumor cells mimic this interaction and escape from our immune system which otherwise would have been destroyed, this phenomenon is known as "tumor evasion".

PD-1 binds on the surface of cancer cells to programmed cell death ligand-1 (PD-L1), which suppresses the T lymphocyte antitumor functions. Thus, the expression of PD-1 suggests depleted lymphocyte activity, and a high level of PD-1 + tumor lymphocyte infiltration correlates with worse breast cancer survival.[7] PD-L1 expression has been shown in various cancers such as lung, melanoma, ovary, colon, and breast cancers.[8,9]

Several studies indicated that chemotherapy had an immunogenic function. They improve the immunogenicity of tumor cells, or they can modulate the immune cells and thus exert antitumor response.[10] Doxorubicin can induce the immunogenic death of cancer cells by the release of high-mobility group box-1 (HMGB-1) protein from dying cancer cells.[11] Paclitaxel enhances lymphocyte anti-tumor function by activating various cytokines.[12] In view of the clinical applications of the PD-1/PD-L1 axis and after reviewing previous studies, we aimed to find out the difference in the expression of PD-L1 by tumor cells after the administration of NAC which may have significant implications for the treatment of breast cancer.

Methods

Patient cohort

The cohort used in this study consists of 30 patients with locally advanced breast carcinoma (LABC) diagnosed between 2017 and 2019 and received NAC followed by surgery at the Department of General Surgery, Institute of Medical Sciences in Banaras Hindu University. Breast cancer specimens were collected using core needle biopsy prior to the administration of NAC and IHC was performed. Then, three cycles of NAC was given and modified radical mastectomy was performed and this sample was used for IHC this time. Most of our patients (76.7%) received CAF regimen (cyclophosphamide, Adriamycin, and 5-fluorouracil) followed by Taxane-based chemotherapy in 23.3% of individuals. The ethical approval for the study was obtained from the Institutional Ethics Committee and the protocols conformed to the ethical guidelines of the 1975 Declaration of Helsinki. A proper written informed consent was obtained from all the patients. Approval from institute ethical committee was taken. It was done on 24/10/2017.

Estimation of PD-L1 using Immunohistochemistry (IHC)

Tissue microarray (TMA) was built using the most representative areas from each single case. Immunohistochemical staining was done after the preparation of slides from formalin-fixed, paraffin embedded tissues (after antigen retrieval from TRIS-EDTA buffer followed by peroxidase block and power block). Slides were incubated overnight at 4°C with a prediluted primary rabbit-anti-human PD-L1 polyclonal antibody (Abcam, Cambridge, UK). The standard DAB-technique (Optiview DAB IHC Detection Kit, Ventana Medical Systems, Tuscon, AZ, USA) was employed for immunostaining. The results were interpreted using a light microscope (Olympus BX 51, Tokyo, Japan) [Figure 1a-d].

PD-L1 Histo-scoring

The frequency and staining intensity of PD-L1 by tumor cells were analyzed, and PD-L1 expression was quantified using the modified Histo-score (H-score) with a range of possible scores from 0 to 300. PD-L1 expression was dichotomized into two groups according to the frequency distributions of the H-scores, using a cut-off score of ≥100 (H-score 0–99 = negative/low expression and 100–300 = high/positive expression).

Statistical analysis

Statistical analysis was performed using statistical package for the social sciences (SPSS), Version 23.0. IBM Corp., NY). Simple descriptive statistics were used (mean ± standard deviation) for quantitative variables, and frequency with percentage

