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Biochemical Variables in the Blood Serum of Women with
BreastCancer in Kirkuk Governorate.**

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Evaluation of CA15-3 Level with Some Immunological and Biochemical Variables in the Blood Serum of Women with Breast Cancer in Kirkuk Governorate.

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Abstract

Samples for the current research were collected from women with cancer in the second stage of infection with breast cancer who were taking two doses of chemotherapy in Kirkuk Governorate for the period from (January to the end of February) of the year 2024. 80 blood samples, whose ages ranged between (25-45) years, were collected from clinic visits. . External samples were divided into two groups:

- Patients group: It included (50) samples of blood from women with breast cancer with treatment 2 doses of chemotherapy.
- Control group: It included (30) blood samples from healthy women.

Then, blood was collected from a group of patients and healthy women and was separated by a centrifuge. Then the studied variables were measured, which included (cancer antigen- CA15-3 , interleukin-10- IL-10 , CXCL12, calcium, zinc, iron, estrogen, progesterone, and Xanthine oxidase). Current research results showed a significant elevated in each of the levels (cancer antigen- CA15-3, interleukin-10, CXCL12, calcium, iron, Estrogen, progesterone, and Xanthine oxidase) also the result

showed a significant decrease in progesterone hormone and zinc level in blood serum of patients with B.C simile to the healthy women .

Keywords: Breast cancer, interleukin 10, progesterone and estrogen, mineral

1-Introduction

Cancer is one of the diseases resulting from abnormal growth of cells and thus leads to the formation of cells that do not obey the normal rules of cell division. In addition, cancer is a disease in which it is difficult to control cell proliferation ⁽¹⁾. Cancer, as it is known, is an umbrella term for more than 100 unique types of malignant tumors in various tissues throughout the human body .Breast cancer is one of the diseases that results in cell division, and these cells can spread to different parts of the body. More than 1,668 cases were diagnosed in Baghdad Governorate in 2018 for breast cancer patients. (3).

The cancer antigen CA15-3 is a protein that is a natural product of breast tissue. In the event of a cancerous tumor in the breast, the concentration of CA15-3 may rise as the number of cancer cells in the body increases .In many patients with breast cancer, there is an increase in CA15. -3 When it passes into the bloodstream, it is identified, which makes it useful as a tumor marker to monitor tumor development. The normal level of CA 15-3 is less than 25 units/cm³. It is elevated with tumors, diseases, or other conditions, such as colon tumor, rectal tumor. Lung tumor, hepatitis, and benign breast diseases ⁽⁴⁾.

IL-10 is an essential cytokine for regulating lymphatic homeostasis. These cytokines stimulate similar responses from lymphocytes, but play markedly divergent roles in lymphatic biology in vivo. It is an anti-inflammatory cytokine that regulates the immune response that IL-10 expression in metastatic cancer cells can regulate the function of cell-mediated inflammatory responses. IL-10 can be considered a potential biomarker for the prediction and prognosis of human cancers ⁽⁵⁾.

Stromal cell-derived factor-1 (SDF1), also known as CXCL12, is a biomarker for the diagnosis of breast cancer. In addition to high expression of CXCL12, it is positively related to estrogen receptor-positive status, human epidermal growth factor receptor-negative status, and small body size. Tumor (6) Primarily by bone marrow stromal cells, it has thus been named stromal cell-derived factor-1 (SDF-1) . On the other hand, calcium has been found to be related to breast cancer, as it was found that the cancer cells themselves affect calcium metabolism, leading to an increase in its levels in the

blood. Also it has been found that zinc, which is one of the minerals necessary for growth and has a relationship with breast cancer, must be available in food or nutritional supplements to reduce the incidence of chronic diseases, including cancer ⁽⁷⁾. It has been found that zinc has an immune function linked to cellular signaling pathways, which provides an important role for zinc in cancer patients, as its function lies in controlling tumors ⁽⁸⁾.

