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PD-L1 expression in Triple Negative Breast Cancer: a study of an Iraqi population

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ABSTRACT

Introduction. Breast cancer is the second most common cause of cancer death in women. Breast cancer awareness has increased due to mammography screening.

Aim. The study aims to evaluate the prevalence of the anti-programmed death-ligand 1 (PD-L1) expression in triple-negative breast cancer (TNBC) cases and to correlate it with clinicopathological parameters.

Material and methods. This retrospective study investigates 44 triple-negative breast cancer cases. PD-L1 expression was measured by an immunohistochemical technique using Dako kits, PD-L1 IHC 22C3 pharm Dx on 44 paraffin block samples from Duhok Municipal Laboratories. If the specimen has a combined positive score (CPS) of 10 or higher, it expresses PD-L1. Age groups, grades, types, stages, and lymph node status are studied.

Results. The mean age of the 44 patients was 47.7 years. 54.5% of the patients were in the middle age group, 63.6% were in grade III, 88.6% had invasive ductal carcinoma, and 75% were negative for PD-L1. 63.6% of the patients had the nuclear protein Ki67 (Ki-67) less than 20. 70.5% of the patients were in stage T2, and 45.5% had N1 lymph node status. There is a significant association between PD-L1 and Ki67. All patients with positive PD-L1 had Ki67 more than 20, while only 15.2% of the patients with negative PD-L1 had Ki67 more than 20.

Conclusion. Most TNBC patients are middle age, have grade III, and 75% have negative PD-L1. There is a significant association between PD-L1 and Ki-67. All patients with positive PD-L1 have Ki67 of more than 20.

Introduction

Breast cancer is the most common type of cancer in women and the second most common cause of death from cancer in women [1]. In recent years, the use of mammography for screening has made more people aware of breast cancer [1]. The prognosis for breast cancer has improved dramatically as a result of treatments that target the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2) [2]. However, tumours that do not express ER, PR, or HER2 are called "triple-negative breast cancers" (TNBC). Triple-negative breast cancer (TNBC) affects only about 13 out of every 100,000 women diagnosed with breast cancer each year. It accounts for about 15% of all invasive breast cancers, has a poor prognosis, and cannot be treated well [3, 4]. The CD274 gene on chromosome 9 encodes a 40 kDa

transmembrane protein known as programmed death ligand-1 (PD-L1). Natural killer cells, macrophages, myeloid dendritic cells, B cells, epithelial cells, and vascular endothelial cells are all typical tissue cell types that contain it [5]. The PD-1 (Programmed death receptor 1)/PD-L1 signalling pathway aids in helping tumours evade the immune system, according to recent studies in various epithelial tumours, due to crosstalk between PD-1 on tumour-infiltrating lymphocytes (TIL) and PD-L1 in tumour cells [5]. Expression of PD-L1 on their cells is a crucial defence mechanism for tumours against the immune system [5]. Researchers have shown PD-L1 expression in many types of cancer, including melanoma, renal cell carcinoma, non-small cell lung cancer, colorectal cancer, gastric cancer, pancreatic cancer, and types of breast cancers [6]. Therapies that block PD-1/PD-L1 could be used to treat these tumours [6]. However, little information exists about how PD-L1 is expressed in breast cancer. There are different opinions about how PD-L1 expression might affect the outcome of breast cancer. Some studies say that PD-L1 is beneficial [7–9], while others suggest its detrimental role [10] or no effect at all [11]. The Ki67 protein is usually found only in growing cells [12]. During interphase, Ki67 is primarily found in the nucleolar cortex, but during mitosis, it moves to where the chromosomes are packed together [13, 14].

Aim

The study aims to evaluate the prevalence of PD-L1 expression in TNBC cases and to correlate it with clinicopathological parameters.

