RESEARCH ARTICLE

Updating of PD-1, PDL-1 and CD8+ in Benign and Malignant Breast Cancer in Iraqi Women

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ABSTRACT

The study conducted at Center for the Early Detection of Breast Tumors at Medical City's oncology teaching hospital carried 90 patients who participated in this study. The research was carried out between February 15, 2021, and July 20, 2021. The diagnosis was made by the consultant medical team using the triple assessment technique, which includes physical breast examination, ultrasonography, mammography, and fine needle aspiration cytology.

Women patients clasified into 3 groups (benign tumor, control and malignant tumor) benign B (34 women) divided in/to two sub groups such as (benign premenopausal age B1 (17) and benign postmenopausal age B2 (17) and malignant M (34), malignant premenopausal age M1 (17) and malignant postmenopausal age M2 (17), and control group C includes (11) premenopausal age C1 and (11) C2. An ELISA approach was used to measure the level of soluble PDL, PD L1, and CD8 expression in serum.

PD1 shows significant increase ($p \leq 0.05$) in pre- and post-menopausal malignant groups M5, M6 when compared with C1, C2, B1, B2. PDL-1 levels were non-significant ($p \geq 0.05$) in pre and postmenopausal control and benign groups (C1, C2, B1 and B2) while they were increased significantly ($p \leq 0.05$) in M1, M2 in comparison with B2,C1,C2, B1. CD8 in the M1 increased significantly ($p \leq 0.05$) when compere with group1, group 2, group bening1, group benign 2 and malignant 2.

Conclusions from the current study that the values of PD1and PDL1in pre and postmenopausal malignant women breast cancer showed an increase compere with the other groups, the levels of CD8+ increase in the premenopausal malignant breast cancer women in comparison with other groups.

Keywords: Malignant Breast, Iraqi Women, PD-1, PDL-1, CD8+ .

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INTRODUCTION

As the largest cause of cancer-related death, breast cancer (BC) is a significant global public health issue.[1] BC is a malignancy that is defined by the uncontrolled growth and spread of abnormal breast cells. In 2018, roughly (2.1) million women worldwide were diagnosed with cancer, accounting for 24.2% of new cases and 627,000 (15%) fatalities.[2] Breast cancer affects more than half of the world's population, with sixty of fatalities occurring in low- and middle-income nations (LMICs). Age-standardized breast cancer incidence varies widely by population around the world, from 19.3/100,000 in Eastern Africa to 89.9/100,000 in Western Europe. The main cause of this variation is variability in reproductive and menstrual features,[3] programmed death-1 (PD-1) and ligand-1 (PD-L1) are type I transmembrane glycoproteins belonging to the immunoglobulin superfamily, which has been named as a programmed cell death-1 receptor in relation to the apoptosis program.[4] Human PD-1 gene, also known as CD279, was located in the chromosome 2q37.35 with relative molecular weight of 55 kDa and composed of extracellular domain, transmembrane domain, and intracellular domain.[5] PD-L1 was widely found on The B lymphocytes, monocytes, NK macrophages, and vascular endothelial cells. It was also upregulated in tumor cell lines, such as cancer of ovaries, lymphoma, and malignant melanoma, indicating a strong link between the occurrence and progression of malignancies.[6] Human breast cancer can be controlled by CD8+ T-cells, however immunosuppressive lymphocytes,

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Table 1: Comparison between difference groups in PD-1, PDL-1 and CD8

Group	*Mean ± SE*		
	PD-1 (pg/mL)	*PDL-1 (pg/mL)*	*CD8+ (u/mL)*
C1	5.47 ± 0.73^{b}	8.31 ± 1.32^{b}	370.54 ± 27.15^{b}
C2	6.81 ± 0.90^{b}	6.86 ± 1.12^{b}	373.96 ± 27.15^{b}
B1	6.43 ± 0.71^{b}	6.59 ± 0.47^{b}	386.96 ± 45.25^{b}
B2	8.25 ± 1.04^{b}	6.58 ± 0.63^{b}	424.15 ± 37.67^{ab}
M1	109.77 ± 30.13^{a}	272.12 ± 65.39^{a}	543.67 ± 63.76^{a}
M2	93.26 ± 34.43^{a}	185.58 ± 48.80^{a}	482.89 ± 55.99^{ab}
LSD value	61.09*	108.89 *	141.62 *

The meanings of the means that contained distinct letters in the same column varied greatly. * ($p \leq 0.05$).

such as CD4+ T-regulatory cells and macrophages, diminish this effect. Intriguingly, the immunoglobulin-chain presently has demonstrated to have the same predictive and prognostic significance in tumor as a single marker.[7] The presence of CD8+ T-cell infiltrates is linked to a better prognosis. Poorer outcomes have been linked to tumor-associated macrophages and CD4+ T-cells, which also comprise T-regulatory cells.

