Association Serum Level Of PD-1 Receptor with Demographic, Clinical Pathological, Hormone Receptors, Molecular and Histological Subtypes in Breast Cancer Patients

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Abstract
Background: The critical role of immune checkpoints Programmed cell death receptor. Although (PD-1) has been extensively documented in a variety of malignant tumors, the basic regulatory mechanisms in breast cancer are still unclear. The objective of this study was to investigate the change in serum PD-1 concentration in pre-treatment and post-treatment groups, as well as the correlation with hormone receptors, molecular and histological subtypes. Method: The study included 82 female breast cancer patients diagnosed before beginning treatment (pre-treatment group), 60 female breast cancer patients diagnosed after undergoing treatment (post-treatment group), and 60 healthy individuals as controls. The sPD-1 levels are measured using ELISA technique. Data on tumors were retrieved from an electronic database at the oncology centre. Results: The highest levels of sPD-1 were detected in the pre-treatment groups, followed by post-treatment groups compared to the healthy group. The age group (40-69 years) had the highest level of PD-1 (6.51±1.68ng/ml) compared to other age groups. The serum level of PD-1 was significantly associated with age (p=0.01), family history (p=0.0001), and menopausal status (p=0.04) in the pre-treatment group. The level of PD-1 was significantly associated with the histological type of tumor (p=0.01), with a higher level seen in invasive tumor types. Furthermore, PD-1 levels were higher in the invasive lobular carcinoma subtype (p=0.03). In the post-treatment period, there was a significant association between PD-1 level and estrogen receptor status. PD-1 levels were higher in estrogen receptor-negative than in estrogen receptor-positive (1.354±0.121 vs. 0.896±0.0773 ng/ml, P =0.01). The higher level of sPD-1 (7.40±1.26ng/ml) was found to be associated with the Ki-67value (>40%) in patients with breast cancer, and the lower level of sPD-1 (5.49±1.77ng/ml) was discovered in the Ki-67value (<20%). Significantly, there is a positive correlation between sPD-1 level and Ki-67 expression (p=0.004). Conclusion: There was a significant association of serum PD-1 level with invasive carcinoma, particularly in invasive lobular carcinoma of patients with breast cancer, and a positive correlation with proliferation marker Ki-67 expression.
Keywords: Programmed cell death receptor (PD-1), Breast cancer, ER, PR, HER2, Histological type.

1. Introduction

The immune system in our bodies performs an essential function in protecting us from different diseases, including cancer cells 1. Immune suppression is known to be a trademark of cancer. Various receptors and their ligands, known as checkpoints, regulate this process 2,3. PD-1 was first described in the early 1990s as a type of immune checkpoint receptor constantly expressed on activated T cells and is inhibitory in nature by interaction with its ligands PD-L1 and PD-L2; it conserves healthy cells from excessive inflammatory or autoimmune reactions 4,5. Even though the importance of the immune checkpoints PD-1 and PD-L1 in a variety of malignant tumors has been well established, the fundamental regulatory mechanisms in breast cancer are obscure. 6. In many countries, breast cancer is one of the most prevalent malignant tumors and the leading cause of cancer-related mortality among women 7,8. Multiple hereditary elements, environmental variations, and their complex interplay cause breast cancer 9,10. Concerning cancer cells' ability to evade the immune system, the discovery of the PD-1 (programmed death 1) and PD-L1 (programmed death-ligand 1) axis has been one of the most promising discoveries in cancer therapy in latest years 11,12. PD-1 is a highly vital component of the immune system. It is a type I transmembrane glycoprotein that belongs to the immunoglobulin CD28 superfamily 5,13. Breast cancer treatment with anti-PD-1 therapies was efficient, especially for patients with triple-negative breast cancer (TNBC) 14. Nivolumab is a PD-1 inhibitor that restores the anti-tumor activity of T cells by binding to PD-L1 on the surface of T cells. This blocks the immunosuppressive signaling pathway that is initiated by PDL-1/2 4,15. At the molecular level, breast cancer patients were classified into luminal A, luminal B, HER-2 positive, and triple negative groups according to the status of

