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Assessment of the HER2, PDL1 and Oxidative Stress Levels at the Menopausal Status of Newly Diagnosed Breast Cancer Patients

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Abstract
Objectives: This study aimed to estimate the levels of HER2, PD-L1 and oxidative stress in newly diagnosed breast cancer patients. Also, to estimate the probability of using HER2 and PD-L1 as a predictive marker on the occurrence of breast cancer in addition to study the effect of menopausal status at the level of HER2, PD-L1, oxidative stress and breast cancer risk factor.
Methods: This study included 125 newly diagnosed breast cancer patients (53 premenopausal and 72 postmenopausal) from Oncology and Nuclear Medicine Hospital in Mosul, Iraq, and 100 apparently healthy women as a control group (44 premenopausal and 56 postmenopausal), during the period from Jan. 2021 to Jun. 2021. The ages of patients and control are matched, and it is ranged from 30–60 years. In this study we estimate the level of Human Epidermal Growth Factor Receptor 2 (HER2), Programmed Death -Ligand 1 (PD-L1), total antioxidant capacity (T-AOC), arylesterase activity, uric acid level, malondialdehyde (MDA) level, peroxidase activity, lactoperoxidase activity and iron level at breast cancer patients and control.
Results: The results show that there is a significant elevation in the level of HER2, PD-L1, malondialdehyde, peroxidase, lactoperoxidase and iron, and a significant decrease in the level of T-AOC, arylesterase and uric acid in the serum of breast cancer patients (pre and postmenopausal) compared with the control group (pre and postmenopausal). Also, increase the level of HER2 and oxidative stress at postmenopausal status for the control and patient groups. While PD-L1 level does not affect by menopausal status for both control and patient groups.
Conclusion: The level of HER2, PD-L1, and oxidative stress was significantly increased in newly diagnosed breast cancer patients compared with the control group at the same menopausal status. Increase breast cancer risk factor at the postmenopausal status.
Keywords: Breast neoplasms, epidermal growth factor, oxidative stress

Introduction

Breast cancer eventuates when the tissue cells of the breast become atypical and divide without control, these atypical cells shape a big lump of tissue, which later develops into a tumor.[1] It is the second leading cause of death after lung cancer.[2]

Breast cancer is affected by many risk factors, such as gender, being older, obesity, menopausal status, full-term pregnancy, abortion, hereditary factors and hormonal factors.[3] The menopausal status is an important risk factor for breast cancer.[4]

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase-activated transmembrane glycoprotein receptor.[5] It is a member of human epidermal growth factor (EGF) receptor family, which includes the EGFR (HER1), HER2 (also known as ErbB2), HER3, and HER4 receptors.[6] It is encoded by the proto-oncogene HER2, which can be found on chromosome 17q21's long arm.[7]

In the majority of these difficult breast tumors, HER2 is the primary oncogenic driver.[8]

Because HER2 is an orphan receptor (no specific ligand for HER2 has been identified),[9] when expressed at very high levels on the cell surface, it relies on heterodimerization with other HER receptors or homodimerization with itself to activate the HER2 signaling pathways.[10,11]

HER2 dimerization causes autophosphorylation of tyrosine remnants within the receptor's cytoplasmic domain, causing activation of a number of downstream signaling pathways, specifically phosphoinositide-3-kinase (PI3K) and mitogen-activated protein kinase (MAPK),[12] which control cellular function like cell migration, differentiation, proliferation, apoptosis, cell cycling, and angiogenesis.[13]

Programmed Cell Death Ligand 1 (PD-L1) is a transmembrane protein that serves as a co-inhibitory agent of the immune response, when combined with it is a receptor (PD-1), it can stop PD-1 positive cells from proliferating, decrease their cytokine release, and trigger apoptosis.[14]

Both cancerous cells and immune cells such as T and B lymphocytes, macrophages, and dendritic cells can express this immunological checkpoint molecule.[15]

The PD-1/PD-L1 pathway regulates inflammation and maintains peripheral T-lymphocyte tolerance.[16] The expression of PD-L1 by tumor cell controls the activity of T cells, boost immunosuppression and tumor escape.[17]

Oxidative stress is an imbalance between the formation of the reactive oxygen species by the cells and the potency of the biological system to detoxifying them.[18] Adequate levels of reactive oxygen species are required for cell function and survival in healthy conditions.[19] When ROS levels rise, they start to damage important cellular structures like proteins, lipids, and nucleic acids.[20] Growing both empirical and clinical proof proposes that the oxidative stress play a role in breast cancer tumorigenesis and progression.[21]

Materials and Methods

Ethical Approval

This study was approved by the ethical committee of the Nineveh health direction training center and human development, ministry of health and environment, Iraq, approved this

study. Informed written consent was obtained from all the participants before sample collection.