Through the high level of immune variables and minerals, the aim of current research is detremantion the level of cancer antigen CA15-3 along with some immune and biochemical variables in the serum of patients with breast cancer in Kirkuk Governorate.

2-Materials and Methods

1-2-Collection of specimens

The samples for the current research were collected from women in Kirkuk Governorate for the period from (January to the end of February) of the year 2024. 80 samples, whose ages ranged between (25-45) years, were collected from visits to outpatient clinics, and the samples were divided into two groups:

- Patients group: It included (50) samples of blood from women with breast cancer-B.C with treatment 2 doses of chemotherapy.
- Control group: It included (30) blood samples from healthy women.

After that, blood was collected from both groups (patients and healthy people) and separated using a centrifuge. Then the biochemical and immunological variables were measured, which included (CA15-3, IL-10, CXCL12 , Ca , Zn , Iron , estrogen , progesterone , Xanthine oxidase).

2-2-Estimation the level of CA15-3 in a group of patients and healthy people

The Sandwich ELISA method was used as a method to measure the level of CA15-3, using a measurement kit designated for them and from the Chinese company Sun Long Biotech

2-3-Estimation the levels of (IL-10, CXCL12, estrogen, progesterone, and Xanthine oxidase in a group of patients and healthy people)

The level of inflammatory cytokines (IL-10, CXCL12), and the level estrogen and progesterone hormone , and Xanthine oxidase were estimated, According to the ELISA

Sandwich approach, the ELISA technique was utilized to measure the quantity of variables. from the Chinese company (Melsin Medical).

2-4-Estimation of calcium concentration in a group of patients and healthy people

The calcium level was estimated according to a kit prepared by ASSEL S.R.I an Italian company.

2-5-Estimation of zinc concentration in blood serum in a group of patients and healthy controls

A concentration of zinc in the serum of blood was estimated using a diagnostic kit prepared by Biovision.

2-6-Estimation of iron concentration in a group of patients and healthy people

The iron level was calculated using a colorimetric method that converts trivalent iron ions into ferric ions in a weakly acidic medium. It forms a colored complex of the iron (II) ion with Ferrozine ⁽⁹⁾.

2-7-analysis Statistic

SPSS statistical program was used to find a mean \pm SD. The averages were also determined for a group of patients with B.C compared to the (healthy people) using a T-test and at the probability level ($P \leq 0.001$).

4-Results and Discussion

4-1Estimation of levels of immunological and biochemical variables for samples studied in both groups:

1-The table below shows mean \pm S.D of the immunological and physiological parameters for samples studied in both groups.

| Groups | Mean \pm SD | |
|-----------------------|------------------------------------|-------------------------------------|
| | Control n=30 | Patients n=50 |
| Parameter | | |
| CA15-3 (U/ml) | 8.01\pm0.47 | 34.24\pm10.92 |
| IL-10 (Pg/ml) | 139.70\pm35.51 | 257.52\pm79.93 |
| CXCL12 (Pg/ml) | 154.48\pm75.22 | 693.04\pm233.13 |

| | | |
|-----------------------------|------------------------|-----------------------|
| Ca (mg/dl) | 7.86 ± 0.49 | 12.16 ± 1.22 |
| Zn (mg/dl) | 67.94 ± 4.36 | 45.10 ± 4.27 |
| Iron (µmol/L) | 140.95±25.13 | 195.11±43.21 |
| Estrogen (pg/ml) | 235.512± 50.231 | 145.678±30.412 |
| Progesterone (pg/ml) | 0.412±0.0561 | 1.043±0.302 |
| XO ng/ml | 5.714±1.123 | 10.231±2.421 |

P ≤ 0.001

The results of present study showed a significant rise in each of the levels (CA15-3, IL-10, CXCL12, Ca, Iron, xanthine oxidase, progesterone) and a significant decrease in the concentration of estrogen and zinc level in blood serum of patients with women infected breast cancer simile control group at its level probability $P \leq 0.001$, as in the following figures.