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biomarkers such as estrogen receptor (ER), progesterone receptor (PR), and epidermal growth factor receptor 2 (HER2) 16. Various groups of patients with breast cancer can acquire a therapeutic benefit by matching chemical and hormonal treatment plans 17,18. Breast carcinoma is caused by several different etiological factors. However, the disease is caused by molecular changes at the cellular level 19,20. The most prevalent type of breast cancer is adenocarcinomas and can be non-invasive (limited to the ductal or lobular epithelium with no basement membrane penetration) or invasive (ductal or lobular carcinoma with involvement of the basement membrane) 21. The sPD-1 level, which has received less attention, might be crucial for anti-tumor immune response. According to the findings of many studies in which participants with breast cancer were compared to healthy controls, those with breast cancer showed greater levels of the protein sPD-1. This indicates that higher serum levels of PD-1 may increase immunosuppression and therefore be considered undesirable prognostic indicators 22,23. As a matter of fact, Iraq has a high incidence of breast cancer and has seen an uptick in recent years 8. However, data on sPD-1 expression in Iraqi breast cancer patients is lacking. As a result, our study is the first in Iraq to address these characteristics.

So, the current study intended to investigate the level of the sPD-1 receptor in breast cancer patients by first measuring their serum levels in the pre-treatment and post-treatment groups compared to healthy subjects to better understand the prognostic value of PD-1 receptor in breast cancer. Secondly, to investigate whether there are any associations between PD-1 and various factors, including demographics, clinical pathology histological types, molecular subtypes, and hormone receptors. And finally, to demonstrate whether the PD-1 and these characteristics are correlated in any way.

2. Methods and Material

Study design and setting

At the Oncology Center in ThiQar, Iraq, a retrospective study was conducted between October 2021 and March 2022. The study included 202 females in total, ranging in age from (23 to 87) years. Among them, 142 had breast cancer, 82 participants did not receive any treatment (pre-treatment group), and 60 participants received different types of treatment protocols (post-treatment group) through a period range (9-192) weeks. The remaining 60 healthy females as a control group. Demographic data, histological classification (invasive or non-invasive), histological subtype (carcinoma in situ, invasive ductal carcinoma, invasive lobular carcinoma, and mixed invasive ductal lobular carcinoma), and molecular subtype of patients were reported.

Ethical approval

Written informed consent was obtained from all patients and healthy controls. The Continuing Medical Education section in ThiQar Health Department and the Scientific Committee at the University of Basra's College of Pharmacy both approved this study.

Inclusion criteria

Females between the ages of (23 and 87) with various stages of breast cancer agreed to participate in the study while visiting the ThiQar Oncology Center either before beginning treatment or with various treatment protocols.

Exclusion criteria

Male breast cancer patients, people who had been receiving chemotherapy for more than four years, and people who declined to take part in the study were all excluded.

Data collection

All data were subsequently gathered from the ThiQar governorate's oncology center's electronic database. Age, body weight, BMI, family history, menopausal status, histological type, histological subtype, and molecular subtype were all included.

Immunohistochemistry staining assay

ER, PR, HER2 status, and Ki-67 status were obtained from the database of the pathology department at Al-Hussain teaching hospital, which was determined by an immunohistochemistry score by specialized histopathologists. Primary antibody ER (DAKO, Denmark, clone 1D5), PR (DAKO, Denmark, clone PgR636), HER2 (Quartett, Germany, clone QR003), and Ki-67 (DAKO, Denmark, clone MIB-1). ER and PR positivity was defined as the presence of immunoactivity in at least one percent of tumor cell nuclei.

HER2, overexpression was determined by immunohistochemistry score of +3 (uniform and intensity membrane staining of >10% tumor cells) or appositive in situ hybrid result. The differential expression of receptors led to the identification of four distinct molecular subtypes of breast cancer, including luminal cancers, which classify into luminal A (ER+ and PR+/HER2-) and luminal B (ER+ and/or PR+/HER2- or ER+ and/or PR+/HER2+). A triple negative (basal-like) tumor was defined as a tumor that was ER, PR, and HER2 negative. HER2 enriched subtype (ER-, PR-, HER2+) 24.

