Evaluation of Fetuin-A and Antioxidant Levels in Breast Cancer Patients

Thamer Karim Jumaa Al-Saeedi, Abdulsalam Tawfeeq Salih Alsamarai, Ahmed Zuhair Al-Samarrai

Abstract

The current study was conducted on (90) samples from a sample of women with breast disease. And G2 included 30 samples of breast-infected women who used the treatment. And the control group (the healthy ones) had (30) blood samples. The ages of the groups ranged between (25-74) years, as pictures were collected from the cancerous tumors hospital of a city in Baghdad governorate, between 1/1/2022 to 1/1/2023. after which the serum was obtained, then it was placed in small test tubes and kept in the refrigerator at a temperature of -20 ° C for the purpose of measuring the levels of Fetuin -A, antioxidants and some minerals to know their effect on patients with breast cancer.

The results of the current research showed that there was a significant increase in the blood serum of group G1, with a decrease in the serum of group G2 in the serum of Fetuin -A patients compared with the control group, with a significant decrease in the level of glutathione in the serum of all groups. While the level of malondialdehyde increased significantly ($P \leq 0.05$) in the serum of group G1 and G2 compared with healthy subjects, and calcium showed no significant differences in the serum of all groups.

Key words / Breast cancer , Fetuin -A, antioxidant , Calcium.

Introduction

Breast cancer is a life-threatening disease that may affect a woman's sense of self-respect, self-love, and her sense of femininity [(1)]. Breast cancer is the most common non-cutaneous malignant tumor [(2)]. Which carries milk to the nipple [(3)], it occurs in women and men, but the percentage of infection in women is very large compared to males, as every 200 infection in women corresponds to one infection in men [(4).]

Breast cancer is a group of diseases that result in lumping as a result of abnormal changes and divisions in the cells of the breast tissue [(5)]. It is a multifaceted disease with both environmental and genetic causes and shows a large degree of heterogeneity between and within tumors [(6)]. Breast cancers most often begin in the milk glands (lobules) or in the ducts that connect the lobules to the nipple [(8,7)].

No symptoms appear in the first period of breast cancer through which breast cancer can be distinguished, because the tumor is small, but when the size of the tumor grows, it can be felt through (its size) [(9)], a change in the skin of the breast and fluid secretion from the nipple [(10)], And among the factors that develop breast cancer is a change in the sequence of nitrogenous bases, or it may be genetic reasons, but there is a mutation of 5-10 that is inherited from the parents, or the mutation may be a result of aging or the result of surrounding factors, and these factors can cause genetic abnormalities in the cell [(11)].

Fetuin -A is a glycoprotein that belongs to the cysteine superfamily of protease inhibitors [(12)]. As it has a molecular weight

of 52 kDa, it is synthesized mostly by the liver and is later excreted into the bloodstream. High amounts of Fetuin were described in fetal calf serum and showed that its concentration decreases with the age of the animal [31].

Fetuin-A protein has a close relationship with the development of heart disease and arteries [14], and it was found to have a significant increase in patients with myeloma compared to healthy subjects [15]. As well as its relationship to Diabetes mellitus, Kidney, Cancer and inhibition of ectopic calcification [16]. Fetuin was also found to be associated with breast cancer [17].

Antioxidants have a role in breast cancer, as glutathione is involved in many metabolic processes, and its deficiency leads to cellular risk as a result of oxidative damage [18]. It was found that disturbances in the balance of the oxidized form and the reduced form of glutathione have an important role in tumor initiation and development, and may have an important role in the extent of response to treatment [19].

In addition, oxidative stress is the state in which the concentration of free radicals increases. Therefore, these radicals are very effective as they interact with the components of the cell, the most important of which are the nucleic acids that form an important part of the cell nucleus, leading to the oxidation of these acids and bringing about changes that may be repairable within the biological system [20]. Malone dialdehyde is an indicator of oxidative stress as well as the incidence of some diseases, as the MDA level rises in women with breast cancer, and it rises in patients after radiotherapy [21].