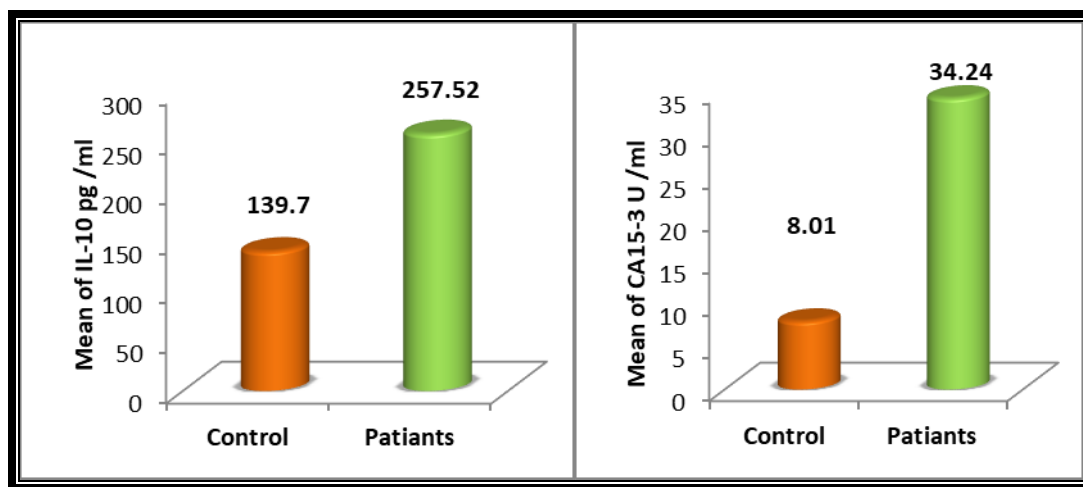
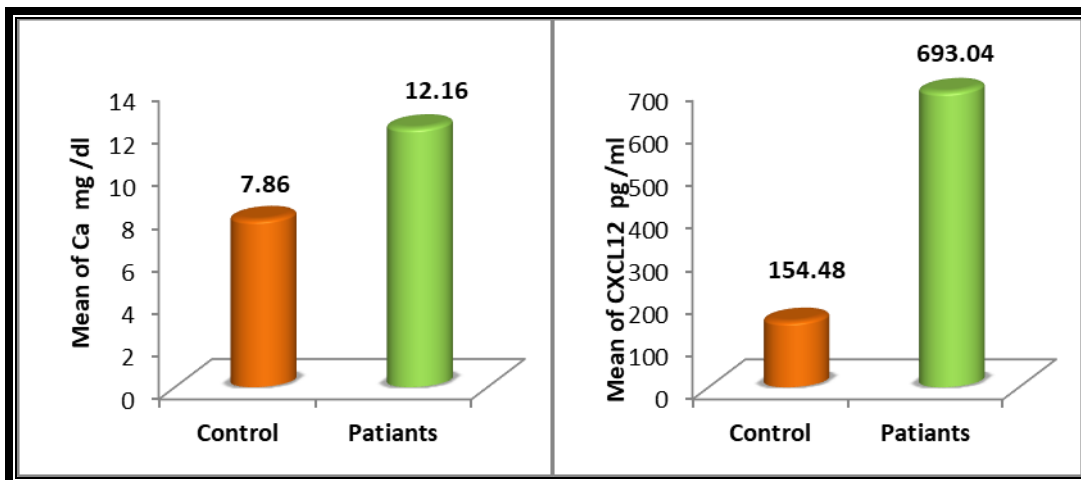