Sample collection

Three ml of whole blood samples were withdrawn by venipuncture from the breast cancer patients and healthy control during their visit to the Oncology department. Then serum was separated by centrifuging the blood samples at 1,000–2,000 x g for 10 minutes in a refrigerated centrifuge. Serum samples were then aliquoted in micro centrifuge tubes and stored at −80 °C for use.

Quantification of sPD-1 level by ELISA assay

The serum level of PD-1 protein was quantified using commercially available immunoassay kits (EKISA kit, Catalog No: E-EL-H1534, Elabscience, USA) with a sensitivity of (0.10ng/mL). The concentration of PD-1 in each sample was determined from a linear standard

curve of known PD-1 concentrations ranging from 0.16 to 10 ng/mL by using (an Elisa microplate washer, Human, Germany and Elisa microplate reader, Bio Tek, USA). The manufacturer's protocol was strictly followed during the assays.

3. Statistical analysis

All the statistical analysis was performed by Statistical Package for the Social science for Science (SPSS) version 24.0 software. The Kolmogorov-Smirnov (k-s) test was used to check the normality of the data. Continuous variables were presented as mean±SD and/or median (minimum and maximum). The categorical variables were presented as frequency and percentage (n, %). Comparison of continuous variables between 2 groups was done by Independent t-test, and comparison between more than two groups was done by One-way ANOVA. Some Statistical analysis was performed using a student's t-test with Graph Pad Prism 6 software (Graph Pad Software, Inc., La Jolla, CA, USA) for measuring PD-1 level with the molecular subtypes of breast cancer and hormone receptors status for both pre-treatment and post-treatment groups. The correlation of PD-1 with different demographic, and hormone receptors was calculated by Pearson's and Spearman correlation analysis. Difference in means between the groups were considered to be significant at 2-tailed p-value ≤0.05.

Table 1: Demographic and clinical pathological characteristics for pre-treatment and post-treatment groups patients

Characteristics	Pre-treatment (N=82) N(%)	Post-treatment (N=60) N(%)	P
Age (year)			
20-39	24 (29.3)	9 (15)	0.03
40-69	46 (56.1)	48 (80)	
≥70	12 (14.6)	3 (5)	
Mean±SD	49.97±14.79	51.45±9.23	0.1
Range	(23-87)	(36-70)	
BMI			
Underweight	0	0	0.13
Normal	18 (22)	6 (10)	
Overweight	31 (37.8)	33 (55)	
Obese	33 (40.2)	21 (35)	
Family history of breast cancer in a first-degree relative			
Positive	10 (12.2)	3 (5)	0.33
Negative	72 (87.8)	57 (95)	
Menopausal state			
Premenopausal	48 (58.5)	21 (35)	0.02
Postmenopausal	34 (41.5)	39 (65)	
Histologic type			
Non-invasive (Carcinoma in situ)	2 (1.4)	0	0.17
Invasive ductal Carcinoma	76 (92.7)	54 (90)	
Invasive lobular Carcinoma	4 (4.9)	3 (5)	
Mixed(Invasive ductal lobular carcinoma)	0	3 (5)	
Molecular subtype			
Luminal A	22 (26.8)	15 (25)	0.21
Luminal B	22 (26.8)	27 (45)	
TNBC	26 (31.7)	12 (20)	
Her2 enriched	12 (14.6)	6 (10)	
ER			
Positive	36 (43.9)	39 (65)	0.04
Negative	46 (56.1)	21 (35)	
PR			
Positive	42 (51.2)	42 (70)	0.054
Negative	40 (48.8)	18 (30)	
Her2/neu			
Positive	16 (19.5)	12 (20)	0.949
Negative	66 (80.5)	48 (80)	
Ki-67			
< 20%	42 (53.7)	33 (55)	0.84
20-40%	26 (29.3)	18 (30)	
> 40%	14 (17.1)	9 (15)	
P value is significant at level 0.05			

