Estimation of Programed Death Ligand_1 Concentrations in Serum and Tissue Among Iraqi Breast Diseases

Noor AL_huda Razakh Huadi, Frial G. Abd, Liqaa Y. Mohsen
Department of Biology, University of Babylon, College of Sciences, Hilla, Iraq

Abstract

Background: A programed death ligand L1 is the particular PD-1 ligand that is primarily found in lymphoid, epithelial, and myeloid cells. In order to activate the PD-1/PD-L1 pathway, which suppresses the production of cytokines and controls immune function, PD-1 must bind to PD-L1. **Objectives:** Estimating the concentration of PDL_1 in serum and breast tissue of patients with breast tumors. **Materials and Methods:** Case–control study included 100 women (17–60 years old) undergoing breast surgery at Babylon Province's Al-Hilla Teaching Hospital and Al-Fayhaa Al Ahly Hospital provided blood and breast tissue samples. Hospital histology lab performed histological confirmation of breast diseases, including benign and malignant tumors. Twenty blood samples were taken as controls from women and men who appeared to be in good health. The enzyme-linked immunosorbent assay was used to determine PDL_1 in both patient and control serum and in the cell supernatant from the patient's breast tissues. **Results:** The mean level of PDL_1 in serum of patient was 549.37 ng/L while control was 594.22 ng/L with found significant differences where P value was 0.05. While tissue of patients was 464.97 ng/L. The results appeared concentrations of PDL-L1 were significantly higher in serum compared with tissues at $P \geq 0.05$. The results found no significantly differences in concentrations of PDL_1 among types of diseases in sera of patients except in fat necrosis patients. Perhaps as a result of our small sample size, the difference in PD-L1 expression between the various subtypes was not statistically significant. **Conclusion:** This study found concentration of PDL-1 decreased with primary breast diseases and it might be diagnostic marker.

Keywords: Breast cancer, PDL-1, prognostic marker

Introduction

In the world, breast cancer is the most frequently diagnosed malignant tumor in women and the leading cause of cancer-related deaths. Around the world, the prevalence of breast cancer is steadily rising.[1] One of the characteristics of cancer cells is their ability to use a variety of tumor-mediated escape mechanisms to evade the immune response.[2,3] Through immunoediting mechanisms that result in tumor selection that is, resistant to immune effectors and the development of an immune-suppressive state within the tumor microenvironment, tumors evade immune surveillance.[2,3] The tumor uses a variety of mechanisms to evade the immune system. The using of immune checkpoints to inhibit the cell-mediated immune response and established a state of immune tolerance is currently identified to be a role in immune evasion mechanism in cancer cells.[3] Immune checkpoints mechanisms are suppressor immunoreceptors that act to maintain self-tolerance and resist over-stimulation of immune response.[4,5] Several immune checkpoints have been determined in cancers, however, of most importance are the programmed death-1 and its ligand programmed death-ligand 1.[4,5]

Programmed death ligand 1 also known CD274 or B7-H1, belongs to the B7 family and is expressed on B lymphocytes, T lymphocytes, monocytes cells, antigen-presenting cells, and epithelial cells.[6] Structurally, PD-1 consists of an IgV-like extracellular domain, a cytoplasmic domain that carries a cytoplasmic immunoreceptor tyrosine–based

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1727_23

Address for correspondence: Mrs. Noor AL_huda Razakh Huadi, Department of Biology, University of Babylon, College of Sciences, Hilla 51002, Iraq
E-mail: noor.razaq3555@gmail.com

Submission: 23-Nov-2023 **Accepted:** 15-Jan-2024 **Published:** 24-Sep-2024

How to cite this article: Huadi NAR, Abd FG, Mohsen LY. Estimation of programed death ligand_1 concentrations in serum and tissue among Iraqi breast diseases. Med J Babylon 2024;21:659-63.