In addition, calcium may contribute to the formation of breast cancer, as it was found that the high level of calcium in postmenopausal women could lead to breast cancer [22]. Since the increase of Fetuin -A and oxidative stress in the human body may be threatening to the immune system and thus exposure to breast cancer, therefore, the current research aimed to evaluate the level of Fetuin -A and antioxidants in patients with breast cancer.

Material and Methods

Study Samples:

The current study was conducted on (90) blood samples, which were divided into three groups:

- **G1 included 30 samples from women with breast cancer.**
- **G2 included 30 samples of women with breast cancer who took a single dose of treatment.**
- **The control group (the healthy ones) had (30) blood samples.**

The ages of the groups ranged between (25-74) years, as the samples were collected from the cancerous tumors hospital affiliated to the Medical City in Baghdad Governorate, for the period between 1/1/2022 to 1/1/2023. Where he collected about 5 cm3 of blood from patients and healthy people, and the samples were divided according to the type of test, after placing them in Jell tubes with a tight cover and free of anticoagulant and leaving the blood at a temperature of 25 °C until it coagulated and then placed in a centrifuge for a period of time. 10 minutes at a speed of 3000 cycles / minute, after which the serum was obtained, then it was placed in small test tubes and kept in the refrigerator at a temperature of -20 ° C for the purpose of measuring the levels of antioxidants, Fetuin -A and some

minerals to know their effect on breast cancer.

-Estimation of Fetuin_A levels in the blood serum

The concentration of Fetuin_A in the serum was estimated through a ready-made assay kit. The ELISA technique is an enzyme-linked immunoassay in the solid phase based on the principle of competitive binding, as the holes of the plate covered with the Fetuin_A antibody are incubated with the Fetuin_A present in the serum and the Incubation DIluent (INC-BUF) in Room temperature with stirring. During the incubation period, a fixed amount of Fetuin_A conjugated with Fetuin_A competes in the serum of the sample to observe a specific number of binding sites on the antibody to Fetuin_A, and then we wash the pits, and after the washing step, detection is made The conjugate Fetuin_A with conjugate (ENZ-CONJ) and specimen Dilunet (SAM-DIL) The conjugation of ENZ-CONJ and the etch-bound SAM-DIL is gradually accompanied by an increase in LH concentration, and after waiting an hour the unbound ENZ-CONJ and SAM-DIL are removed and the pits are washed after Then a chromogenic solution is added and incubated at room temperature with stirring for 15 minutes, which leads to the appearance of a blue color, then the color turns yellow by adding a stop solution. A standard curve is obtained by plotting the concentration of the standard against the absorbance. The intensity will be The color is directly proportional to the amount of Fetuin_A in the sample.

-Estimation of GSH and MDA level in the blood serum

The level of antioxidants represented by (glutathione, malondialdehyde) was estimated, as the ELISA technique was used to measure the level of antioxidants according to the ELISA Sandwich technique, as the Microelisa plate for each was coated with the appropriate antibody (GSH.MDA), after which the standard solution or samples were added to Etch the appropriate Microelisa stripplate and conjugate it with the specific antibody, then add the glutathione-specific HRP-Conjugate reagent and Malone dialdehyde to each hole of the Microelisa plate well and incubate it, and then it is washed well and then add chromogen solution A, B the color of the liquid will turn blue. After adding the stop solution, the color turns yellow, and then the absorbance is measured at a wavelength of 450 nm, as the value of the intensity of the absorbance is proportional to the concentrations of glutathione and malondialdehyde in blood serum [23,24].

-Estimation of Calcium level in the blood serum

The concentration of calcium in blood serum was measured according to the ready-made assay kit by the Spanish company LiNER [25], as the method depends on the interaction of calcium with the red complex compound as a complex component in the basic solution Ocresol phtaleine in which we measure the concentration of calcium.

Statistical analysis:

The statistical program SPSS was used to analyze the results obtained, if the arithmetic mean and the standard deviation SD were used for the data under study, and the T-test was used to compare the biochemical variables between the two groups of patients and control at the level of probability $P \leq 0.05$.