MATERIALS AND METHODS

Information Collection

A questionnaire included some information about age , weight, age at marriage, average lactation term etc.

The study had 90 women patients who came for an early breast cancer screening. To ensure that none of the women had an infection, whether benign or malignant, we took a sample from each patient and ran tests on it Their ages ranged from 23 to 70. Three groups of women patients were formed (benign tumor, malignant tumor and control). Benign B 34 female divided in/to two sub groups include benign premenopausal and postmenopausal age (17 women) and Malignant M (34 women) divided in to premenopausal and postmenopausal age included (17 female) and control group include 11 premenopausal and postmenopausal age C1, C2. Between February 15, 2021 and July 20, 2021, the women were sent to the center for early breast tumor detection at the oncology teaching hospital in Medical City. A triple assessment technique was used to make the diagnosis, which was made by the specialist medical professionals. In an aseptic setting, 5 mL of blood were taken and placed in a disposable 5 mL syringe. Blood was placed in a tube with plain material, allowed to clot for 15 minutes at 4°C, and then centrifuged for 10 minutes at 5000 rpm. Sera were divided, preserved in Eppendorf tubes, and there after kept in a deep freezer until they were examined for a biochemical analysis.[8]

PD1 and PDL-1 Measurement in Blood Serum

Using CUSABIO ELISA kits, the concentration of PD1 and PDL-1 in the serum was determined. Results are automatically calculated by a highly sensitive ELISA reader at the conclusion of the test. In less than five minutes, the optical density of each one well was determined using a microplate reader set to 5400 nm.

Normal Values PD1: Premenopausal (2.079–9.72 pg/mL), postmenopausal, (2.901–10.401 pg/mL),

Normal Values PDL-1: Premenopausal (0.279–12.222 pg/mL), postmenopausal, (2.901–10.401 pg/mL)

Measurement of CD8+ in Blood Serum

Cluster of differentiation (CD8+) utilizing CUSABIO ELISA Kits, concentrations in the serum were determined. Results of the experiment are automatically calculated by a highly sensitive ELISA reader. The optical density at reading at 450 nm wavelength,

Normal Values: Premenopausal (259.732–499.035) u/mL postmenopausal (215.263–502.463) u/mL

Statistical Analysis

The statistical analysis system eight application was used to determine the impact of various research parameter components. The least significant difference (LSD) test (ANOVA) was carried out to significantly compare the means. The study's estimation of the correlation coefficient with variables.

RESULT AND DISCUSSION

Table 1 is presented the levels of PD1 and PDL-1 in the study groups PD1 values were non-significant difference ($p \geq 0.05$) among C1 (5.47 ± 0.73); C2 (6.81 ± 0.90); B1 (6.43 ± 0.71) and B2 (8.25 ± 1.04) while it was increased significantly ($p \geq 0.05$) in M1 (109.77 ± 30.13) and M2 (93.26 ± 34.43) when compere with C1, C2, B1, B2. Levels PDL-1 were non difference significant ($p \geq 0.05$) among C1 (8.31 ± 1.32); C2 (6.86 ± 1.12); B1 (6.59 ± 0.47); B2 (6.58 ± 0.63), while it was increased significantly ($p \leq 0.01$) in M1 (272.12 ± 65.39); M2 (185.58 ± 48.80) in comparison with C1, C2, B1, B2. The values of CD8+ in C1 (370.54 ± 27.15); C2 (373.96 ± 27.15); B1 (386.96 ± 45.25); B2 (424.15 ± 37.67) and M2 (482.89 ± 55.99) non-significant difference ($p \geq 0.05$). The B2 (424.15 ± 37.67) and M2 (482.89 ± 55.99) demonstrate that the difference is not significant with C1, C2, B1. The M1 (543.67 ± 63.76) increased substantially ($p < 0.05$) when compere with C1; C2; B1, B2 and M2.

Table 2 shows non-significant link between CD8 and PD-1 (0.18), while there was significant ($p < 0.05$) correlation between CD8+ (0.51) and PDL-1 (Figure 1).

In Table 1, the values PD1 and PDL-1were increased significantly in groups (M1; M2) due to ability of cancer cell to produce this protein to evading the immunity therefore we can use the PD1 and PDL-1 as a marker for diagnosis of the breast cancer.