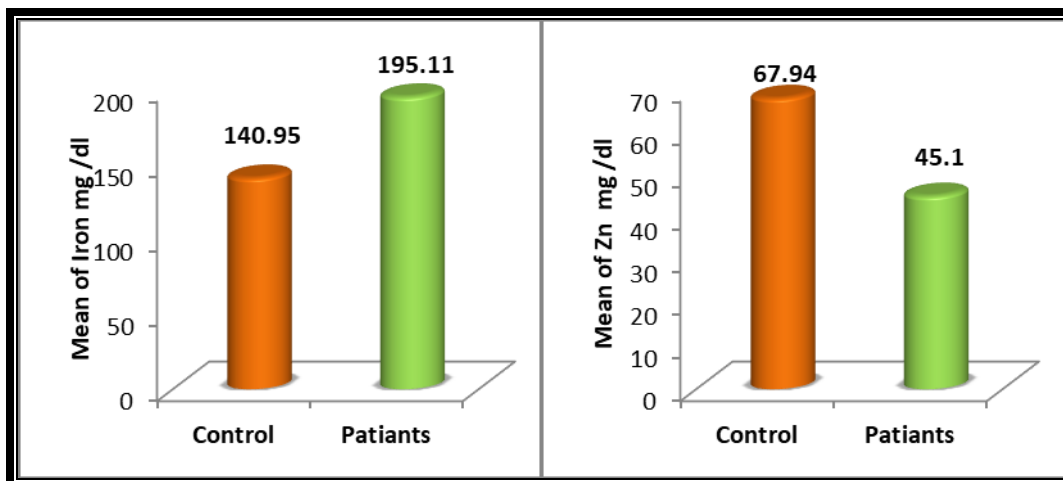
Fig (1):-Level of CA15-3 in all group

Fig (2):-Level of IL-10 in all group



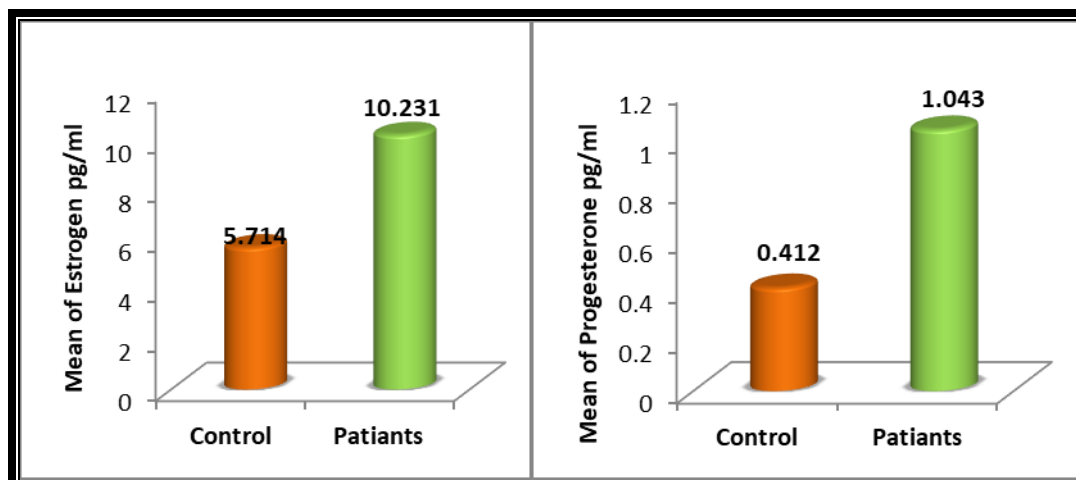
Fig(3):-Level of CXCL12 in all group

Fig(4):-Level of Ca in all group



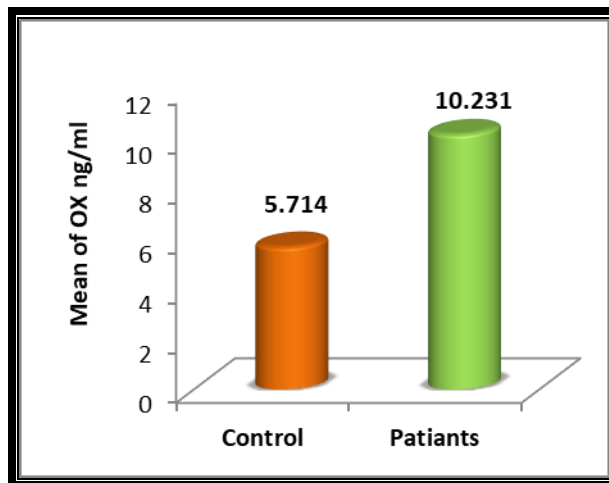
Fig(5):-Level of Zn in all group

Fig(6):-Level of Iron in all group



Fig(7):-Level of Estrogen in all group

Fig(8):-Level of progesteron in all group



Fig(8):-Level of XO in all grou

Discussion

Cancer antigen (CA15-3) is one of the important diagnostic variables for breast cancer. The results of the research are consistent with the results of ⁽¹⁰⁾ who indicated high levels of cancer antigen in patients suffering from breast cancer, as it is considered the most important diagnostic variable and can be used as one of the tumor markers, so it is a marker with high specificity and sensitivity for the disease, as most Studies have shown that it is a biochemical marker for breast cancer patients ⁽¹¹⁾.

As for IL-10, it is considered a cytokine that has been known for a long time in immunology, Particularly concerning its impact on T and B cells, not to mention that its absence results in the death of immature immune cells and that it is essential for the growth of both B and T cells. It's interesting to note that some research has strongly implied that IL-10 could have a function in immunology as well as potentially having a direct or indirect impact on cancer. ⁽¹²⁾, especially breast cancer, through its work to inhibit apoptosis and stimulate the formation of blood vessels. In tumors. IL-10 is an anti-inflammatory cytokine and can inhibit inflammatory responses by antagonizing co-stimulatory molecules expressed in APC. In addition, IL-10 may contribute to the development of breast cancer ⁽¹³⁾.

In addition, the concentration of the chemokine CXCL12 increased in female patients simle to a healthy women, as results of a this study agree with a findings of Emilia ⁽¹⁴⁾, Marina ⁽¹⁵⁾, and Dinesh ⁽¹⁶⁾. In their study, they confirmed the elevated concentration of chemokines in patients with breast cancer, especially the chemokine CXCL12, which