Results and discussion:

- **Measurement of the levels of biochemical variables for the samples under study:**

Table (1): shows the mean - standard deviation of the biochemical variables of the samples under study

Parameters	Mean ±S.D			P
	Control	G1	G2	
Fetuin-A ng/ml	54.643±11.449c	103.605±14.434b	32.345±11.901b	≤0.05
GSH(ng/ml)	8.206±1.082a	3.812±0.430b	3.986±1.066b	≤0.05
MDA (ng/ml)	5.486±1.607c	13.962±2.956b	19.976±5.565a	≤0.05
Ca(mg/dl)	8.749±0.323a	8.022±0.319c	8.252±0.408b	≤0.05

Significant P ≤ 0.05

• The different letters indicate the presence of significant differences, but the similar ones, there are no significant differences.

• G1 refers to the group of women with breast cancer without a dose.

• G2 refers to the group of women diagnosed with breast cancer for a single dose.

-Estimation of the Fetuin-A in the blood serum

Table (1-1) shows the average ± standard deviation of the fatwain level, as it reached (54.643 ± 11.449) ng / ml in patients of the first group G1 and (103.605 ± 14.434) ng / ml in patients in group G2, compared to (127.140 ± 21.425) ng/ml in healthy women in the control group. The results showed that there was a significant increase in the level of fatwain in the blood serum of groups G1 and G2 compared to healthy women and at the level of probability (P≤ 0.05).

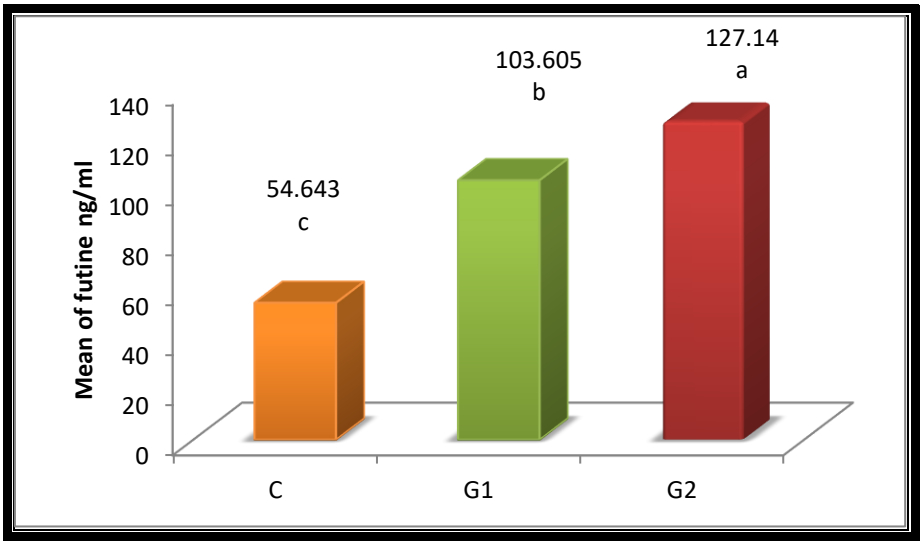

Figure (1): The level of the Fetuin-A in the blood serum of the samples under study

Cigdem (26) indicated in his study that there were no significant differences in the level of Fetuin in patients with breast cancer in the early stages of infection, so

he concluded in his study that he did not find any correlation between the levels of Fetuin and patients, and it was found that Fetuin -A is an important marker in the microenvironment tumorigenic CSCs and metalloproteinases [27, 28]. Fetuin-A was found to be a biomarker in the serum of colorectal cancer patients [29]. Moreover, a study conducted in Mexico in breast cancer patients found that autoantibodies in the blood against fetuin-A protein were useful as serum biomarkers for early-stage breast cancer screening [30]. So it appears that Fetuin-A is a chemoattractant in the blood that also promotes the invasion of breast cancer tumor cells [31].

On the other hand, a study in a rat model reported that deficiency in Fetuin -A may reduce PyMT transgenic breast cancer tumors by more than 60% [32]. Because Fetuin -A is an abundant serum protein, it is considered an important factor of metastasis in breast cancer in humans. It has been reported that Fetuin -A deficiency resulted in failure of breast tumor development in murine models [33].

