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Immunohistochemical Expression of Cyclin D1 and PD-L1 With TILs Density in Prediction of the Response to Neoadjuvant Chemotherapy Treatment in Invasive Ductal Carcinoma of the Breast

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Abstract

Introduction: Breast cancer is the most common malignancy affecting women. Cyclin D1 overexpression is one of the basic genetic alterations which implicated in its carcinogenesis. PD-L1 acts as a promising biomarker emerging in several tumor types.

Aim: To evaluate the immunohistochemical (IHC) expression of cyclin D1 and PD-L1 in invasive ductal carcinoma of the breast of no special type (IDC-NST) in different molecular subtypes, and to analyze the correlation between their expression with stromal tumor-infiltrating lymphocytes (TILs) density and response to neoadjuvant chemotherapy (NAC).

Materials and Methods: This prospective study was conducted in Faculty of Medicine, Zagazig University, Egypt. A total of 80 patients diagnosed with IDC-NST were studied from January 2017 to May 2019. Specimens taken were 30 core biopsies before receiving NAC protocol, and 50 mastectomy specimens not received NAC. Data was statistically analyzed using SPSS 22.0 software.

Results: PD-L1 expression was detected in 30.1% in tumor cells and in 22.5 % in TILs. There was a significant association between PD- L1 expression and stromal TILs ($p<0.001$).TILs density was high in 47.5 % of the cases mainly in triple-negative breast cancer. Cyclin D1 expression was observed in 56.3%. There was a significant association between PD-L1 expression and TNBC, and a significant association between luminal breast cancer and cyclin D1 expression. PD-L1 and cyclin D1 expression was significantly correlated with response to NAC.

Conclusion: Cyclin D1 and PD-L1 may act as predictive biomarkers for response to neoadjuvant chemotherapy and can be targeted in future therapeutic approaches.

Keywords: Breast cancer; Cyclin D1; PD-L1;Tumor-infiltrating lymphocytes;Neoadjuvant chemotherapy

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Introduction

Breast cancer is the most common malignancy affecting women worldwide [1]. PD-L1 is T-lymphocyte-inhibitory molecule and a member of the B7 family, several investigations have recently demonstrated that PD-L1 expression may have a key role in the interaction of tumor cells with the host immune response, and may function as a mechanism of adaptive immune resistance [2]. PD-L1 is expressed in both tumor cells and TILs [3]. PD-L1 acts as a promising biomarker emerging in several tumor types; patients whose tumors overexpress PD-L1 by immunohistochemistry (IHC) have improved clinical outcomes with anti-PD-L1 directed therapy [4]. Many previous studies reported that breast cancers have unregulated PD-L1 on the tumor cell surface [3,5,6]. TILs can predict a response to a given treatment. It can serve as an excellent surrogate for monitoring cancer response to treatments. Therefore, these are most useful when assessed before the initiation of treatment with NAC. Also, TILs are relevant in decision-making regarding immunotherapy selection in various solid tumor types [7]. Some types of aggressive breast cancer do not respond to hormonal or targeted therapy such as triple negative breast cancer (TNBC). PD-L1 expression in TNBC has been shown to range from 40 to 65% in several studies [8]. The mammalian cell cycle is driven by a complex interplay between cyclins and their associated cyclin-dependent kinase (CDK) partners, and dysregulation of this process is one of the hallmarks of breast cancer. Cyclin D1, a cell cycle regulator that has a critical job in cell cycle progression from the G1 phase to the S phase through interaction with CDk4 and CDk6 [9]. Cyclin D1 protein was recognized by IHC in around 65-70% of breast carcinoma in several studies [10]. Over expression of cyclin D1 in breast cancer

might be related to a good prognosis as it is conveyed in ER-positive subtypes [9]. This study aimed to evaluate the immunohistochemical expression of PD-L1 in both tumor cells and stromal TILs and cyclin D1 expression in tumor cells in IDC- NST with its different molecular subtypes and to analyze the relationship between their expression with stromal TILs density and response to NAC treatment received.