regulates breast tumor growth and enhances a entry of cancer cells by increasing blood vessel permeability and expanding leaky tumor blood vessels. Overexpression of CXCL12 by breast cancer cells can promote in vivo invasion and recruitment of macrophages to the underlying tumor. CXCL12 overexpression also leads to increased microvascular density, which may also be mediated by Connective tissues are associated with the tumor and contribute to changing tumor architecture ⁽¹⁷⁾.

As for calcium, it showed a significant increase, as our results agree with Hassan (2011) ⁽¹⁸⁾, who discovered that breast cancer patients had higher Ca concentrations than those in the control healthy. The majority of observational studies assessing dietary calcium intake provide evidence for the preventive effect of calcium against breast cancer⁽¹⁹⁾. Controlled trials revealed that Ca supplementation did not lower postmenopausal women's overall risk of benign proliferative breast disease, which is a precursor to breast cancer. ⁽²⁰⁾. It has been demonstrated that circulating calcium, which is involved in numerous biological functions, can support the hypothesis that Ca protects against breast cancer, is inversely associated with breast cancer risk. Increased cellular calcium levels after an rise in serum calcium may account for this protective effect. Serum calcium may have an impact on several cellular processes, such as the cell cycle and cell death. ⁽²¹⁾.

As for zinc, it showed a significant decrease, as the results agree with the results of Arooj and others (2012) ⁽²²⁾, .They found that zinc levels decreased in women with B.C compared to healthy group. Zinc deficiency can be linked with malignant tumors ⁽²³⁾, and precise function of zinc in cancer ⁽³⁹⁾. However, zinc is known to be essential for over 100 different metabolic role ⁽²⁴⁾. It is required for DNA synthesis by altering binding of histones F and F3 to DNA to affect RNA synthesis ⁽²⁵⁾ Whereas Zn deficiency and Zn supplements indicate inhibition and stimulation responses to tumor growth, adding to this funaction of zinc in human cancer. It turns out that there is a significant decrease in the concentration of zinc in the serum of women with breast cancer compared to the control group. This low in zinc concentration can be explained by an elevated demand on cancerous tissue due to increased cellular uptake and enzymatic activity by tumors ⁽²⁶⁾.