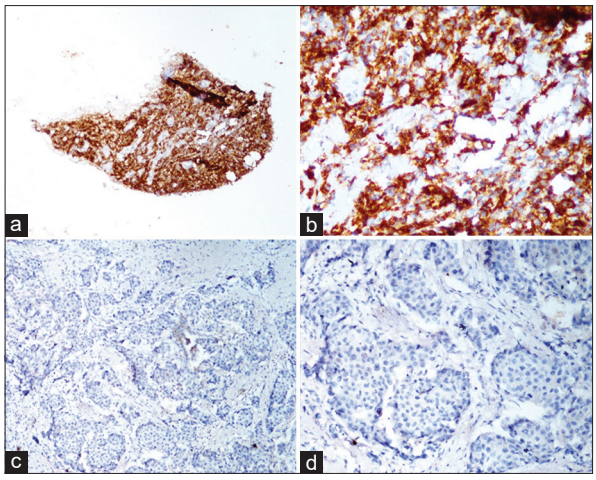

Figure 1: (a) Photograph of PD-L1 expression in breast cancer tissue specimen showing diffuse positivity of PD-L1 seen after IHC staining on 10X field. (b) Photograph of PD-L1 expression in breast cancer tissue specimen showing membranous and cytoplasmic positivity of PD-L1 seen on tumor cells after IHC staining through a 40X field. (c) Photograph of PD-L1 expression in breast cancer tissue specimen without any staining seen after IHC staining (PD-L1 negative) through a 10X field. (d) Photograph of PD-L1 expression in breast cancer tissue specimen without any staining seen after IHC staining (PD-L1 negative) through a 20X field

distribution for categorized variables. All the clinical and related parameters studied during observation period were compared using Chi-square test for parametric variables. Statistical analysis was performed using the paired and unpaired Student's t test, the Wilcoxon test, and the Mann–Whitney U test, with the comparison of absolute and relative values for cells obtained before and after NAC. The value of $P < 0.05$ was considered as the significant difference for comparison.

Results

The mean age of the patients in our study was 51.37 ± 11.37 years (ranging from 30 to 71 years). Majority (33.3%) of the patients were aged 41–50 years followed by >60 years (26.7%) and 51–60 years (23.3%), respectively. There were 29 women and only 1 male patient. 13 (43.3%) of our patients had T2-stage primary tumor followed by T4 stage in 12 (40%) patients as per TNM staging of AJCC 8th 2017. In addition, majority of them (63.3%) were in the cN1 stage. Triple negative breast cancer (TNBC) patients were seven in number (23.3%). The demographic and clinicopathologic characteristics of the patients are shown in Table 1.

The response of NAC according to RECIST criteria showed that most of patients (83.3%) showed complete response followed by those with partial response (13.3%) after NAC; one patient (3.3%) was stable and did not show any decrease or increase in size.

Of the 30 cases, there were 11 (36.7%) patients who were PD-L1 positive before the administration of NAC. After giving three cycles of NAC only four (13.3%) patients remained PD-L1 positive while the rest of them, i.e., seven patients out of 11 changed from positive to negative with a significant *P* value of 0.036 [Figure 2].

Table 1: Demographic and clinicopathologic characteristic of patients

	Number (Percentage)
Age, years	51.37±11.379 (range 30-71)
Gender	
Male	1 (3.3)
Female	29 (96.7)
BMI	
<18.5	1 (3.3)
18.5-24.9	22 (73.3)
25-29.9	7 (23.3)
Symptoms	
Breast Lump	30 (100.0)
Breast Pain	21 (70.0)
Discharge from Nipple	8 (26.7)
Ulceration of breast	3 (10.0)
Anorexia	5 (16.7)
Weight loss	3 (10.0)
Age of menarche	
<15 years	18 (60.0)
≥15 years	11 (36.7)
Other (Male patient)	1 (3.3)
Menopausal status	
Premenopausal	10 (33.3)
Postmenopausal	19 (63.4)
Other (Male patient)	1 (3.3)
Tumor Stage	
II A	2 (6.7)
II B	11 (36.6)
III A	5 (16.7)
III B	12 (40.0)
Histological grade	
Grade I (Low)	14 (46.7)
Grade II (Intermediate)	16 (53.3)
Estrogen receptor	
Positive	18 (60.0)
Negative	12 (40.0)
Progesteron receptor	
Positive	18 (60.0)
Negative	12 (40.0)
HER2/neu	
Positive	16 (53.3)
Negative	14 (46.7)
TNBC (Triple Negative Breast Cancer)	7 (23.3)

Upon comparing the clinicopathological parameters with PD-L1 expression before NAC, all of the PD-L1 positive patients (n = 8, 72.7%) were in the T4 category followed by T2 category in two (18.2%) patients. Most of the PD-L1 negative patients (n = 11, 57.9%) were in T2 category which showed significant association (p = 0.020). Also among PD-L1 positive patients, nine (81.8%) patients were in stage IIIA & IIIB while in negative patients, 11 (57.9%) patients were in Stage IIA & IIB which showed a significant association (p = 0.034). Sixteen (84.2%) ER positive patients and 16 (84.2%) PR positive patients who were negative for PD-L1 expression showed significant