Materials and Methods

Patients and tissue specimens

Eighty patients with IDC-NST were enrolled in this prospective study, during the period from January 2017 to May 2019. This study was carried out at pathology, general surgery, and clinical oncology departments, Faculty of Medicine, Zagazig University, Egypt. The diagnosis of breast cancer was achieved thorough clinical examination followed by mammography, ultrasonography, and eventually a core biopsy. Specimens taken were 30 core biopsies from patients before receiving neoadjuvant chemotherapy protocol (AC-paclitaxel); 4 cycles of doxorubicin (60 mg/m^2 day1/21 days) and cyclophosphamide (600 mg/m^2 Day1/21days) followed by 4 cycles of paclitaxel (175 mg/m^2 IV.3 hours day1/21 days, and 50 mastectomy specimens (modified radical mastectomy or breast-conserving surgery with axillary lymph node dissection) not received neoadjuvant chemotherapy protocol. In this study, we excluded all other special types of breast cancer, patients who have other malignancies and patients who have metastatic breast cancer. The clinico-pathological data were collected by general surgeons and pathologists. The tumors were graded according to the Nottingham modification of the Bloom- Richardson system [11]. The ER, PR, and HER2 staining were obtained as described in patients' reports. Molecular classification of patients was selected as follows: 20 luminal A, 20 luminal B, 20 triple-negative and 20 HER2-neu enriched type.

Evaluation of response to neoadjuvant chemotherapy

The clinical and pathological responses to NAC of post chemotherapeutic treatment mastectomy specimens of the 30 cases were classified according to the established WHO criteria [12]. Clinical response to NAC was assessed using ultrasonography and computed tomography. A clinical complete response (CR) was defined as the disappearance of all known tumor. Clinical partial response (PR) was a 50% or more decrease in total tumor size. Reduction of less than 50% in tumor size, without a 25% increase in tumor size was considered as stable disease (SD). Clinical progressive disease (PD) was defined as a 25% or greater increase in tumor size. The pathological response was evaluated by examination of H&E slides of the post-mastectomy specimens of the 30 cases; it was scored as pathological complete response (pCR) or residual disease [12]. The pathological complete response was defined as the complete disappearance of an invasive tumor or an in-situ component in breast tissue [13].

Evaluation of tumor-infiltrating lymphocytes (TILs)

TILs were assessed in hematoxylin and eosin-stained sections, carefully following the guidelines published by the International TILs Working Group to standardize TILs evaluation with a positivity cutoff set as 1% of the stroma [14]. Their recommendations focused on stromal TILs density. Cases were defined as TILs-high for ≥ 50% stromal TILs, and as TILs-low for <50% stromal TILs [15].

Immunohistochemistry

Immunohistochemical staining was carried out using the EnVision (USA) method. Tissue sections (3-5 μm) were deparaffinized in xylene and rehydrated ingraded alcohol. To block endogenous peroxidase, slides were incubated for 10 minutes in 0.3% hydrogen peroxide. Dako target antigen retrieval solution (pH 6.0) was applied for 20 min. Then the slides were incubated for 30 min at room temperature with a rabbit monoclonal antibody to PD-L1(PD-L1Rb, isotope IgG, Clone CAL10 1:100 dilution, Biocare medical 4040 Corporation, pike lane,concord, USA, Catalogue number 94520), and a rabbit monoclonal antibody to cyclin D1 (ready to use, Clone SP4, catalogue number 94538, Thermo Scientific/DAKO Corporation, Fermont, USA). The reaction was visualized by incubating the sections with diaminobenzidine (DAB) for 15 minafter that Mayer's hematoxylin was used.