In addition, iron showed a significant rise in the group of patients, as its results are consistent with the findings of Rozoqi ⁽²⁷⁾ and Salih ⁽²⁸⁾, who showed in their study a higher concentration of iron in patients with breast cancer simile to the healthy women . One indicator of cancer, according to studies, is an abnormal iron balance. Cancer cells

require a lot more iron than regular cells do because they have greater metabolic and reproductive rates than normal cells. This increases oxidative stress in the cells. Moreover, concurrent modulation of antioxidant defenses by cancerous cells may be necessary for their survival. This regulation may include increased expression of several antioxidant genes and the activation of antioxidant transcription factors. ⁽²⁹⁾.

Increased iron metabolism depletes intracellular iron stores by either employing iron-chelating compounds or by mimicking self-regulatory mechanisms, including microRNAs, and is linked to malignant transformation, cancer growth, and medication resistance. Furthermore, hepatitis, a virus that can be generated in cancer cells and provide an alternate anti-cancer strategy, can be brought on by iron overload and result in controlled cell death ⁽³⁰⁾.

Also a results showed a reduction in estrogen hormone in the sera of women patients, and this may be due to the treatment. It was found that a subgroup of patients with a high concentration of estrogen receptor protein and HER2 negative benefited from the drug tamoxifen, which may lead to a decrease in total cholesterol, which is the main source of estrogen ⁽³¹⁾. Therefore, estrogen receptor status may be altered in 5% of chemotherapy groups. Ghufran ⁽³²⁾ also indicate that there was an rise in estrogen level before treatment, but it decreased after radiotherapy. The results of a study are also consistent with a results of Wassan ⁽³³⁾, and Mousa ⁽³⁴⁾. In their study, they showed an increase in the level of progesterone in sera of patients with B.C compared with the healthy women, as a reason for the increase is due to the changes that occur in the secretory phase preceding the menstrual cycle, and periodic changes occur in the lining of the uterus and cervix, while the follicle-stimulating hormones and estradiol regulate the secretion of progesterone in a way. Indirectly, it increases luteinizing hormone receptors on ovarian cells responsible for secreting progesterone ^(35,36).

On the other hand, it was found through results of current research that there was elevated in the levels of xanthine oxidase, as results of study agree with Thamers results ⁽³⁷⁾, who indicated an increase in the effectiveness of the enzyme thymine oxidase. Therefore, the reason for the increase in the enzyme may be the result of an imbalance of redox and oxidation in the cells that occurs as a result of Oxidative stress is found in many cancer cells compared to normal cells, and thus the imbalance of oxidative stress may be related to the stimulation of tumors ⁽³⁸⁾ The research results of Ismail et al. demonstrated that surface cell markers CD86/CD80 play an active role in the development of certain types of cancers, including brain cancer. ⁽³⁹⁾ Nanoparticles can

also be used on MCF-7 breast cancer cell lines, similar to zinc oxide (ZnO) nanoparticles, which have shown clear results on cancer cells. ⁽⁴⁰⁾

Conclusion :- It is concluded from the results of the current research that breast cancer is one of the types of cancers widespread in the world and causes death for many women, as it was found to have a correlation with inflammatory cytokines, including interleukin 10 and chemokines, as increasing their levels may be considered a diagnostic variable for B.Cr, in addition to the level of hormones. The female sex hormones, including progesterone and estrogen, have a relationship with breast cancer, in addition to the activity of the enzyme xanthine oxidase, which may be an important diagnostic indicator for the development of the disease.

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