- Estimation of GSH level in blood serum:

Table (1-1) shows that the mean ± standard deviation of the glutathione level was (3.812 ± 0.430) nanomoles / liter in patients with the first group (G1) and 3.986 ± 1.066 n mol / L in patients with the second group (G2), compared to (8.206 ± 1.082) nanomoles / L in healthy women, the results showed a significant decrease in the level of glutathione in the blood serum of groups G1, G2, compared to the healthy group, at a probability level (P≤0.05), as in Figure (2).

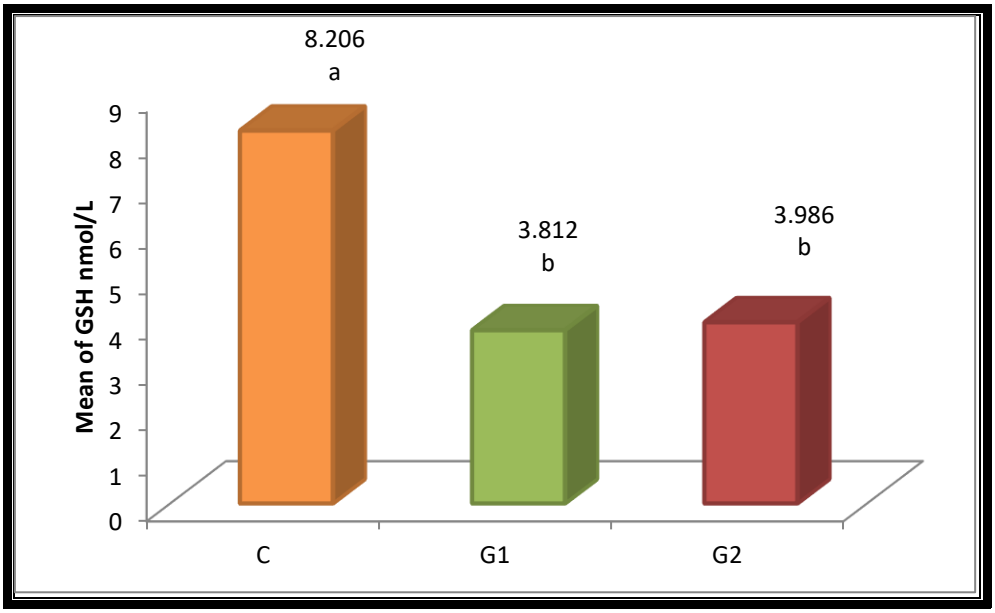

Figure (2): The average level of GSH in the blood of the samples under study

Through the results, we notice a decrease in the level of glutathione in patients with breast cancer, so the results of the current study agree with the findings of Enrico [34] and Luke [35]. In the forms of reduced glutathione and oxidized glutathione, the processes of glutathione synthesis, transport, utilization, and metabolism are tightly controlled to maintain intracellular glutathione and redox homeostasis [36]. As for cancer cells, they need a greater level of ROS than normal cells. To promote

excessive metabolism and reproduction [37, 38].

Fatemeh [39] indicated that patients with breast cancer who were treated with chemotherapy Adriamycin at a concentration of 60 mg / m2 and Cytoxan at a concentration of 600 mg / m2 showed that the results after 3 cycles of chemotherapy showed a decrease in the concentration of antioxidants, so it was found that chemotherapy in breast cancer patients It may lead to radical changes in the levels of the oxidation system / antioxidants in the body. The decrease in glutathione levels in group G1 and G2 is due to its depletion as a result of oxidative stress caused by cancer cells in G1 and due to chemotherapy in group G2.

On the other hand, it was found through the results also that there was a decrease in glutathione levels in patients with breast cancer as a result of taking treatment, as it was noted that there was a relationship between an increase in the level of glutathione and resistance to chemotherapy in many types of cancer [40]. A weakened antioxidant defense system, including glutathione, can sensitize cancer cells to current chemotherapy treatments. As well as a moderate decrease in the level of GSH would be an effective strategy to improve the sensitivity of cancer cells to chemotherapies. Therefore, depletion of cellular GSH in cancer cells will make them more vulnerable and sensitive to oxidative stress and chemotherapy. Cysteine insufficiency, glutamate insufficiency, or pharmacological and genetic inhibition of the system can also reduce the resistance of cancer cells to chemotherapies [41].