Analysis of PD-L1 Immunostaining

PD-L1 positivity is evaluated in both tumor cells and TILs present within the breast stroma. PD-L1 positivity (membranous and/or cytoplasmic) defined as ≥1% of tumor cells and as ≥1% positive stromal TILs. The staining intensity is disregarded [16,17]. The expression of PD-L1 in tumor cells was evaluated as follows: negative expression (<1% positive tumor cells), low expression (≥1-49% positive tumor cells), high expression (≥50- 100% positive tumor cells), and the expression of PD-L1 in TILs was scored as follows: negative expression (<1% positive TILs), positive expression (from ≥1% positive TILs) [18,19].

Analysis of cyclin D1 Immunostaining

Nuclear cyclin D1 positivity is evaluated, which based on the percentage of positive tumor cell nuclei. The expression was evaluated as negative (no positive nuclei), scored as low expression (1 - <10% positive nuclei), moderate expression (≥10-50% positive nuclei), and high expression (>50-100% positive nuclei) [20,21].

Statistical analysis

All data was collected, tabulated and statistically analyzed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Categorical data were compared using the chi-square test. The trend of change in the distribution of relative frequencies between ordinal data was compared using the chi-square test for trend, p-value <0.05 was considered statistically significant.

Ethical approval

The study was carried out following the Code of Ethics of the World Medical Association (Helsinki Declaration of 1975, as revised in 2000) for studies involving humans [22]. Institutional Review Board (IRB) of the faculty of Medicine Zagazig University affirmed this study protocol (No. 3498). Written informed consent was obtained from all participants.

Results

The clinicopathologic parameters of the studied cases (N=80)

Showed in the below table (Table 1).

The immunohistochemical expression of PD-L1 and cyclin D1 in the studied cases (N=80)

PD-L1 expression was observed in 30.1% (24/80) cases in the tumor cells, and in 22.5% (18/80) cases in stromal TILs. Cyclin D1 expression was detected in tumor cell in 56.3% (45/80) cases (Table 2).

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Table 1: The clinicopathological parameters of the studied cases (N=80).

Clinicopathological parameters	All studied patients (N=80)	
	No.	%
Age (years)		
Mean ± SD	55.33±10.91	
Median (Range)	55 (35–80)	
≤50 years	31	38.8%
>50 years	49	61.2%
Tumor size in mammogram by millimeter (mm)		
Mean ± SD	39.08±15.73	
Median (Range)	40 (10–70)	
Grade		
Grade I	2	2.5%
Grade II	29	36.3%
Grade III	49	61.3%
Lymphovascular invasion		
Absent	35	43.8%
Present	45	56.3%
Intraductal components		
Absent	52	65%
Present	28	35%
Necrosis		
Absent	53	66.3%
Present	27	33.8%
Lymph node metastasis		
Negative	10	12.5%
Positive	70	87.5%
Stage		
Stage I	7	8.8%
Stage II	22	27.5%
Stage III	51	63.7%
ER/PR status		
Negative	40	50%
Positive	40	50%
Her2/neu		
Negative	45	56.2%
Positive	35	43.8%
Ki-67 index		
≤14%	23	28.7%
>14%	57	71.3%
Molecular subtype		
Luminal A	20	25%
Luminal B	20	25%
HER2 enriched	20	25%
Triple negative	20	25%
Stromal TILs density		
Low	42	52.5%
High	38	47.5%

Table 2: The immunohistochemical expression of PD-L1 and cyclin D1 in the studied cases (N=80).

Immunohistochemical staining	Site of the expression	Expression	All studied patients (N=80)	%
PD-L1	Tumor cells	Negative	56	70%
		Low	9	11.3%
		High	15	18.8%
PD-L1	Stromal TILs	Negative	62	77.5%
		Positive	18	22.5%
Cyclin D1	Tumor cells	Negative	35	43.8%
		Low	3	3.8%
		Moderate	10	12.5%
		High	32	40%

High stromal TILs were detected in H&E slide sections in 47.5% (38/80) cases in the figure (a) (Figure 1), and low stromal TILs were detected in 52.5% (42/80) cases.