Blood Sample

This study included 125 newly diagnosed breast cancer patients from Oncology and Nuclear Medicine Hospital in Mosul, Iraq, and 100 apparently healthy women as a control group. Their ages ranged from (30–60) years. Patients and control were divided into two groups based on their menopausal status as premenopausal and postmenopausal. The premenopausal group is comprised of 53 breast cancer patients and 44 control. While the postmenopausal group is comprised of 72 breast cancer patients and 56 control. The samples were collected during the time period from the beginning of Jan. 2021 to the end of Jun. 2021.

Peripheral venous blood samples (5 ml) were collected from all study participants and incubated directly at 37°C for 10 min, after that centrifuged at 3500 rpm for 12 minutes. The serum was separated and frozen in aliquots at –20°C until used.[22]

Laboratory Analysis

- HER2 and PDL-1 concentration: was measured by using Bioassay technology laboratory kit (Shanghai, China), which based on enzyme linked immunosorbent assay (ELISA) technique.
- Total antioxidant capacity (T-AOC): was measured by using Solarbio Science & Technology kit (Beijing, China), which based on spectrophotometer method.[23]
- Arylesterase activity: was measured by using enzymatic hydrolysis of phenyl acetate to produce phenol and acetic acid.[24,25]
- Malondialdehyde (MDA): was measured by using thiobarbituric acid was used to assess malondialdehyde.[26]
- Peroxidase activity: was measured by using enzymatic oxidation of hydrogen peroxide by peroxidase.[27]
- Lactoperoxidase activity: was measured by using enzymatic oxidation of pyrogallol to purpurogallin.[28]
- Iron concentration: was measured by using Biolab kit (Maizy, France), which is based on reducing Fe^{+3} to Fe^{+2}.[29]
- Uric acid concentration: was measured by using Biolab kit (Maizy, France) which is based on enzymatic oxidation of uric acid by uricase.[30]

Statistical Analysis

Data were analyzed by using the SPSS Statistics 22.0 software package (SPSS version 22.0, IBM, USA).

Results

Effect of the Menopausal Status at HER2 Level

The mean levels of HER2 were (6.87 ± 0.31, 8.18 ± 0.50 ng/ml) in the pre and postmenopausal status for the control group, respectively, but the mean levels of HER2 were (11.72 ± 0.33, 14.4 ± 0.48 ng/ml) in the pre and postmenopausal status for the patient group, respectively.

Also, the results in Figure 1 show there is a significant increase (*P*-value <0.05) in the level of HER2 at both pre and postmenopausal patients compared with the pre and postmenopausal control group.

Figure 2 show there is a significant elevation (*P*-value <0.05) in the level of HER2 at postmenopausal status compared with the premenopausal status of both control and patient groups.

Effect of the Menopausal Status at PD-L1 Level

The mean levels of PD-L1 were (145.17 ± 4.05, 149.58 ± 8.06 ng/ml) in the pre and postmenopausal status for the control group, respectively, but the mean levels of HER2 were (237.81 ± 10.7, 225.06 ± 8.18 ng/ml) in the pre and postmenopausal status for the patient group, respectively.

Also, the results in Figure 3 show there is a significant increase (*P* value < 0.05) in the level of PD-L1 at both pre and postmenopausal patients compared with the pre and postmenopausal control group.

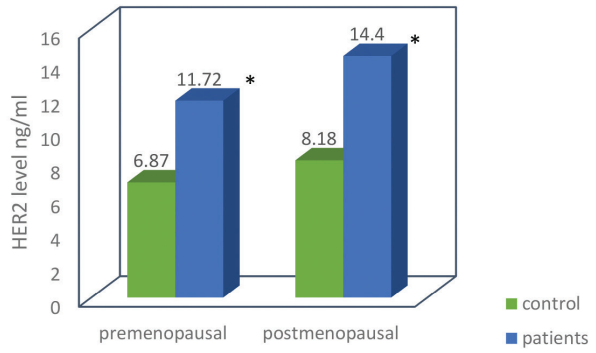

Fig. 1 **HER2 level at pre and postmenopausal status for control and patient group. *Significant at *P* < 0.05.**