Material and methods

The study is a retrospective of 44 triple-negative breast cancer cases. The samples were collected as paraffin blocks from different laboratories in Duhok city. PD-L1 expression was assessed with an immunohistochemical method using Dako kits, PD-L1 IHC 22C3 pharm Dx. PD-L1 expression was determined by combined positive score (CPS), and the sample was considered positive if CPS was equal to or greater than 10 [15]. Patients who test PD-L1 positive are those whose tumours have a combined positive score (CPS) of at least 10. A score ranging from 0 to 100 is obtained by dividing the total number of PD-L1-expressing tumour cells (TC), lymphocytes, and macrophages by the total number of alive TC. Study variables are; age, grades, types and stages of cancer and lymph node status. SPSS 22 was used for the statistical analysis. Frequencies and percentages were calculated for categorical data. Mean, median, and standard deviation were calculated for continuous data. Chi-square analysis was used to calculate the degree of correlation between two variables. A significant p-value is less than or equal to 0.05.

Results

The mean age of the patients was 47.7 years (47.7 ± 14). 54.5% of the patients were in the middle age group. 63.6% of the patients were in grade III, most (88.6%) had invasive ductal carcinoma, and 75% were negative for PD-L1. 63.6% of the patients had Ki-69 less than 20. 70.5% of the patients were in stage T2, and 45.5% had N1 lymph node status. **Table 1** shows the detailed data. There is no significant association between PD-L1 and age groups, grades, or types, as shown in **Table 2**. There is no significant association between PD-L1 status and stage. As shown in **Table 3**, there is also no significant association between PD-L1 status and lymph node status. As shown in **Table 4**, there is a significant association between PD-L1 status and the Ki-67 percentage score. 100% of patients with positive PD-L1 expression had Ki67 more than 20, while only 15.2% of patients with negative PD-L1 had Ki67 more than 20.

Discussion

Most triple-negative breast cancers are highly malignant tumours that affect young women. It accounts for 10%–20% of all breast cancers [16, 17]. Because they proliferate quickly, they are usually found at a late stage when they are diagnosed [3]. Chemotherapy drugs such as anthracyclines, taxanes, ixabepilone, and platinum-based drugs are currently used to treat these tumours, but no single drug works well in all tumours [18]. There is

Table 1. Distribution of patients by age groups, grades, types and PD-L1 status.

Variables		Frequency	Percentage
Age (years)	Young adult (less than 30)	12	27.3
	Middle age (30-59)	24	54.5
	Old (60 and more)	8	18.2
Grade	I	2	4.5
	II	14	31.8
	III	28	63.6
Types	Adenoid	1	2.3
	Inflammatory breast carcinoma	4	9.1
	Invasive ductal carcinoma	39	88.6
PD-L1	Negative	33	75.0
	Positive	11	25.0
Ki-67	<20	28	63.6
	>20	16	36.4
Stages	T1	5	11.4
	T2	31	70.5
	T3	8	18.2
Lymph node status	N0	13	29.5
	N1	20	45.5
	N2	9	20.5
	N3	2	4.5

Table 2. Association between PD-L1 and (age groups, grades, types).

Variables		PD-L1		P-value
		Negative	Positive	
Age groups	Young adult	8; 24.3%	4; 36.4%	0.6
	Middle age	18; 54.5%	6; 54.5%	
	Old	7; 21.2%	1; 9.1%	
	Total	33; 100.0%	11; 100.0%	
Grades	I	2; 6.0%	0; 0.0%	0.31
	II	12; 36.4%	2; 18.2%	
	III	19; 57.6%	9; 81.8%	
	Total	33; 100.0%	11; 100.0%	
Types	adenoid	1; 3.1%	0; 0.0%	1.000
	IDC	32; 96.9%	11; 100 %	
	Total	33; 100%	11; 100%	

P-value ≤ 0.05 (significant).

Table 3. Association between PD-L1 and (stages, lymph node status).

		PD-L1	
		Negative	Positive
Stage	T1	3; 9.1%	2; 18.2%
	T2	23; 69.7%	8; 72.7%
	T3	7; 21.2%	1; 9.1%
Total		33; 100.0%	11; 100.0%
Lymph node status	N0	10; 30.3%	3; 27.3%
	N1	12; 36.4%	8; 72.7%
	N2	9; 27.3%	0; 0.0%
	N3	2; 6.0%	0; 0.0%
Total		33; 100.0%	11; 100.0%

P-value = 0.5 (not significant).