Membranous and/or cytoplasmic PD-L1 expression was detected in tumor cells with 18.8% (15/80) cases showed high expression the figure (b, c and d) and 11.3% (9/80) cases showed low expression (e) (Figure 1). Negative PD-L1 expression was observed in 35 cases. All cases with PD-L1 expression in stromal TILs showed also its expression in tumor cells in the below figure (f). No PDL-1 stain was observed in normal breast tissue while it was observed in associated in situ intraducal carcinomas with a percentage of 39.2% (11/28) cases.

High nuclear cyclin D1 expression was detected in 40% (32/80) cases (a,b), 12.5% (10/80) cases showed moderate cyclin D1 expression (c), and 3.8% (3/80) cases showed low expression (d) (Figure 2).

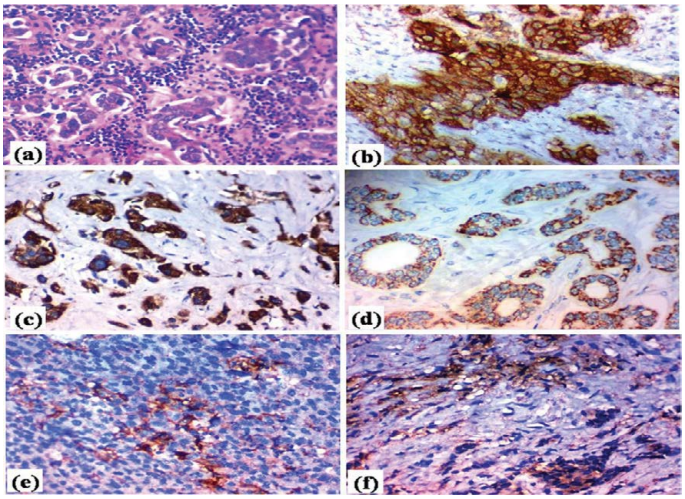

Figure 1: Stromal TILs and PD-L1 expression in IDC-NST: (a) A case of TNBC grade III showing groups of tumor cells surrounded by high stromal TILs density (H&E, ×400). (b) IDC grade III showing strong membranous PD-L1 expression in tumor cells (IHC, ×400). (c) IDC grade III showing strong cytoplasmic and membranous PD-L1expression in tumor cells (IHC,×400). (d) IDC grade II showing membranous PD-L1 expression in tumor cells lining the tubules (IHC,×400). (e) IDC grade III showing low and focal PD-L1 expression(IHC,×400). (f) IDC grade III showing PD-L1 expression in both tumor cells and stromal TILs(IHC,×400).H&E: Hematoxylin and Eosin, IHC: Immunohistochemistry

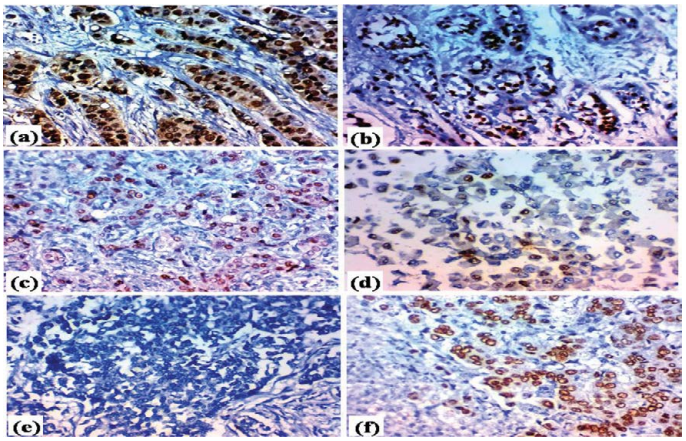

Figure 2: Cyclin D1 in expression in IDC-NST: (a) IDC grade III showing strong cyclin D1 nuclear expression (IHC, ×400). (b) IDC grade II with tubular formations showing strong nuclear cyclin D1 expression (IHC, ×400).(c) IDC grade III showing moderate cyclin D1 nuclear expression (IHC, ×400). (d) IDC grade III showing low cyclin D1 nuclear expression (IHC, ×400). (e) IDC grade III negative for cyclin D1 (IHC, ×400). (f) IDC grade III surrounded by stromal TILs showing cyclin D1 nuclear expression only in tumor cells (IHC, ×400). H&E: Hematoxylin and Eosin, IHC: Immunohistochemistry

Negative cyclin D1 expression was observed in 35 (12.5%) cases (e). Cyclin D1 expression was detected only in tumor cells with no expression in stromal TILs (f) (Figure 2).