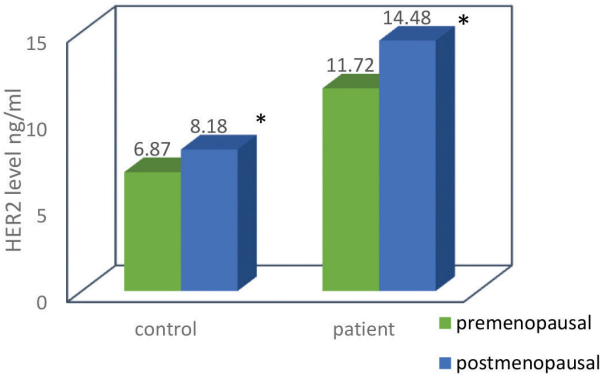

Fig. 2 **HER2 level at menopausal status for control and patients. *Significant at *P* < 0.05.**

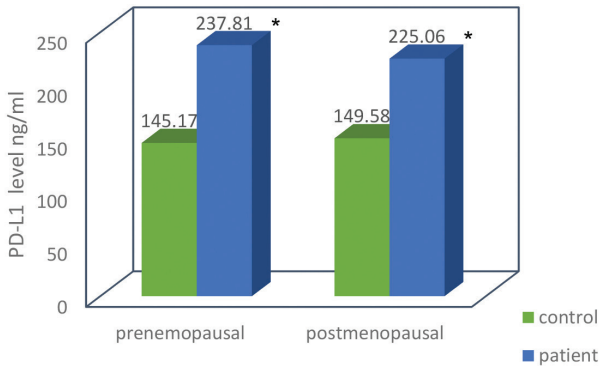

Fig. 3 **PD-L1 level at pre and postmenopausal status for control and patient group. *Significant at *P* < 0.05.**

Figure 4 showed that the PD-L1 level had not changes at menopausal status for both patients and control group.

Effect of the Menopausal Status at Antioxidant Level

The results in Table 1 demonstrated that levels of T-AOC, arylesterase and uric acid was significantly decreased ($P < 0.05$) in both pre and postmenopausal patients compared with the pre and postmenopausal control group.

The result from Table 1 also show there is a significant reduction in the levels of antioxidant parameters at postmenopausal status compared with premenopausal status for both control and patient group.

Effect of the Menopausal Status at Oxidant level

The result in Table 2 showed there is a significant elevation ($P < 0.05$) in the level of oxidant parameters (MDA, peroxidase, lactoperoxidase, and iron) in patients serum comparing with the control at pre and postmenopausal status.

This results also clarified elevation of the oxidative stress at postmenopausal status for both patients and control group.

Discussion

The second most frequent cancer in women is breast cancer. In our study, HER2 level was significantly increased in the patients compared with the control group at both pre and postmenopausal status, these results were in agreement with Banys-Paluchowski., et al. and Fabricio et al., which indicate that the serum HER2 level was elevated at those who are HER2 positive breast cancer (+3 score),[31,32] which consider as an indicator for the progression and recurrence of the disease.[33]

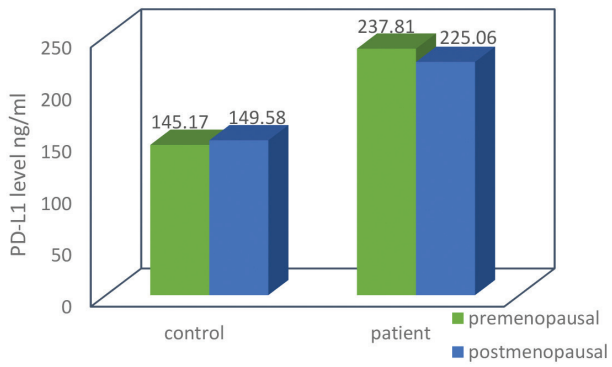

Fig. 4 **PD-L1 level at menopausal status for control and patient.**

In the same as with our result, Di Gioia et al. demonstrate elevated HER2 levels at postmenopausal status.[34]

The high level of HER2 at postmenopausal status causes an increased level of both follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) which they are boosted cell differentiation and proliferation, causing an increase in the risk factor of breast cancer.[4,35]

The high level of HER2 in postmenopausal breast cancer patients also consider as an indicator of the probability of metastatic.[36,37]

PD-L1 is an immune checkpoint and it is overexpressed on the surface of the cancer cell to evade anti-tumor immune responses by interacting with it a is a receptor (PD-1) at the surface of the T-cell, and then inhibit T-cell function.[38,39] And that explains the high level of PD-L1 in the serum of breast cancer patients compared with control.

