Original Article

Evaluation of Superoxide Dismutase and Some Biochemical Parameters as a Vital Biomarker in Breast Cancer Patients

Nabaa Haider Abd Al-Latif[1 *], Firas Taher Maher[1], Nazar A. N. abed[2]

[1] Department of Chemistry, College of Science, Tikrit University, Tikrit 34001, Iraq

[2] College of Dentistry, Al-Mashreq University, Baghdad, Iraq

[*] Corresponding author: nabaa.h.abd@st.tu.edu.iq

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Abstract

Background: breast cancer is defined as the uncontrollable development of breast cells, second highest cause of cancer-related deaths in the world. **Objective:** estimate the activity of (SOD), levels of liver function (ALP, GOT and GPT), prolactin and the level of magnesium in the blood of women breast cancer with compared with control women. **Materials and Methods:** This study was conducted on 90 women (60 patients and 30 controls). Patients were referred to the Oncology Teaching Hospital/Medical City, during the period from 2023 to 2024. **Results:** significantly decrease ($P<0.01$) in the level of (SOD)the level of prolactin hormone at p-value ($P<0.05$) activity in BC patients comparison with healthy women, and significant elevation in the level of : (ALP) and (GPT)at ($P<0.01$) in BC patients comparison with control group while the levels of magnesium and(GOT) were appeared at the normal range $P>0.05$. **Conclusion:** The results showed a decrease in the level of SOD due to oxidative stress and negative changes in prolactin in breast cancer patients compared to the control group, with increased levels of GPT and ALP, which may indicate the presence of a malignant tumor. In contrast, no significant differences were observed in the levels of magnesium and GOT between patients and healthy individuals. This study is considered a vital indicator and a tool for diagnosing and detecting disease.

Keyword: Breast Cancer (BC), Super Oxide Dismutase (SOD), Glutamate oxaloacetate transferase(GOT), Glutamate pyruvate transferase (GPT), Alkaline phosphatase (ALP).

Introduction

Despite advanced treatment breakthroughs, breast cancer remains the second most common desease and lethal tumours in women [1], Breast cancer incidence has increased dramatically during the last four decades. [2] In 2020, there were about 2.3 million new cases of breast cancer and 6,85,000 deaths were recorded in worldwide, with the presence of geographic nations and regions[3]. Breast cancer is also known as a group of heterogeneous physiological and molecular diseases that start in the breast cancer. [4,5] The presence of cancer cells inside the body is often by early symptoms in the patient. Lump, skin changes, nipple discharge, swelling and discomfort in the breast, fatigue, insomnia, digestive problems, and shortness of breath [6,7]. Pathogenic treatment and prognosis evaluate according to the type and extent progression of the malignant tumor and the general health condition of the patient [8,9]. Surgery, chemotherapy, and radiotherapy are all possible setrategic selection. Unfortunately, only 14% of women with breast cancer recover here heath in five years. A recent methodology to cancer staging is the tumor-node-metastasis (TNM) system which presents a prognosis of the tumor depending on the developing of the primary tumor [10]. Stage ZERO cancer is the first stage that can be revealed. It is non-invasive

and benign, and the most common form is called ductal carcinoma in situ (DCIS), denoted to a highly treatable disease that is still confined to where the breast epithelium arises, Stage **I** is the early stage of invasive breast cancer, when the spreading of tumor is still relatively tiny. The size of tumor is 2 cm. The underarm lymph nodes are not affected. Stage **I** and **II** cancers are still described as early-stage cancers[1]. Stage (II) is also limited area of the breast, but it is larger than stage **I** cancers[2]. The tumor is 2 to 5 cm in size, the lymph nodes under the armpit may be affected [9]. Stage (**III**) has spread more extensively in the breast and is biggest than 5 cm areas surrounding the breast, such as fibers and skin, are also affected [3]. Stage (**IIV**) is the most advanced type of cancer and includes its spread around the organs such as the lungs, liver, bones, and other organs, and has no specific size [9].

Superoxide dismutase SOD: It is an enzyme that contribute in the scavenging of superoxide free radicals into ordinary molecular oxygen (O_2) or hydrogen peroxide H_2O_2 [4]. Superoxide is yielded as a byproduct of oxygen metabolism and, if not regulated, leads to numerous kinds of cell damage. Hydrogen peroxide is also harmful and dissociation by other enzymes like catalase[5]. Magnesium $Mg^{2+}$is the fourth most abundant element in the human body ($Ca^{2+}>K^{+}>Na^{+}>Mg^{2+}$) and the second most abundant cation in the body's cells, after potassium. where it participates with calcium and phosphorus in the formation of the skeleton, as well as muscles and soft tissues [9]. Studies have shown alterations in mineral metabolism in breast cancer patients, such as magnesium. [11] These patients had reduced plasma magnesium levels. However, it is vital to note that metal concentrations vary amongst cells depending on the stage of cancer growth. Low magnesium concentrations in the blood were found to be negatively associated with symptoms of oxidative stress in breast cancer patients. [12]

Alkaline phosphatase (ALP) (EC 3.1.3.1) is a hydrolase enzyme that delete of phosphate groups from various compounds, such as nucleotides, proteins, and alkaloids [13]. The high level of ALP may be due to a physiological or pathological cause. Increased of blood Alkaline phosphatase levels are bounded with bile obstruction[14], cholestasis[15], liver disease[16], hepatitis[17,18], and malignancy. Patients with primary and metastatic liver and bone cancers, including colon cancer[19], rectal cancer [20] malignant neoplasms, and breast cancer with bone and liver involvement, have higher serum alkaline phosphatase levels. The determination of alkaline phosphatase activity can help with cancer diagnosis and treatment. [21]