The correlation between stromal TILs density, PD-L1 expression, cyclin D1 expression and molecular subtypes of breast cancer in the studied cases (N=80)

A highly significant correlation was detected between stromal TILs density, PD-L1$^+$ tumor cells, PD-L1$^+$ TILs with the different molecular subtype of breast cancer (p<0.001), with the highest expression in TNBC. TNBC cases showed high stromal TILs density in 90% (18/20) cases, 60% (15/20) cases showed high PD-L1 expression in tumor cells and 65% (13/20) cases showed PD-L1 expression in TILs (Table 3).

Cyclin D1 expression in tumor cells showed a significant correlation with molecular subtypes (p =0.006). luminal A and luminal B types showed the highest expression as 60% (12/20) cases luminal A and 50% (10/20) cases luminal B showed high nuclear cyclin D1 (Table 3).

The correlation between PD-L1 expression in TILs, stromal TILs density and PD-L1 expression in tumor cells in the studied cases (N=80)

A highly significant correlation was detected between PD-L1 expression in TILs, stromal TILs density and PD-L1 expression in tumor cells (p<0.001). All cases with PD-L1 expression in TILs showed its expression in tumor cells. It was detected that 44.7% (17/38) cases with high stromal TILs density showed PD-L1 expression in TILs (Table 4).

The correlation between cyclin D1 expression and PD-L1 expression in studied cases(N=80)

No significant correlation was noted between cyclin D1 expression in tumor cells and PD-L1 expression in both tumor cells and stromal TILs (p=0.129, p=0.468 respectively) (Table 5).

The correlation between stromal TILs density, PD-L1 expression,cyclin D1 expression in core biopsy specimens with clinical and pathological response to NAC (N=30)

Although 44.4% (12/27) cases with high stromal TILs density showed a complete clinical and pathological response to neoadjuvant chemotherapy, yet it doesn't reach a statistically significant value. A statistically significant association was found between complete clinical and pathological response to chemotherapy with PD-L1 expression in TILs (p<0.001 for both), and also with PD-L1 expression in tumor cells (p<0.001, p=0.001 for complete clinical and pathological response respectively) (Table 6). Cyclin D1 expression in tumor cells of pretreatment core biopsy specimens showed a significant correlation with a complete clinical and pathological response after neoadjuvant chemotherapy (p=0.027, p=0.010 respectively) (Table 6).

Table 3: The correlation between stromal TILs density, PD-L1 expression, cyclin D1 expression and molecular subtypes of breast cancer in the studied cases (N=80).

	Molecular subtypes								Test§	p-value
	Luminal A (N=20)		Luminal B (N=20)		HER2 enriched (N=20)		Triple negative (N=20)			
	No.	%	No.	%	No.	%	No.	%		
Stromal TILs density										
Low	19	95%	14	70%	7	35%	2	10%	27.966§	**<0.001**
High	1	5%	6	30%	13	65%	18	90%		**(HS)**
PDL1 + tumor cells										
Negative	18	90%	20	100%	13	65%	5	25%	39.260§	**<0.001**
Low	2	10%	0	0%	4	20%	3	15%		**(HS)**
High	0	0%	0	0%	3	15%	12	60%		
PDL1 + TILS										
Negative	20	100%	20	100%	15	75%	7	35%	32.401§	**<0.001**
Positive	0	0%	0	0%	5	25%	13	65%		**(HS)**
Cyclin D1 expression in tumor cells										
Negative	8	40%	10	50%	9	45%	8	40%	22.931§	**0.006**
Low	0	0%	0	0%	1	5%	2	10%		**(S)**
Moderate	0	0%	0	0%	7	35%	3	15%		
High	12	60%	10	50%	3	15%	7	35%		

Where: §Chi-square test, p<0.05 is significant. (S): Significant, (HS): highly significant.