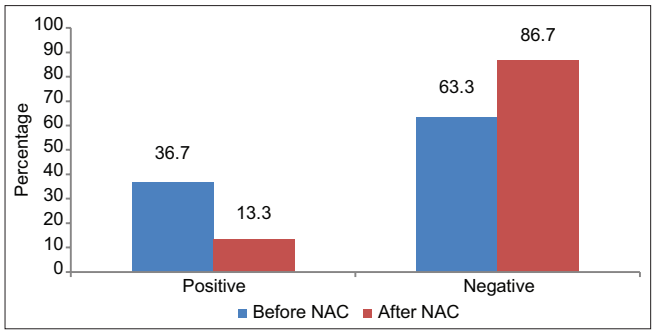

Figure 2: Comparison of change in PD-L1* on administration of NAC *Here positive and negative is based on PD-L1 expression in IHC which was calculated using Histo Score which ranged from 0-300. Negative implies values between 0-99 while positive implies values from 100-300

	Before NAC	After NAC	*p*
PD-L1*			
Positive	11 (36.7%)	4 (13.3%)	**0.036**
Negative	19 (63.3%)	26 (86.7%)	

association ($p < 0.001$). Her2-neu status and histological grade of tumor were also compared with PD-L1 expression but did not show any significant association [Table 2]. After NAC, among negative PD-L1 patients, 18 (69.2%) were ER positive and 18 (69.2%) PR positive, while none of them were positive in PD-L1 positive patients. Other parameters such as primary tumor (T), tumor stage, histological grade, and Her2-neu status were also compared but they did not show any significant association [Table 3].

Discussion

Locally advanced breast cancer (LABC) is a heterogeneous group of patients with uncertain prognosis and a 5-year survival rate of 50%–80%.[13] Among developing countries, the prevalence of LABC is higher than in Western countries, possibly due to the late-stage disease when diagnosed.[14] NAC is one of the standard therapy options for the management of LABC. The objectives of preoperative systemic therapy in LABC include early eradication of distant subclinical micrometastases and downstage of the primary tumor to enable operability. This method has the added benefit of making an *in vivo* measurement of chemotherapy responsiveness to tumors.

It was clearly evident in this analysis that most of our participants were 41–50 years of age. This is slightly below the age of 52 in Western countries.[15] This is further confirmed by a study conducted by Surakasula *et al.*, who also reported breast cancer in India as opposed to their Western counterparts in a younger age group.[16] Only seven (23.3%) patients in our sample had triple negative breast cancer, the result was close to different studies, i.e., the prevalence of triple negative breast cancer to be in the range of 10–30%.[17]

PD-L1 has been shown to be directly involved in the protection of cancer cells from destruction by activated T lymphocytes by inhibiting anti-cancer immune response.[18] The interaction

Table 2: Comparison of clinico-pathological parameters with expression of PD-L1 before NAC

	PD-L1		*p*
	Positive (*n*=11)	Negative (*n*=19)	
	No. (%)	No. (%)	
Primary Tumor (T)			**0.020**
T1	0 (0)	0 (0)	
T2	2 (18.2)	11 (57.9)	
T3	1 (9.1)	4 (21.1)	
T4	8 (72.7)	4 (21.1)	
Tumor Stage (dichotomised)			**0.034**
Stage II A & II B	2 (18.2)	11 (57.9)	
Stage III A & III B	9 (81.8)	8 (42.1)	
Histological grade			0.156
Grade I (Low)	7 (63.6)	7 (36.8)	
Grade II (Intermediate)	4 (36.4)	12 (63.2)	
Estrogen receptor			**<0.001**
Positive	2 (18.2)	16 (84.2)	
Negative	9 (81.8)	3 (15.8)	
Progesterone receptor			**<0.001**
Positive	2 (18.2)	16 (84.2)	
Negative	9 (81.8)	3 (15.8)	
HER2/neu			0.510
Positive	5 (45.5)	11 (57.9)	
Negative	6 (54.5)	8 (42.1)	

Table 3: Comparison of clinico-pathological parameters with expression of PD-L1 after NAC