switch motif and an inhibitory motif, and PD-L1 is made up of immunoglobulin V– and immunoglobulin C–like extracellular domains, a transmembrane, and a short cytoplasmic domain. The extracellular domains of PD-L1 and PD-1 can interact to cause PD-1 to change conformation, which phosphorylates (activates) ITSM and ITIM and lowers the TCR signal.[7] In addition to mediating immunosuppression in triple-negative breast cancer, soluble PD-L1 also induces regulatory B cell differentiation and can transduce inhibitory signals to effector T cells.[8]

PD-1 binding to PD-L1 inhibits T cell activation and function.[3] Under typical circumstances, the PD-1/PD-L1 pathway is activated to prevent overt immune responses and to preserve tolerance to self-antigens.[7] However, in the tumor microenvironment, tumoral PD-L1 expression is linked to a reduced immune response.[9] Many solid cancers, such as lung cancer,[10] renal cell carcinoma,[11] glioblastoma,[12] melanoma,[13] bladder cancer,[14] colorectal cancer,[15] and breast cancer,[16] have overexpressed PD-L1 on their tumor cells. Cancer cell-intrinsic PDL-1 signals present novel drug discovery targets and also have potential as reliable treatment response biomarkers.[17,18] This study's objective is to determine the amounts of PDLI in the serum and tissue of Iraqi patients suffering from breast cancer.

Materials and Methods

Subjects

About 100 specimens (blood and tissue) were enrolled in this study, age between 17 and 60 years, among male and female with breast surgery during July 2023 to November 2023 at AL_Hilla Teaching Hospital and AL_Fayhaa Al_Ahly Hospital, Babylon Province, Iraq. For either malignant or benign tumors, the women and men underwent lumpectomy breast procedures.

Tissue collection

The tissue obtained by clinical surgery for analysis was combined outside the marginal region, approximately 5 cm away from the tumor. Post circumcision, the fresh tissue was immediately placed in a sterilized plane tube or urine cup it contain a normal saline solution and it was cut, homogenized by using a sterile surgical scalpel and wooden sticks within 30 min of collection. The solution centrifuged for 5 min at 3500 rpm, then solution was placed into Eppendrof (Hamburg, Germany) tubes at freeze until used.[19]

Blood collection

About 100 blood samples were taken from tumor-bearing women and men, and 20 blood samples—5 men and 15 women—were taken as controls, 5 mL of blood was drown by venipuncture using disposable syringes, then blood was placed in disposable tube (gel tube), then centrifuged at 3000 rpm for 10 min. Before being used, sera samples were carefully placed into Eppendorf tubes and kept at a deep freeze.

Estimation of programmed death ligand 1

PDL_1 concentrations in sera and tissues were determined, as per the manufacturing company (BT LAB, Shanghai Korain Biotech, China), that uses the assay for enzyme-linked immunosorbent. Kits were used for the quantification of human PDL_1. An equation that fits the standard curve was used to calculate the test's result.

The analysis of the current study was performed by using Statistical Process for Social Sciences (SPSS) version 23. Results are expressed as (mean ± SD) paired *t* test was used to analyze the differences between systemic and mucosal immune response of the breast tumors. Independent samples *t* test was used to compare patient and control systemic. ANOVA test between groups. Correlation test between immunological markers *P* value below 0.05 were considered to be statistically significant.[20]

Ethical approval

On July 15, 2023, The Babil Health Directorate's Ethical Committee approved the study protocol. Furthermore, the patients' verbal consent was obtained before taking the sample. During the sampling, precautions were taken to ensure the safety of the participants. This work was also carried out by the Iraqi Ministry of Health's Ethics Committee and followed all national rules.

Results

The women afflicted with breast tumors divided according to the age groups. The higher percentage found in age group 17-30 years was 50%, while percentage patient with age group 31-50 years was 30%, and less than percentage with age group 51-60 was 20%. These results of the present study were agreement with other studies[21,22] who recorded that the age group of ≤20 years the highest tumor rate compared with other age. This study agree with study in Baghdad[22] in ratio of breast tumors according to marital status. Late marriage or unmarried women was more likely to developed to breast cancer because increased estrogen in their body but in this study, samples were taken from married women are compared with un married women were lower risk of breast cancer [Table 1].