4. Results

The clinical and pathological characteristics of patients with breast cancer in terms of demographics. According to (table1), the majority of participants were between the ages of 40 and 69, and there was a significant difference in age distribution between pre-treatment and post-treatment groups (P=0.03). But no significant difference in BMI level was found between the groups (P= 0.13). The vast majority of patients in the pre-treatment group (40.2%) were obese, while the post-treatment group (55%) was overweight. In terms of their first-degree relative's breast cancer family history, there was no statistically significant difference between the groups (p=0.33). As shown in table 1, the majority of patients in both groups had a negative family history of breast cancer, with 87.7% and 95%, respectively. Moreover, there were appreciable differences in the state of menopause between pre-treatment and post-treatment groups (p=0.02). The majority of participants in the post-treatment group (65%) were postmenopausal, compared to 41.5% in the pre-treatment group. Contrasting the post-treatment group, the majority of those in the pre-treatment group were premenopausal. Furthermore, there was no significant difference between the groups in the

distribution of the histological, molecular subtype, PR, HER2 status, and Ki-67 expression. But ER status varied significantly between groups (P=0.04). ER positivity was higher in the post-treatment group (65%) than in the pre-treatment group (43.9%).

BMI: body mass index, ER: Estrogen receptor, PR: Progesterone Receptor, HER2: Human Epidermal Growth Factor Receptor 2, TNBC: Triple Negative Breast Cancer, N: Number, %: Percentage.

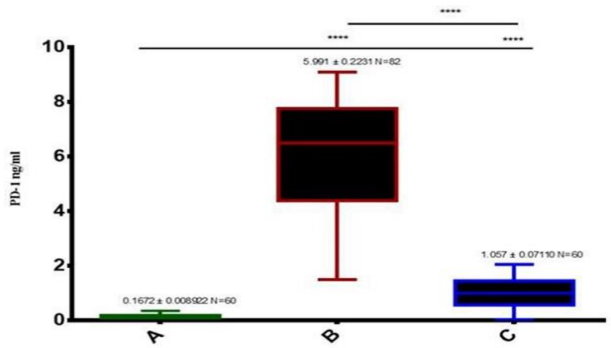

Figure 1. PD-1 serum levels in different groups were compared.

Comparison serum levels of PD-1 receptor among pre-treatment, post-treatment and healthy groups. There are clear significant differences in levels of sPD-1 expression among pre-treatment, post-treatment and healthy groups. The higher level of sPD-1 was found in pre-treatment group followed by the post treatment group compared to control group (5.991±0.2231, 1.057±0.0711, 0.1672±0.0089 ng/mL) respectively. (p<0.0001), as depicted in Figure 1.

A: Healthy Control; B: Pre-treatment group: post-treatment group

Association level of serum PD1 with demographic features. Serum expression of PD-1 was found to be significantly associated with age (p=0.01). The highest level of sPD-1 was found in the age group (40-69 years) compared to other groups (6.51±1.68ng/ml). BMI had no significant association with sPD-1 level. A significant association was observed between the expression of sPD-1 receptor and family history (p<0.0001). Patients with a positive family history had a significantly lower level of sPD-1 (3.85±1.24ng/ml) than those without a family history (6.28±1.62ng/ml). There was a significant association between sPD-1 and menopausal status (p=0.04). Premenopausal patients had significantly lower levels of sPD-1 compared to post-menopausal patients (5.656±1.717, 6.44±1.74) as shown in table 2.

Table 2: Association expression of serum of PD-1 with demographic features in pre- treatment group patients with breast cancer

Parameter	PD-1		
Age groups	Mean	SD	P ≤*
20-39	5.11	1.59	0.01
40-69	6.51	1.68	
≥70	5.71	1.77	
BMI			
Under weight	-		0.57
Normal	5.87	2.05	
Overweight	5.78	1.65	
Obese	6.23	1.72	
Family History			
Positive	3.85	1.24	0.0001
Negative	6.28	1.62	
Menopausal status			
Premenopausal	5.656	1.717	0.04
Postmenopausal	6.44	1.74	
P value is significant at level of 0.05.			