-Estimation of the MDA level in the blood serum

Table (1 shows that the mean ± standard deviation of the malondialdehyde level was (13.962 ± 2.956) µ mol / L in patients of the first group G1 and (19.976 ± 5.565) µ mol / L in patients of the second group G2, compared to (5.486 ± 1.607) µ mol/L in healthy women.

The results showed a significant increase in the level of malondialdehyde in the blood serum of groups G1 and G2, compared to the healthy group, at a probability level (P≤0.05), as in Figure (3).

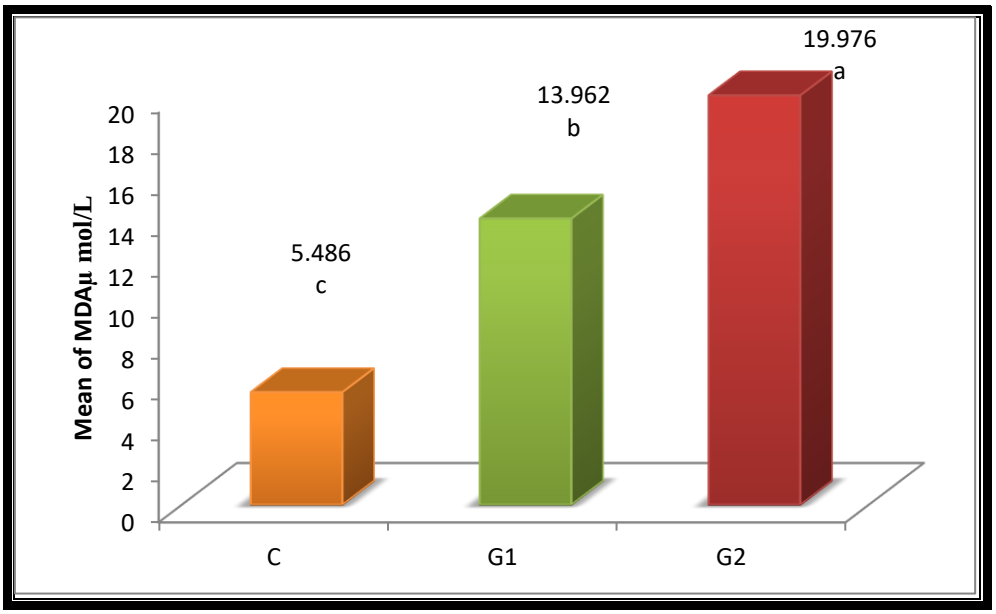

Figure (3): The level of MDA in the blood serum of the samples under study

The results showed a significant increase in the level of malondialdehyde in patients with breast cancer compared with the group of healthy women, as the results of the current study agree with the results of Lilo and his group [42] and Khalaf and his group [43] Nsaif, , who showed in their study a high level of MDA in the serum of breast cancer patients compared with the control group. Maher (saad et al., 2020) [44] showed that serum MDA levels are a marker of lipid peroxidation caused by oxidative stress, as it was found in his study that malondialdehyde levels were significantly lower in healthy subjects compared to breast cancer patients.

MDA is one of the types of oxidative stress that is a major cause of the initiation and development of breast cancer and is often associated with a high risk of cancer, as its level is elevated in patients with breast cancer. It is commonly associated with vital metabolism-boosting antioxidants [46,45]. Therefore, the results also agree with what Araz [47] said.

Oxidative stress is an important and major factor for the development of cancer, as it is associated with the generation of reactive oxygen species for the development of cancer, as cancer cells show high levels of ROS and a strong regulation of redox homeostasis to maintain a low level of oxidative stress [48]. On the other hand, the results showed a significant significant increase in the G2 group after taking the treatment, as the results of the current study agree with the results of Gupta and his group [49]. And those who showed in their study the high level of MDA in women with breast cancer after chemotherapy compared to a group of healthy women. While [50] indicated in his study that there was a decrease in the level of malondialdehyde during the different doses of treatment, including the first, second and third doses, and this is not consistent with the results of the current study.