In the microenvironment of the tumor, the tumor cells' expression of PD-L1 improve tumor cell immune evasion by raising inhibitory signals in order to preserve immunological tolerance Therefore, the enzyme-linked immunosorbent assay (ELISA) was utilized in this study to estimate programed death ligand 1 (PDL_1) and quantify the concentrations of b human PDL_1. The standard curve fit equation was used to compute the test results [Figure 1]. The mean level of PDL_1 in serum of

Table 1: Socio-demographic characteristics for breast tumor patients

Groups	Percentage (%)
Age (years)	
17-30	50%
31-50	30%
51-60	20%
Sex groups	
Male	15%
Female	85%
Marital status	
Married	80%
Unmarried	20%

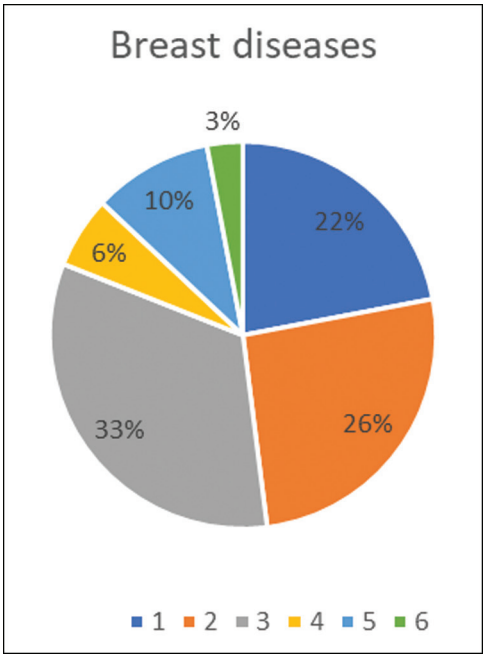

Figure 1: The percentage of breast diseases (1) ductal invasive, (2) fibrocystic change, (3) fibroadenoma, (4) granulomatous mastitis, (5) gynecomastia, and (6) fat necrosis

Table 2: Comparison of the PDL_1 between patients and control in serum

Serum PDL-1	Concentration, M ± SD (ng/L)	*P* value (sig)
Patients	549.3740 ± 93.22678	0.05*
Control	594.2200 ± 64.93154	

*Significant at $P \geq 0.05$

Table 3: Comparison of the PDL_1 between patients serum and patients tissue

PDL-1 in patients	Concentration, M ± SD (ng/L)	*P* value (sig)
Serum	549.3740 ± 93.22678	0.00
Tissue	464.9728 ± 77.81894	

*Significant at $P \geq 0.05$

Table 4: Concentration of PDL_1 in patients sera with breast disease according tumors types

Types of breast diseases	PDL_1 concentrations in serum, M ± SD (ng/L)
Invasive carcinoma	591.60 ± 74.993 (a)
Fibrocystic change	529.56 ± 84.537 (a)
Fibroadenoma	533.54 ± 91.178 (a)
Granulomatous mastitis	516.009 ± 74.930 (a)
Gynecomastia	530.406 ± 127.528 (a)
Fat necrosis	730.100 ± 17.416 (b)

Similar letters in the same column indicate that there is no significant difference ($P > 0.02$; ANOVA_Duncan)

Table 5: Concentrations of PDL_1 in patients tissues according type of diseases

Types of breast diseases	PDL_1 concentrations in Tissues, M ± SD (ng/L)
Invasive carcinoma	478.724 ± 121.901 (a)
Fibrocystic change	473.940 ± 82.072 (a)
Fibroadenoma	459.614 ± 55.776 (a)
Granulomatous mastitis	497.960 ± 56.961 (a)
Gynecomastia	485.640 ± 43.021 (a)
Fat necroses	424.089 ± 25.211 (a)

Similar letters in the same column indicate that there is significant difference ($P > 0.56$; ANOVA_Duncan)

patients was 549.37 ng/L while controls was 594.22 ng/L with found significant differences where *P* value was 0.05 as in Table 2.