BMI: Body mass index

Association sPD-1 expression with the histological classification of breast cancer in pre-treatment group. Statistically significant association of sPD-1 level (p=0.01) with histological classification (invasive or non-invasive) was observed. The sPD-1 expression was significantly higher (6.06±1.69) in the patients with invasive tumour as compared to patients with non-invasive tumours (2.75±1.77). Also our results confirmed there was significant association between sPD-1 level and other histological subtypes of tumor (Invasive ductal carcinoma, Invasive lobular carcinoma ductal carcinoma in situ (p=0.03). Particularly, Patients with Invasive lobular carcinoma had highest level of sPD-1 (6.27±1.77) as presented in Table 3.

Table 3: Association sPD-1 expression with the histological classification breast in pre-treatment group patients

Parameters	PD-1		
Histological classification	Mean	SD	P ≤*
Invasive carcinoma	6.06	1.69	0.01
Non-invasive(Carcinoma in situ)	2.75	1.77	
Histological subtype			
IDC	6.05	1.71	0.03
ILC	6.27	1.66	
DCIS	1.6	0.14	
P value is significant at 0.05 level			

IDC: Invasive Ductal Carcinoma, ILC: Invasive Lobular Carcinoma, DCIS: Ductal Carcinoma In Situ, SD: Stander Deviation.

Association sPD-1 expression with hormone

receptors ER, PR and HER2 in pre-treatment and post-treatment groups. No significant association was observed between sPD-1expression and hormone receptors (ER, PR and HER2/neu status) for both pre-treatment and post -treatment groups. But, there was found significant association between sPD-1 level and ER status in post-treatment. The higher level of sPD-1 was found in ER negative than ER positive (1.354±0.121; 0.896±0.0773 ng/ml) respectively, P =0.01 as shown in figure 2.

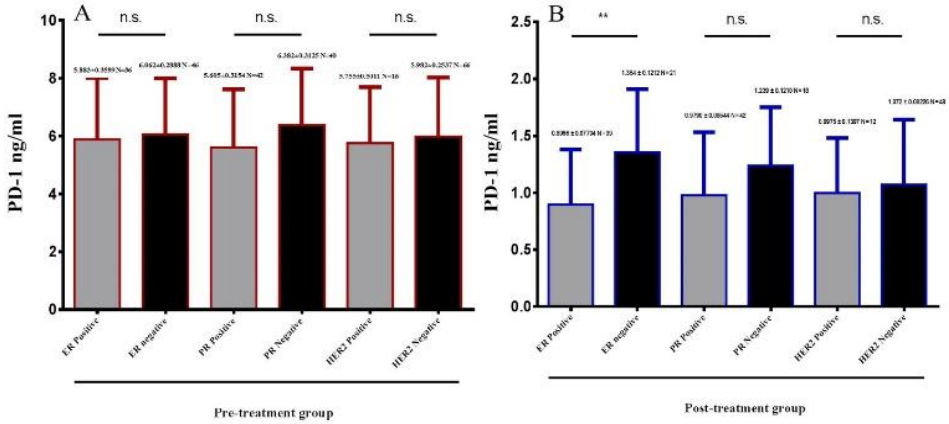

Figure 2. Association sPD-1 expression with hormone receptors ER, PR and HER2 in pre-treatment and post-treatment groups

Association sPD-1 level with Ki-67 expression in pre-treatment group. The results proved that there is significant association between Ki-67 expression with levels of sPD-1 (p=0.002). It was found that the Ki-67value (>40%) in breast cancer patients was linked with higher level of sPD-1(7.40±1.26ng/ml),and the lower level of sPD-1(5.49±1.77ng/ml) was found in Ki-67value (< 20%),Table 4.

Table 4: Association sPD-1 level with Ki-67 expression in pre-treatment group.			
Parameter	PD-1		
Ki-67 expression	Mean	SD	P ≤*
< 20%	5.49	1.77	0.002
20-40%	5.90	1.61	
>40%	7.40	1.26	
P value is significant at 0.05 level.			