The immune checkpoint inhibitor programmed cell death-1 receptor (PD-1) is found on the surface of cell of immunity . It is largely triggered by the protein PD-L1, found in all human cells.[9] The PD-1/PD-L1 pathway is crucial for maintaining peripheral T-lymphocyte tolerance and inflammatory control. By increasing the expression of PD-L1 on the tumor cell surface, the PD-1/PD-L1 inhibitory pathway can be used to

Table 2: Correlation coefficient between PD-1, PDL-1 with CD8+

Parameters	*Correlation coefficient-r*
	CD8
PD-1 (pg/mL)	0.18 NS
PDL-1	0.51 **
	** (p≤0.05), NS: Non-Significant.

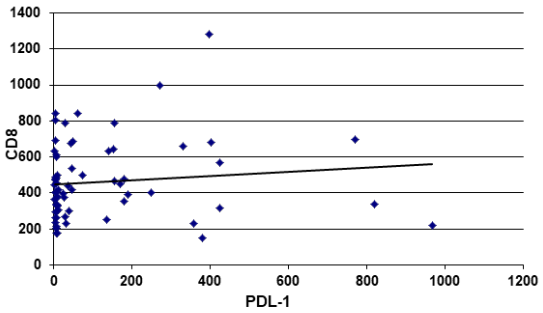

Figure 1: Relationship between PDL-1 and CD8 +

quiet the immune system; however, the rise in both biomarkers was only seen in solid tumors.[10] PD-L1 has been demonstrated to promote carcinogenesis and invasiveness *in-vivo,* as well as making cancer cells are decrease vulnerable to CD8+ T-cells.[11] A significant PD-1 ligand known as programmed death-ligand 1 (PD-L1), a 40-kDa transmembrane glycoprotein, was shown to be more prevalent in premenopausal breast cancer patients than in postmenopausal patients.

T-cell apoptosis, and T-tolerance cells can all be brought on by the interaction of PD-L1 and programmed death 1,[12] resulting in tumor aggravation and evasion of the host immune response. The increase in PD-1 concentration in the group of patients with malignant tumor due to the ability of cancer cells to display PD-1 which plays a role in suppressing immune cells against cancer cells, but this matter is unclear in benign tumor cells compared to the control group.[13]

The values of CD8+ increased in premenopausal M1 and postmenopausal malignant M2 the incidence of cancerous tumors in some cells, especially in M1 causes a marked increase of CD8+ due to the cytotoxic cell attacking the cancerous cells, causing an increase in the review of CD8+.

CD8+ is essential part of adaptive immunity and it is necessary for effective tumor elimination and can control CD8+ T-cells human breast cancer.[13] The findings of this study show that the cytotoxic T-cell population plays a prognostic role in breast cancer. This indicates that cytotoxic T lymphocytes have clinically considerable anticancer efficacy in human breast carcinoma.[14]

Better survival results have been associated with increased CD4 T cell infiltration and it was[15] revealed in TNBC patients, an increase in CD4 and CD8 T cells was associated with a good prognosis.[15] We found a considerably greater frequency of CD8+ in sera from early stage BC in M1 premenopausal malignant and M2 postmenopausal malignant.[16] The findings imply that overall CD8+ T cell counts do not remain constant in the course of tumor and may serve as a useful tumor marker, with CD8+ T cells increased with Egyptian females with breast tumor.

Furthermore, T cells vary between early and late stages of breast cancer, implying that CD8+ T cells are controlled by various mechanisms. Infiltrating CD8-cytotoxic lymphocytes with homeostasis intervention in breast cancer.[16] Our findings imply that the cell-mediated immune response plays a significant role in the development of breast cancer. Table 2 and Figure 1 shows there were non-significant ($p > 0.05$) correlation between CD8 and PD-1 (0.18), while there was significant ($p < 0.05$) correlation between CD8 (0.51) and PDL-1. The PD-1 (programmed cell death -1) PD-L1 axis is a critical immune response modulator that plays a important role in regulating the early the cytotoxic immunological reaction. The expression of PD-L1 by cancer tissue, or the microenvironment of tumor has been described in a different of malignancies. According to preclinical research, suppressing the PD-L1 and PD-1 axis in the tumor microenvironment may improve tumor regression. Several other drugs that target PD-1 or PD-L1 have demonstrated notable response rates in clinical trials in a variety of tumor types.[17,18]

CONCLUSION

It is concluded from the present study that the levels of PD1and PDL1 in pre- and post-menopausal malignant women breast cancer showed an increase in comparison with other groups, the levels of CD8+ increase in the premenopausal malignant breast cancer women in comparison with other groups.

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