The mean level in serum of patient was 549.37 ng/L while tissue of patient was 464.97 ng/L. The results appeared concentrations of PDL-L1 were significantly higher in serum compared with tissues at $P \geq 0.05$ in Table 3.

The types of breast diseases that enrolled in this study as in Figure 1. The results found fibroadenoma was higher percentage 33% and followed with fibrocystic change was 26% then ductal invasive was 22%. The concentrations of PDL_1 in serum among types of tumors were no significantly differences expect with fat necrosis. It was in Invasive carcinoma 591.60 ng/L, fibrocystic change 529.58 ng/L, fibroadenoma 533.54 ng/L, granulomatous mastitis 516.00 ng/L, gynecomastia 530.41 ng/L and fat necroses 730.10 ng/L as shown in Table 4.

Its concentrations in Invasive carcinoma 478.72 ng/L, fibroadenoma 459.61 ng/L, fibrocystic change 473.94 ng/L, granulomatous mastitis 497.96 ng/L, gynecomastia 485.64 ng/L, and fat necrosis 424.0 9 ng/L as shown in Table 5.

In the present study express the Correlation between serum and tissue was negative correlation as in Table 6 and Figure 2 from results found that PDL-1 concentrations were significantly higher in sera compared with tissues.

Table 6: Correlation between PDL_1 in serum and tissue in patients

PDL	Serum	Tissue
Serum	1	−0.029
Pearson correlation		0.867
Sig. (2-tailed)		
Tissue		1
Pearson correlation	−0.029	
Sig. (2-tailed)	0.867	

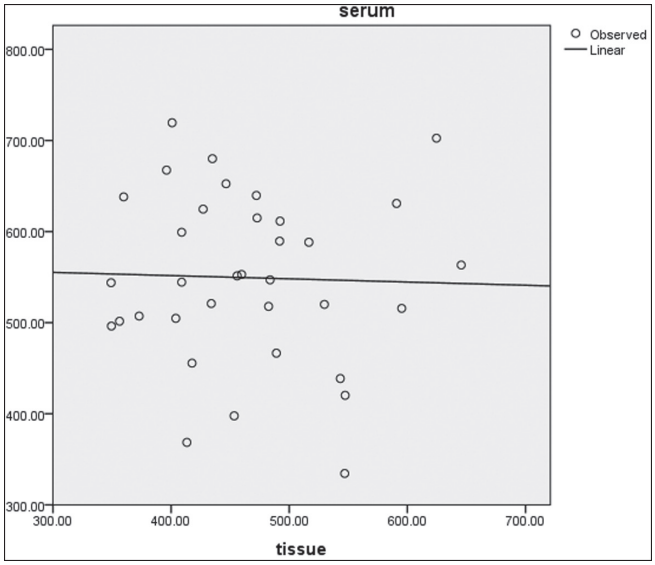

Figure 2: Correlate between PDL-1 in serum and tissue of breast patients

Discussion

The results found concentrations of PDL_1 were increased in breast disease patients compared with apparently healthy controls this agreed with several studies which indicated the PD-1/PD-L1 expression status in patients with breast cancer, although the findings were inconclusive. A retrospective analysis of 1091 patients with invasive breast cancer revealed that 27.0% of the patients (295/1091) had PD-L1 high levels and 73.0% of the patients (796/1091) had PD-L1 low levels. Kornepati *et al.*[17] but Tsang *et al*.[23] discovered that the transcription of programmed death-1 was rising in tumor tissue. Generally speaking, these tissues also showed an increase in PDL-1 expression, though this was not statistically significant.