Table 4. Association between PD-L1 and Ki67.

Variables		PD-L1		P-value
		Negative	Positive	
KI67	<20	28; 84.8%	0; 0.0%	0.0001
	>20	5; 15.2%	11; 100.0%	
	Total	33; 100.0%	11; 100.0%	

P-value ≤0.05 (significant).

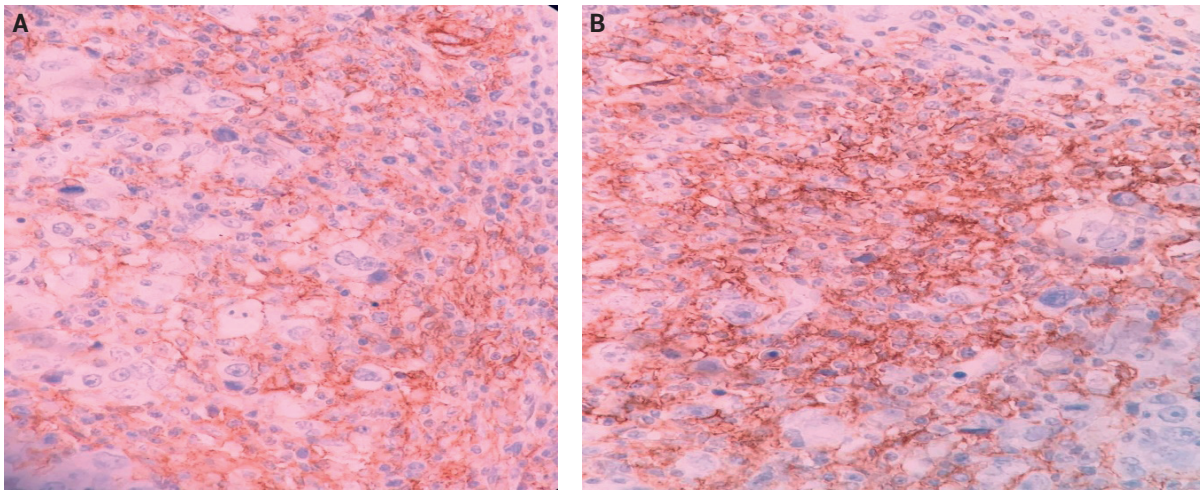

Figure 1. A) PD-L1 expression by tumour cells and associated inflammatory cells. B) PD-L1 expression by tumour cells.

much variation in how PD-L1 immunohistochemistry assays are performed and interpreted in the literature. H scores, 1% cut-offs, and graded scoring systems (0–3) are commonly used to measure PD-L1 expression in tumours [5, 11, 18, 19]. People have used 5% as a cut-off to see if PD-L1 is present in the tumour microenvironment [11, 19]. More extensive studies are needed to identify the optimal cut-off value and the antibody to use as a gold standard [20]. The presence of PD-L1 in tumours was observed to correlate with a higher Ki-67 proliferation index [p = 0.017]. Further life expectancy studies should confirm this finding [5, 7]. o correlation was observed between PD-L1 expression in the tumour or tumour microenvironment and age, tumour size, tumour grade, lymph node metastasis, the presence of Lymphovascular invasion (LVI) or Ductal Carcinoma In-situ (DCIS), recurrence, or metastatic status. However, PD-L1 expression in tumours has not been associated with these factors (age, tumour size, tumour grade, lymph node metastasis, the presence of Lymphovascular invasion (LVI) or Ductal Carcinoma In-situ (DCIS), recurrence, or metastatic status) in several studies [5, 7, 11, 19, 21]. Triple-negative breast cancer is a tumour type with no targeted treatment; new treatment options are needed. PD-L1 in and around these aggressive tumours may be a reason to treat them with anti-PD-L1 therapies (PD-L1 monoclonal antibodies) [20].

Conclusion

Most TNBC patients are in the middle age group and have grade III. Therefore, this study's low prevalence of PD-L1 positivity may be related to the high CPS cut-off.

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Conflict of interest statement
The authors declare no conflict of interest.

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