Comparison of s PD-1 level in different molecular subtypes in pre-treatment and post treatment groups patients with breast cancer. No significant association was observed in levels of sPD-1 among different molecular subtype (Luminal A, Luminal B, TNBC and HER2) in pre-treatment group. But the significant difference in level of sPD-1 among molecular subtype was found when compared between both groups (p<0.0001). The higher level of sPD-1(1.460±0.0178ng/ml) was found in HER2 enriched type of tumor than other types in post treatment group and in pre-treatment group the higher sPD-1 level was in triple-negative breast cancer as shown in figure3

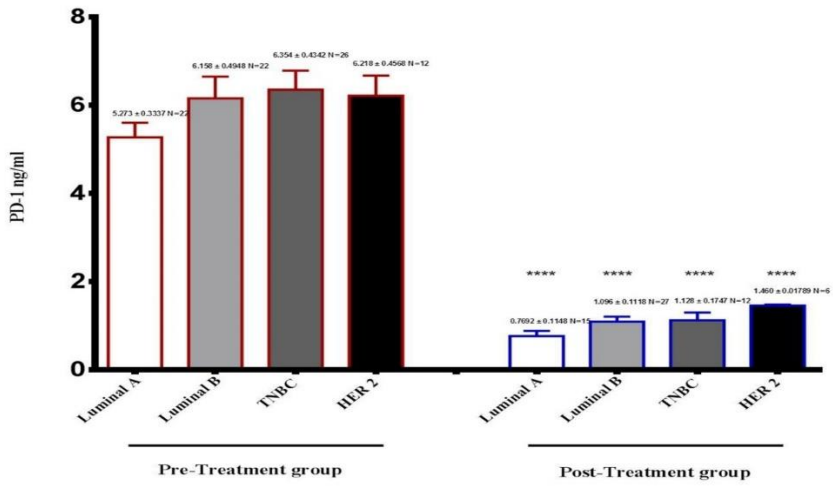

Figure 3: Comparison of s PD-1 level in different molecular subtypes in pre-treatment and post treatment groups patients with breast cancer

Correlation of sPD-1 level with different demographic characters in pre-treatment group. The

result as shown in (table 4), indicate that serum level of sPD-1 was positively correlate with family history and menopausal state (r=0.423, p=0.0001, r=0.244, p=0.027) respectively. No significant correlation of sPD-1 with age, body weight.

Table 5: Correlation sPD-1 level with different demographic characters in pre-treatment group						
		Age	BW	BMI	Family history	Menopausal state
PD-1	r	0.196	0.077	0.116	0.423	0.244
	P	0.077	0.527	0.307	0.0001	0.027
P value significant at ≤0.05 level.						

Pearson correlation for age, BW: body weight, BMI: body mass index. Family history and menopause by Spearman correlation.

Correlation serum level of PD-1 with immunohistochemistry in pre-treatment group. The table 6 illustrated that there was positive correlation between sPD-1 protein and Ki-67antigen (r=0.396, p=0.0047). While there no significant correlation between sPD-1 expression and hormone receptors ER, PR, HER2 as shown in (table 5)

Table 6: Correlation of sPD-1 expression with IHC in pre-treatment group Of breast cancer patients					
		ER	PR	HER2	Ki-67
PD-1	Rho	0.003	-0.126	0.032	0.396
	P	0.977	0.260	0.776	0.0047
ER: Estrogen Receptor; PR: Progesterone Receptor; HER2: Human Epidermal Growth Receptor 2					