The concentrations of PDL_1 in serum were higher significantly increased than tissue, This finding might be consistent with[24,25] hypothesis, which states that in order for cancer cells to migrate to regional lymph nodes, they have to be able to successfully avoid immune surveillance. In these cells, the PD-1/PD-L1 signaling pathway is elevated.

The results found no significantly differences in concentrations of PDL_1 among types of diseases in sera of patients except in fat necrosis patients. Perhaps as a result of our small sample size, the difference in PD-L1 expression between the various subtypes was not statistically significant. Lou *et al*. found that while PD-L1 was absent from the surrounding normal breast tissue, it was expressed in specimens of invasive ductal breast cancer found that while PD-L1 was absent from the surrounding normal breast tissue, it was expressed in specimens of invasive ductal breast cancer.[26,27] In line with another study, there was no discernible variation in the proportion of PD-1-positive T cells in the peripheral blood of patients with breast cancer, regardless of the disease's stage or molecular makeup, when compared to the healthy controls.[28] A specific target for cancer therapy has been identified as the PD-1/PD-L1 pathway, whereby high-affinity anti-PD-1 or anti-PD-L1 antibodies can activate and proliferate T cells again and reverse tolerance. Clinically, the identification of patients who could benefit from immune therapy has been done using the expression of PD-L1 on tumor cells. Regarding this, the PD-L1 inhibitor medication atezolizumab has been approved by the US FDA.[29,30]

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Smolarz B, Nowak AZ, Romanowicz H. Breast cancer-epidemiology, classification, pathogenesis and treatment (review of literature). Cancers (Basel) 2022;14:2569.
2. Vinay DS, Ryan EP, Pawelec G, Talib WH, Stagg J, Elkord E, *et al*. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. Semin Cancer Biol 2015;35:S185-98.
3. Ayoub NM, Al-Shami KM, Yaghan RJ. Immunotherapy for HER2-positive breast cancer: Recent advances and combination therapeutic approaches. Breast Cancer (Dove Med Press) 2019;11:53-69.
4. He X, Xu C. Immune checkpoint signaling and cancer immunotherapy. Cell Res 2020;30:660-9.
5. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012;12:252-64.
6. Khan AR, Hams E, Floudas A, Sparwasser T, Weaver CT, Fallon PG. PD-L1hi b cells are critical regulators of humoral immunity. Nat Commun 2015;6:5997.
7. Fife BT, Pauken KE, Eagar TN, Obu T, Wu J, Tang Q, *et al*. Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. Nat Immunol 2009;10:1185-92.
8. Li X, Du H, Zhan S, Liu W, Wang Z, Lan J, *et al*. The interaction between the soluble programmed death ligand-1 (sPD-L1) and PD-1^{+} regulator B cells mediates immunosuppression in triple-negative breast cancer. Front Immunol 2022;13:830606.
9. Yu H, Boyle TA, Zhou C, Rimm DL, Hirsch FR. PD-L1 expression in lung cancer. J Thorac Oncol 2016;11:964-75.
10. Choueiri TK, Fay AP, Gray KP, Callea M, Ho TH, Albiges L, *et al*. PD-L1 expression in nonclear-cell renal cell carcinoma. Ann Oncol 2014;25:2178-84.
11. Nduom EK, Wei J, Yaghi NK, Huang N, Kong L-Y, Gabrusiewicz K, *et al*. PD-L1 expression and prognostic impact in glioblastoma. Neuro Oncol 2016;18:195-205.