Table 4: The correlation between PD-L1 expression in TILs, stromal TILs density and PD-L1 expression in tumor cells in the studied cases (N=80).

	Total	PD-L1 expression in TILs				Test§	p-value
		Negative (N=62)		Positive (N=18)			
		No.	%	No.	%		
Stromal TILs density							
Low	42	41	97.60%	1	2.40%	20.525§	**<0.001 (HS)**
High	38	21	55.30%	17	44.70%		
PD-L1 expression in tumor cells							
Negative	56	56	100%	0	0%	61.109§	**<0.001 (HS)**
Low	9	5	55.60%	4	44.40%		
High	15	1	6.70%	14	93.30%		

Where: §Chi-square test, p<0.05 is significant. (HS): Highly Significant.

Table 5: The correlation between cyclin D1expression and PD-L1 expression in studied cases (N=80).

	Total	Cyclin D1 expression in tumor cells								Test	p-value
		Negative (N=35)		Low (N=3)		Moderate (N=10)		High (N=32)			
		No.	%	No.	%	No.	%	No.	%		
PD-L1 positive tumor cells											
Negative	56	27	48.20%	2	3.60%	6	10.70%	21	37.50%	2.305‡	0.129 (NS)
Low	9	5	55.60%	0	0%	1	11.10%	3	33.30%		
High	15	3	20%	1	6.70%	3	20%	8	53.30%		
PDL1 positive TILs											
Negative	62	29	46.80%	2	3.20%	6	9.70%	25	40.30%	2.541§	0.468 (NS)
Positive	18	6	33.30%	1	5.60%	4	22.20%	7	38.90%		

Where: §Chi-square test. ‡Chi-square test for trend, p<0.05 is significant. (NS): significance.

Table 6: The correlation between stromal TILs density, PD-L1 expression, cyclin D1expression in core biopsy specimens with clinical and pathological response to NAC (N=30).

	Total	Clinical response								Test	P-value	Pathological response				Test	P-value
		CR (N=12)		PR (N=8)		SD (N=6)		PD (N=4)				PCR (N=12)		RD (N=18)			
		No.	%	No.	%	No.	%	No.	%			No.	%	No.	%		
Stromal TILs density																	
Low	3	0	0	2	66.7	0	0	1	33.3	5.000§	0.172 (NS)	0	0	3	100	2.222§	**0.255 (NS)**
High	27	12	44.4	6	22.2	6	22.2	3	11.1			12	44.4	15	55.6		
PDL1 in tumor cells																	
Negative	10	0	0	2	20	4	40	4	40	14.650‡	**<0.001 (HS)**	0	0	10	100	10.667‡	**0.001 (S)**
Low	5	2	40	3	60	0	0	0	0			2	40	3	60		
High	15	10	66.7	3	20	2	13.3	0	0			10	66.7	5	33.3		
PDL1 in TILS																	
Negative	12	0	0	4	33.3	4	33.3	4	33.3	14.919§	**<0.001 (HS)**	0	0	12	100	13.333§	**<0.001 (HS)**
Positive	18	12	66.7	4	22.2	2	11.1	0	0			12	66.7	6	33.3		
Cyclin D1 expressionin tumor cells																	
Negative	13	2	15.4	4	30.8	5	38.5	2	15.4	4.888‡	**0.027 (S)**	2	15.4	11	84.6	6.704‡	**0.01 (S)**
Low	2	1	50	1	50	0	0	0	0			1	50	1	50		
Moderate	7	3	42.9	2	28.6	0	0	2	28.6			3	42.9	4	57.1		
High	8	6	75	1	12.5	1	12.5	0	0			6	75	2	25		

Where: §Chi-square test. ‡Chi-square test for trend, p<0.05 is significant. S: Significant; HS: highly Significant; NS: not significant; CR: clinical complete response; PR: partial clinical response; SD: stable disease; PD: persistent disease; PCR: pathologic complete response; RD: residual disease.