5. Discussion

Cancer cells can develop mechanisms to downregulate immune responses in order to evade the immune system by overexpressing particular components of the immune checkpoint system, such as PD-L1. The upkeep of an immunosuppressive tumor microenvironment in humans has been shown to depend heavily on the interaction between PD-1 receptor and its ligand 20. Thus, the microenvironment of the tumor is becoming an increasingly important factor in cancer research because of its capacity to control several checkpoint mediators and so regulate the development of the tumor. 23. As an outcome, the development of novel analysis techniques and molecular drugs targeting PD-1/PD-L1 in breast cancer patients may be facilitated by a deeper comprehension of the PD-1/PD-L1 pathway, which may be attained by studying it in more details. When sPD-1 levels in three groups were compared, sPD-1 levels significantly varied between the three groups. The level of sPD-1 in the pre-treatment group was significantly greater than in the post-treatment group and the control group. Serum level of PD-1 was also significantly higher in post-treatment cases than in healthy controls suggesting that both breast cancer groups had immune suppression and benefit from antiPD-1 therapy. Several studies supported these results 22,25, proposing that elevated serum PD-1 levels may promote immunosuppression and are therefore to be considered adverse prognostic factors6,23. Exactly the contrary, Yao et al. showed no statistically significant change in PD-1 serum levels between the pre- and post-treatment groups 22. Since that chemotherapy is a typical treatment for cancer, and that its cytotoxic effects might vary to varying degrees. Immunogenic cell death is one of the processes by which chemotherapy induces the immune system to detect and destroy malignant cells. Other anticancer drugs, such as cyclophosphamide at high dosages, have immunosuppressive effects.26. Chemotherapy has the potential to drastically lower the number of immune suppressive cells in the peripheral blood and the tumor microenvironment (TME), as well as lower the number of inhibitory factors of anti-tumor immune response. On the other hand chemotherapy can result in the elimination or decrease of virtually all immune cells. 27. The heterogeneities within each group were not significant and supported our conclusions although it cannot be excluded that insufficient statistical power might account for these our conclusions because of the biological function of the PD-1or PD-L1 pathway itself and the complicated interaction between cancer cells and the immune system20.

Therefore, characterizing the status of anti-tumor immunity and auxiliary investigating the eligibility of breast cancer patients in Iraq for immunotherapy relies on an understanding of the expression landscape of the target immune checkpoints .The fact that breast cancer patients in Iraq have some distinct demographic differences from those in Western populations 28 and this fact has been shown by some different demographic features for Iraqi patients in the present study. They include a younger age group (40-69) at diagnosis for each pre and post-treatment group, as this age group had the highest percentage of patients. In the pre & post groups. (87.7%, 95%) of first-degree relatives with breast cancer had a negative family history in the pre-treatment and post-treatment groups respectively. Premenopausal had a higher incidence (58%) in pre-treatment, while (65%) postmenopausal was found in post-treatment group. The evidence provided by our findings demonstrates that demographic factors were present to be significantly associated with sPD-1expression as age, family history, and menopausal status. Age was associated with higher sPD-1 expression in females in the age group range (40-69) years, this finding agrees with the results of the previous study 25,29. This might be explained by IFN-γ may activate its receptor-associated JAK1/2

pathway, leading to phosphorylation and nuclear localization of STAT1which have a role in PD-1/PD-L1 signaling in tumorigenesis 30 and by a compensatory up-regulation of PD-1 in a microenvironment where an aggressive immune response threatens the tumor and cases in the present study in a range of higher PD-1 expression maybe with poor prognosis in an advanced stage, high tumor size, more involvement of axillary lymph node and higher grade of the tumor.

The most factor significantly associated with higher sPD-1 expression was negative family history (87.8%); as compared with patients with a positive family history of breast cancer (12.2%) although family history considers a risk factor for the prevalence of breast cancer. Allessio Cortellini et al. reported that positive family history cases had higher sPD-1 expression 31 which is in contrast to our study, this is due to a number of reasons, including the small sample size and the short period of time, in addition to different distribution of sample. Also, pre-menopausal patients with breast cancer (57.5%) had significantly lower PD-1 expression as compared to postmenopausal (42.5%).

In the same scenario, we found that significant association of PD-1 level with the general histological classification of tumor (invasive or non-invasive) and with the histological subtype of the tumor (IDC, ILC, DCIS). Furthermore, cases with invasive lobular carcinoma with the highest level of PD-1 than other subtypes. This is due to that PD-1/PD-L1 pathway is reduced when PD-L1 on the surface of tumor cells links to PD-1 on activated T cells. This results in T cell inhibition and restricts cytotoxic T cells from attacking other cells in the body.

These deactivated T cells stay limited in the tumor microenvironment 3. Additionally, as a result of intralesional heterogeneity, in which there is the possibility of several parts of the tumor presenting different histologic characteristics 32.