	PD-L1		*p*
	Positive (*n*=4)	Negative (*n*=26)	
	No. (%)	No. (%)	
Primary Tumor (T)			
T1	0 (0)	0 (0)	
T2	1 (25.0)	12 (46.2)	0.360
T3	0 (0)	5 (19.2)	
T4	3 (75.0%)	9 (34.6)	
Tumor Stage			
Stage II A & II B	1 (25.0)	12 (46.2)	0.427
Stage III A & III B	3 (75.0)	14 (53.8)	
Histological grade			
Grade I (Low)	2 (50.0)	12 (46.2)	0.886
Grade II (Intermediate)	2 (50.0)	14 (53.8)	
Estrogen receptor			
Positive	0 (0)	18 (69.2)	**0.018**
Negative	4 (100)	8 (30.8)	
Progesterone receptor			
Positive	0 (0)	18 (69.2)	**0.018**
Negative	4 (100)	8 (30.8)	
HER2/neu			
Positive	1 (25.0)	15 (57.7)	0.222
Negative	3 (75.0)	11 (42.3)	

of PD-1/PD-L1 between the tumor cells and T-lymphocytes results in the impairment of both cytokine development and T-lymphocyte apoptosis. Patients with breast cancer already have immune defects such as a lower absolute number of peripheral blood lymphocytes and an increased number of functionally immunosuppressive CD4 + CD25 + T-reg

lymphocytes in both the peripheral blood and tumor microenvironments.[19]

We found the PD-L1 expression in breast cancer specimens to be 36.7% before NAC administration, which is consistent with the study by Ghebeh *et al.*[20] who reported the expression of PD-L1 in 34% of breast cancers. Schalper *et al.*[21] reported the expression of PD-L1 mRNA in 58% of their specimens of breast cancer. Since giving NAC, there was a significant reduction in the expression PD-L1, which had now decreased to 13.3%. Nonetheless, the higher expression of PD-L1 in NAC patients could be due simply to the fact that the patients who receive it typically have LABC. Most of the cases were negative at baseline and remained negative after chemotherapy in a study done by Pelekanou *et al.*[22] They also reported that PD-L1 expression decreased in most of the initially positive PD-L1 tumors following chemotherapy that is in line with our study.

Cytotoxic drugs are widely used to supplement the activation of immune checkpoints, which in some cases can provide immunogenic benefits. Contrary to our study, Peng et. al reported that anthracycline and taxane upregulated PD-L1 and Gal-9 expressions, independent of the effect of IFN-γ, *in vitro* and also that cytotoxic drugs could induce PD-L1 upregulation through the NFκB-dependent pathway in an ovarian cancer model.[23]

Upon comparing PD-L1 with various clinicopathological parameters it was further found that PD-L1 expression was associated significantly with poor prognostic factors like higher tumor grade (72.7% of PD-L1 positive cases were in T4 category), tumor stage (81.8% of PD-L1 positive cases had stage III tumor), and hormone receptor status (81.8% of PD-L1 positive cases were both ER and PR negative). Histological grade and Her-2-neu status did not show any significant association. This in accordance with various other previous studies.[17,24,25]

Once expressed by antigen-presenting cells, PD-L1 generates an inhibitory signal to T-lymphocytes by inducing T-lymphocyte apoptosis.[26] Therefore, by inhibiting the immune system, it is likely that PD-L1 can establish a favorable environment for the tumor to develop. Another likelihood of lower survival may be due to the association of tumors with higher growth rates (large tumor size and high histological grade) or poor differentiation (small histological grade and negative estrogen and progesterone receptors), which is known to reduce the survival of patients with cancer.[27]

We agree that our research has some limitations such as the fact that the use of tissue microarrays (TMAs) may not accurately represent PD-L1 protein expression due to the intratumoral heterogeneity of expression. Also, there are questions about the efficacy of immunohistochemical staining for PD-L1, due to the lack of a standardized procedure for staining and analysis, as well as the variety of antibodies. The relatively small number of patients in each group makes it difficult to draw a conclusion. While the majority of our patients (76.7%) received CAF regimen (cyclophosphamide, adriamycin and 5-fluorouracil), some were given Taxane-based chemotherapy (23.3%), limiting any interpretation of treatment-specific results.

Conclusion

Manipulating the immune system to directly identify and kill tumor cells can be an effective alternative approach to cancer treatment in the days ahead. Blocking the PD-1/PD-L1 pathway has become a recent therapeutic strategy promising in oncology. Taking these findings into account, anti-PD-L1 antibodies combined with chemotherapy agents may be suitable candidates for chemoimmunotherapy. The area of PD-L1 assessment is rapidly evolving and several corresponding and complementary diagnostic applications in various other malignancies are approved by FDA. Also, we conclude that change in PD-L1 expression may serve as an indirect indicator to assess the response of NAC in a patient.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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