Discussion

Breast cancer is the most commonly occurring cancer in women worldwide and an important leading cause of cancer death. There were over 2 million new cases in 2018 [1]. Tumor immune escape means the phenomenon by which tumor cells can grow and metastasize by keeping away from recognition by the immunity [23]. The expression of immunosuppressive molecules or their receptors, including PD-L1 and its receptor, programmed cell death-1 (PD-1), which are known as the immune checkpoints, can inhibit the activation of cytotoxic T lymphocytes which leads to tumor immune escape [24]. Blocking these immune checkpoints became an important target of immunotherapy recently to avoid suppression of the immune system and restore its function. Among these immune checkpoint blockers, PD-1/PD-L1 blockers represent important drugs approved by the FDA in recent years and are currently in clinical trials [24]. PD-L1 expression in tumor cells has been related to the presence of stromal TILs. The presence of TILs may demonstrate immune-mediated host defense against the tumor [3]. Breast cancer was considered less immunogenic as compared with other tumor types. Yet, in the last few years, anti-PD-L1 monoclonal antibodies are emerging as novel immunotherapy in breast cancer, especially in TNBC with promising outcomes [25,26]. PD-L1 protein expression in breast cancer in many previous studies has been observed with a frequency between 15.8% and 30% [27-29]. These results are consistent with the results of the current study as PD-L1 expression was reported in 30.1% in tumor cells of breast cancer and 22.5% in stromal TILs of the tumor micro environment. The current study reported that PDL1 expression in tumor cells and stromal TILs was demonstrated in 75% and 65% of TNBC respectively with highly statistically significant association. These results are near to results observed by Bellucci R, et al. (2015) and Ali HR, et al. (2015) who stated that PD-L1 positive status was significantly different between the molecular intrinsic subtypes with highest values reported in TNBC [30,31]. Mittendorf EA, etal. (2014) and Soliman H, et al. (2014) have demonstrated also that PD-L1 expression is correlated with ER, PR negative cases [32,33].

The present study declared a significant association between high stromal TILs density and TNBC. Our suggestion was supported by the previous studies found by Wang ZQ, et al. (2017), Elghazawy H, et al. (2019) and Herrero-Vicent C, et al. (2017), who reported that stromal TILs were higher in triple-negative type compared to the rest of the studied cases [34-36]. Neoadjuvant (preoperative) chemotherapy is increasingly used in the treatment of early-stage breast cancer. Numerous studies have suggested that the pathological response after the neoadjuvant chemotherapy treatment is an indicator of response [37]. Identifying biomarkers that can predict the pathological response in a group of breast cancer patients is very important [38]. In the current study, we evaluated the association between PD-L1 expression