12. Hino R, Kabashima K, Kato Y, Yagi H, Nakamura M, Honjo T, *et al*. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. Cancer 2010;116:1757-66.
13. Huang Y, Zhang SD, McCrudden C, Chan KW, Lin Y, Kwok HF. The prognostic significance of PD-L1 in bladder cancer. Oncol Rep 2015;33:3075-84.
14. Li Y, Liang L, Dai W, Cai G, Xu Y, Li X, *et al*. Prognostic impact of programed cell death-1 (PD-1) and PD-ligand 1 (PD-L1) expression in cancer cells and tumor infiltrating lymphocytes in colorectal cancer. Mol Cancer 2016;15:55.
15. Baptista MZ, Sarian LO, Derchain SF, Pinto GA, Vassallo J. Prognostic significance of PD-L1 and PD-L2 in breast cancer. Hum Pathol 2016;47:78-84.
16. Dill EA, Gru AA, Atkins KA, Friedman LA, Moore ME, Bullock TN, *et al*. PD-L1 expression and intratumoral heterogeneity across breast cancer subtypes and stages: An assessment of 245 primary and 40 metastatic tumors. Am J Surg Pathol 2017;41:334-42.
17. Kornepati AVR, Vadlamudi RK, Curiel TJ. Programmed death ligand 1 signals in cancer cells. Nat Rev Cancer 2022;22:174-89.
18. Azim HA, Shohdy KS, Elghazawy H, Salib MM, Almeldin D, Kassem L. Programmed death-ligand 1 (PD-L1) expression predicts response to neoadjuvant chemotherapy in triple-negative breast cancer: A systematic review and meta-analysis. Biomarkers 2022;27:764-72.
19. Shaheed TS, Barem WN, Abd FG, Al-Khikani FH. Estimation of systemic and mucosal toll-like receptors 4 and 6 in women with breast tumor. J Med Soc 2023;37:9-12.
20. Al-Rawi, KM. Enter to Static. 2nd ed. Vol. 15. College of Agriculture, Mosul University; 2000. p. 145-9.
21. Carroll JC, Cappelli M, Miller F, Wilson BJ, Grunfeld E, Peeters C, *et al*. Genetic services for hereditary breast/ovarian and colorectal cancers–physicians' awareness, use and satisfaction. Public Health Genomics 2008;11:43-51.
22. Shakir DA. Isolation and Identification of Some Aerobic Bacteria from Solid Tumors of Humans and Animals (M.Sc. Thesis). University of Baghdad; 2008.
23. Tsang JYS, Au W-L, Lo K-Y, Ni Y-B, Hlaing T, Hu J, *et al*. PD-L1 expression and tumor infiltrating PD-1+ lymphocytes associated with outcome in her2+breast cancer patients. Breast Cancer Res Treat 2017;162:19-30.
24. Uhercik M, Sanders AJ, Owen S, Davies EL, Sharma AK, Jiang WG, *et al*. Clinical significance of PD1 and PDL1 in human breast cancer. Anticancer Res 2017;37:4249-54.
25. Yuan C, Liu Z, Yu Q, Wang X, Bian M, Yu Z, *et al*. Expression of PD-1/PD-L1 in primary breast tumours and metastatic axillary lymph nodes and its correlation with clinicopathological parameters. Sci Rep 2019;9:14356.
26. Wais RS, Ali HH. Axillary lymph node involvement in primary invasive breast carcinoma in a center in Baghdad Province. Med J Babylon 2021;18:322-6.
27. Lou J, Zhou Y, Huang J, Qian X. Relationship between PD-L1 expression and clinical characteristics in patients with breast invasive ductal carcinoma. Open Med (Wars) 2017;12:288-92.
28. Setordzi P, Chang X, Liu Z, Wu Y, Zuo D. The recent advances of PD-1 and PD-L1 checkpoint signaling inhibition for breast cancer immunotherapy. Eur J Pharmacol 2021;895:173867.
29. Xu-Monette ZY, Zhang M, Li J, Young KH. PD-1/PD-L1 blockade: have we found the key to unleash the antitumor immune response? Front Immunol 2017;8:1597.
30. Touma HS, Shani WS. Increased transforming growth factor-β and interleukin-17 transcripts in peripheral blood of breast cancer patients with different clinical stages. Med J Babylon 2018;15:145-9.