Salama et al. and Han et al.[40,41] illustrated that the PD-L1 level do not effects by menopausal status and that agrees with our results.

The low level of the thyroid hormone in patients with breast cancer[4] causes increased oxidative stress by the reduction of the level of the total antioxidant capacity[42] and elevation of MDA, peroxidase, lactoperoxidase and uric acid.[43] This agrees with our results.

In our present study, the low level of T-AOC, aryleaterase and uric acid in patients' serum is in agrees with former studies,[44] which indicate to increase the oxidative stress in breast cancer patients.

At postmenopausal status the low level of estrogen which have antioxidant characteristic causes reduction in the levels of antioxidant parameters and then increase breast cancer risk factor.[45]

Also, the high level of iron makes it susceptible to Fenton reaction and then increase the level of reactive oxygen species.[46] The high level of reactive oxygen species causes lipid

Table 1. **The level of antioxidants in the patient and control according to menopausal status**

Clinical parameters	Premenopausal Control Mean ± SE	Premenopausal Patient Mean ± SE	*P*-value	Postmenopausal Control Mean ± SE	Postmenopausal Patient Mean ± SE	*P*-value
T-AOC µmol/ml	0.82 ± 0.04	0.70 ± 0.03	<0.05	0.64 ± 0.03	0.48 ± 0.02	<0.05
Arylesterase U/L	98.69 ± 3.30	59.51 ± 2.13	<0.05	78.10 ± 2.81	39.87 ± 2.40	<0.05
Uric acid mg/dl	4.44 ± 0.21	3.21 ± 0.20	<0.05	3.79 ± 0.19	2.34 ± 0.15	<0.05

Table 2. **The level of oxidants in the patient and control according to menopausal status**

Clinical parameters	Premenopausal Control Mean ± SE	Premenopausal Patient Mean ± SE	*P*-value	Postmenopausal Control Mean ± SE	Postmenopausal Patient Mean ± SE	*P*-value
MDA µmol/L	12.24 ± 0.78	26.01 ± 1.30	<0.05	18.68 ± 0.84	34.96 ± 1.54	<0.05
Peroxidase U/L	77.49 ± 3.60	142.96 ± 5.70	<0.05	95.32 ± 3.71	159.27 ± 8.03	<0.05
Lactoperoxidase U/ml	46.26 ± 1.56	79.00 ± 2.63	<0.05	57.60 ± 2.28	109.50 ± 3.52	<0.05
Iron µg/dl	66.28 ± 2.5	84.35 ± 2.8	<0.05	76.75 ± 3.82	110.78 ± 4.16	<0.05

peroxidation, hence destruction of cell membranes. MDA (the end product of lipid peroxidation) is reflecting the level of oxidative stress.

The high levels of MDA, peroxidase, lactoperoxidase and iron in the serum of breast cancer patients is agreeing with previous studies.[47]

The reduction of arylesterase level and elevation of peroxidase and lactoperoxidase levels reflect the imbalance in oxidant-antioxidant mechanism, which in result increase breast cancer risk factors at menopausal status.

Increase oxidative stress in patients with breast cancer causes activation of the number of transcription factors and then lead to expression of the genes that include growth factor, inflammatory cytokines, chemokines and cell cycle regulatory molecules,[48] and then result in harm to DNA and impede signal transduction pathways responsible for cell proliferation, apoptosis, and angiogenesis.[49]

Conclusion and Recommendation

1. Elevation in the levels of HER2, PD-L1, MDA, peroxidase, lactoperoxidase and iron in the serum of breast cancer patients compared with the control. whereas the level of T-AOC, arylesterase, and uric acid was demotion in the serum of the patients compared with the control groups at the same menopausal status.
2. Elevated HER2 and oxidative stress at postmenopausal women increase the risk factor of breast cancer.
3. Due to the low cost and ease to done we can use the serum level of HER2 and PD-L1 as a predictor of breast cancer incidence and recurrence.

Conflict of Interest

The authors declare that no conflict of interest exists.

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