in pretreatment core biopsy specimens and clinical and pathological response after neoadjuvant chemotherapy. We found a significant association with complete clinical and pathological responses with high PD-L1 expression and with stromal TILs density. These results were consistent with those found by Wimberly H, et al. (2015), Thompson E, et al. (2016), Wu Z, et al. (2019) and Nanda R, et al. (2016) [3,37-39]. This supportsthe finding of the previous studies which reported that PD-L1 expression in the pretreatment core biopsy specimens can act as a surrogate marker for predicting therapeutic effects of chemotherapy regimen for breast cancer patients [40]. Regarding the correlation between stromal TILs density with the clinical and the pathological response, 44.4% of cases with high stromal TILs showed a complete response, but without statistically significant value. Many prospective studies confirmed that higher pretreatment stromal TILs count is associated with a greater probability of pathologic complete response (pCR). These studies validated the relevance of TILs as an additional parameter for prediction of the response to neoadjuvant chemotherapy and as a promising therapeutic strategy [41,42]. This suggests that further international standardization for TILs is recommended. Our results were also consistent with Pelekanou V, et al. (2018) [43], who examined the association of pretreatment TILs count and PD-L1 levels with pCR and stated that higher pre-treatment TIL count and PD-L1 expression are associated with a greater probability of pCR. This study supports the hypothesis that chemotherapy response is partly mediated by activated cytotoxic T cells [42]. There are multiple and varied genetic alterations implicated in breast cancer carcinogenesis. One of the basic genetic alterations is cyclin D1 protein amplification [44]. Cyclin D1 binds to CDK4 and CDK6 inducing hyperphosphorylation of the Rb gene, thereby promoting cellular proliferation [20,45]. Many studies suggested that inhibition of cyclin Dl/CDK activity might be important in considering drugs of future therapies as a new class of anti-neoplastic drugs (CDK 4/6 inhibitors) targeting cell cycle activation by cyclin D1 in breast cancer [46,47]. In this study, cyclin D1 expression was observed in 56.3% (45/80) cases, these results are in line with theresults of Ortiz AB, et al. (2017) and Ahlin C, et al. (2017) who reported that overexpression of cyclin D1 was observed in approximately 50% of invasive breast cancer [48,49]. While the prevalence of cyclin D1 overexpression in a study carried out by Sarkar S, et al. (2015) was 70.9%. This was considerably higher than the results of the present study [50]. This variability of the results might be illustrated by breast cancer heterogeneity. In this study, the correlation between cyclin D1 with molecular breast cancer subtypes was statistically significant. It was detected that 60% (12/20) luminal A subtype and 50% (10/20) luminal B showed high cyclin D1 expression. This finding supports the view of some previous studies in this field, which have stated a positive relationship between cyclin D1 expression and ER, PR positive status in breast carcinoma suggesting that cyclin D1 may be directly or indirectly related to maturation and differentiation of tumor cells [21,49,51]. In the current study, we evaluated the association between cyclin D1 expression in pretreatment core biopsy specimens with the clinical and the pathological response after neoadjuvant chemotherapy. A significant association was detected. These results are in line with the results of the previous study done by Li XR, et al. (2011) and Kurozumi S, et al. (2019) who found that pretreatment cyclin D1 expression was a predictor of response to neoadjuvant chemo-endocrine therapy in breast cancer [52,53]. These findings can support the idea of assessment of the proliferative activity before NAC is significantly linked to tumor response and outcome, and the importance of the selection of breast cancer patients that are likely to benefit from neoadjuvant chemotherapy by using immunohistochemical cyclin D1 expression [52]. Recently, the advantage of testing for high expression of cyclin D1 might provide a base for its use as targeted therapy [54]. On the contrary, Wachter DL, et al. (2013) reported no relation between cyclin D1 expression and response to NAC treatment [55]. Further studies ona large number of cases are needed to confirm our results.To the best of our knowledge, this is the first study that investigates the correlation between immunohistochemical expression of cyclin D1 and PD-L1 in IDC-NST. In the present study, no statistically significant association was found between cyclin D1 expression and PD-L1 expression in both tumor cells and TILs.

Planes-Laine G, et al. (2019) and Zhang J, et al. (2018) discussed the combination of PD-1/PD-L1 Inhibitors with cyclin/CDK4/6 Inhibitors in breast cancer [56,57]. They predicted that CDK4/6 inhibition synergizes with anti–PD-L1 to restore TILs to enhance antitumor immunity and suppress tumor growth, which may improve the outcomes of endocrine therapy either in hormone-sensitive or insensitive disease. They did not, however, perform a trial to prove the correlation in pretreatment biopsies.

Conclusion

PD-L1 expression in breast cancer was correlated with TNBC subtype suggesting its clinical significance to be used as targeted therapy with a future hope for combined immunotherapy and chemotherapy in this group. Cyclin D1 expression was correlated with luminal breast cancers and can be used in the therapeutic approaches. PD-L1 and cyclin D1 may act as predictive cancer biomarkers for response to neoadjuvant chemotherapy.

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