Our outcomes are similar to the results of the majority of prior 13. Similar findings were demonstrated by a few studies: Chenxi Yuan et al. reported that invasive ductal carcinoma was found in a significant fraction of patients' histology, accounting for 91% and there was a presence of a positive PD-1 expression rate in these types of tumor 33. Furthermore, no significant association was found in the level of sPD-1 with hormone receptors (ER, PR) and HER2/neu status for both pre-treatment and post-treatment groups. Except ER status was significantly associated with sPD-1 level in the post-treatment group. Our finding also, demonstrated that there was no correlation observed between sPD-1 levels with hormone receptors. Similar findings were reported by Chenxi Yaun 33. Although, sPD-1 level was higher in negative hormone receptors which is similar to the previous study 3.

Likewise, in addition to the conventional histopathological parameters, the assessment of proliferation is one of the major factors for the treatment decisions in breast cancer patients. Ki-67 protein expression is closely related to the pathological characteristics of breast cancer and can be used as an indicator of high-grade malignancy 34. The results proved that Ki-67 levels were significantly associated with sPD-1 levels suggesting breast cancer present with the most aggressive features showed higher Ki-67, It was found that the Ki-67value (>40%)in breast cancer patients was linked with higher level of sPD-1, and the lower level of sPD-1 was found in Ki-67value (< 20%). in the present study, it was confirmed that Ki-67 is a prognostic factor in breast cancer patients. Ki-67 is a useful prognostic marker, but its predictive role is limited. Millar E.K et al. reported the importance of Ki-67 as an improved marker panel for prognostic evaluation of breast cancer 35. Similarly, another study quoted the Ki-67 as an important prognostic marker and its significant association with the cancer markers like PD-1 36. Ermiah et al. reported that Ki-67 expression was more frequent in tumors with high S-phase fraction (SPF) than low SPF. Patients with high Ki-67 and high SPF had shorter survival times and predicted recurrence than patients with low Ki-67 and low SPF. Since breast cancer is highly heterogeneous, sPD-1 levels may vary among different breast cancer subtypes 37.

Generally, the outcomes of our current study supported the existence of significant associations in molecular subtypes between pre and post-treatment groups. However, no significant association of sPD-1 levels with molecular subtypes was found in the pre-treatment group, which was consistent with previous study 33. TNBC had the highest sPD-1 level compared to other molecular subtypes. The highest sPD-1 level was found in TNBC than in other molecular subtypes, this may be due to more aggressive phenotype and a worse prognosis in operable breast cancer and immunosuppression may be attributed to TNBC malignant biological behavior and triple-negative breast cancer is characterized by the presence of PD-1 expression, which plays a significant role in both the recurrence pathways and the metastasis 4,29. Using PD-1 inhibitors prevents tumor cells from evading the immune system by blocking the PD-1/PD-L1 negative regulatory pathway. Thus, anti-PD-1/PD-L1 targeted immunotherapy may be useful for TNBC patients 13. This result agrees with the finding obtained by several studies 4,22,33. Although, in the post-treatment group, sPD-1 level was higher in HER2 enriched than other subtypes which is bear a resemblance to some studies 3,23. Similarly, according to the findings of Vidula et al., the concentration of sPD-1 was shown to be significantly greater in human epidermal growth factor receptor 2 (HER2)-positive and triple-negative breast cancer (TNBC) molecular subtypes. 38.

6. Conclusion

There was a significant association between sPD-1 and invasive carcinoma, particularly with the worse

histopathological subtype (invasive lobular carcinoma). The sPD-1 expression may vary among different molecular subtypes. However, there was no significant association between the level of sPD-1 and hormone receptors (ER, PR), or HER2, even though the level of sPD-1 was higher in TNBC subtypes with negative hormone receptors, which indicates aggressive features. Furthermore, there was a significant positive correlation with the proliferation marker Ki-67 expression. Elevated sPD-1 levels may promote immunosuppression and thus should be regarded as unfavorable prognostic factors.

Data availability

The authors confirm that the data supporting the results of this study are included within article.

Conflict of Interest

None

Funding statement

None

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