The reason for the high level of MDA in group G2 may be attributed to the decomposition of the membranes of red blood cells as a result of the attack of unsaturated fats in them by free radicals. A study indicated that MDA levels increased in the serum of patients with breast and cervical cancer compared to healthy subjects. They attributed the reason for the increase in lipid peroxidation, which could be attributed to increased generation of reactive oxygen species or inhibition of the antioxidant defense mechanism in metabolically active tissues [51].

-Estimation of the Calcium level in the blood serum

Table (1) shows that the mean ± standard deviation of the calcium level was (8.022 ± 0.319) mg/dl in patients of the first group and 8.252 ± 0.408) mg/dl in patients of the second group (G2), compared to (8.749 ± 0.323) mg. / dl in healthy women, the results showed a significant decrease in the level of calcium in the blood serum of groups G1, G2 compared to the group of healthy women and at the level of probability ($P \leq 0.05$) as in Figure (4).

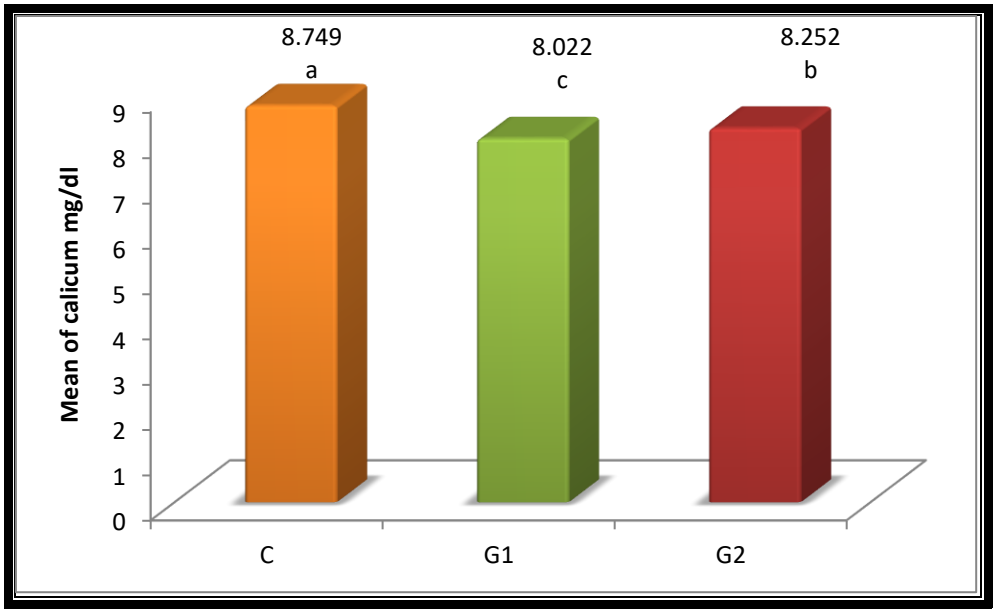

Figure (4) - The level of calcium in the blood of the samples under study.

It is clear from the above results that the level of calcium (Ca) decreased in women with breast cancer compared to the control group. The results of the current study agree with what the researchers Hutchison and Aziz [53,52] said that the low level of calcium in the blood of women with breast cancer confirms what the researchers said that calcium is inversely associated with breast cancer [52, 53]. This protective effect can be explained by By increasing cellular calcium levels after an elevated blood calcium level, which may affect multiple cellular processes including cell cycle and cell death.

The results of the current study do not agree with the results of Hassan [54], who found an increased level of calcium concentration in women with breast cancer compared to the control group. Jasim and others [55] indicated that the level of calcium is high in women with breast cancer, especially if the cancer spreads to the bones, causing osteoporosis and releasing calcium into the blood, where cancer cells secrete themselves like parathyroid hormone, which increases calcium absorption. And increase its concentration in the blood.

The higher calcium in the G2 group may be evidence of a protective effect of calcium against breast cancer mostly from observational studies evaluating dietary calcium intake [56]. Controlled trials showed that calcium supplementation did not reduce the overall risk of benign proliferative breast disease, a precursor to breast cancer, in postmenopausal women [57]. To confirm the proposed protective effect of calcium against breast cancer, it has been shown that circulating calcium, which is involved in many cellular processes [58].

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