There are transaminase enzymes in liver cells. They consist of an aspartate aminotrans-ferase(GOT) and alanine aminotransferase (ALT) [22]. The concentration of Alkaline phos-phatase and alanine aminotransferase is highest in liver cells and in very low concentrations in any other tissue. These enzymes are employed as biomarkers which may be predict the course of the disease in various malignant tumors such as the lung, colon, pancreatic, breast, and kidney[23,24]. The serum GOT/GPT ratio was first used as a disease assessment tool in viral hepatitis research. In recent years, the GOT/GPT ratio has been identified as a biomarker capable of predicting prognosis in renal cell cancer[25] carcinoma. It has been suggested that GOT/GPT may be linked to anaerobic glycolysis. Glucose metabolism has also been linked to urothelial carcinoma (UC), whereas the GOT/GPT ratio has been linked to prognosis in urinary tract cancer. Upper urinary tract cancer [26]revealed

that the pre-treatment GOT/GPT ratio rose statistically considerably from TNM staging of breast cancer and may be an independent prognostic factor for breast cancer. Prolactin (PRL) is a tertiary female sex hormone and a polypeptide hormone secreted mainly by lactotroph cells in the anterior pituitary gland, and it has a major role in a large number of biological processes. [11,12]. Studies demonstrated that prolactin stimulates cell division, activates receptors, and binds to specific ribonucleic acid (RNA) sites. These actions stimulate the transcription of structural genes that produce the synthesis of specific proteins, changing the activity of the cell to turn it into a tumor [27]As a result, prolactin plays a prominent role in the proliferation of cancer cells. The role of prolactin in the pathogenesis of breast cancer is receiving attention prolactin affects the original cell or nearby cells in an autocrine/paracrine and prolactin receptor-mediated manner in the human breast, which can promote DNA synthesis, epithelial cell proliferation, and the generation of breast milk. [13] This study aims to estimate the activity of (SOD), levels of liver function (ALP, GOT and GPT), prolactin and the level of magnesium in the blood of women breast cancer with compared with control women.

Materials and Methods

Collection and preservation of blood Samples:

Blood samples were collected at the Oncology Teaching Hospital/Medical City in Baghdad. From December 1, 2023 until the end of January 2024. Sixty samples were assembled from women with breast cancer and thirty samples from healthy individuals. Using a plastic syringe, approximately ten milliliters of blood was drawn from the vein. The samples were divided into groups, centrifuged at 3500 revolutions for ten minutes, and the samples were stored at -20°C.

Estimation of superoxide dismutase activity:

The activity of superoxide dismutase was evaluated by modified photochemical nitro blue tetrazoline method (NBT) this method including inhibiting of SOD activity by sodium cyanide and the activity of SOD followed by variation of optical activity of Formazine that formed by reducing O2 for NBT dye at 560 nm according to the following equation:

$$\text{SOD activity} = \frac{A0-A1}{A0} \div 50\% \times \frac{System\ Volume}{Sample\ Volume} \times Dilution\ Factor$$

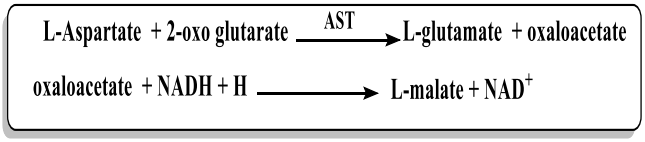
Estimation of alkaline phosphatase (ALP) activity:

The activity of superoxide dismutase was measured by liberating phenol inpresence of 4-aminoantipyrine and potassium ferricynide by randox GOD APA – USA kit at 510 nm according to the following equation:

ALP (IU/L) = (Abs/min) Assay/ (Δ Abs/min) Calibration) × calibrator Concentration

Estimation of Glutamate-Oxaloacetate aminotransferase (sGOT) activity:

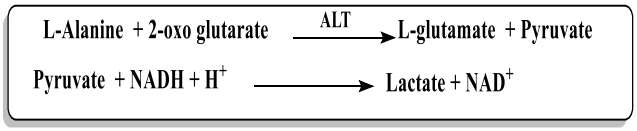
GOT enzyme catalyzing transaminase from aspartate into oxoglutarate to afford glutamate and oxaloacetate, then oxaloacetate converted into L-malate by NADH at 340 nm, according to the following equations:



Estimation of Glutamate – Pyruvate aminotranseferase (ALT) activity:

AST enzyme catalyzing transaminase from L-alanine into 2- oxoglutarate to afford glutamate and pyruvate , then pyruvate converted into Lactate by lactate dehydrogenase (LDH) and NADH the reaction monitored by decreasing of

NADH absorbence at 340 nm . according to the following equations:



Estimation of prolactine hormones (PRL):

The level of prolactine hormone was estimated by immunosorbent assay (ELISA)solide phase enzyme –linked at 450 nm.

Estimation of the concentration of magnesium in the blood serum:

Magnesium ion reacted with xilidil blue in alkaline medium to produce chelating complexes dissolving in water with purple color, the color intensity depending on the magnesium concentration, the level of Mg^{++} monitored at 512 nm and calculated according to the following equation:

$$Magnesium\left(\frac{mg}{dl}\right) = \frac{Abs\ Sample}{Abs\ Standard} \times Standard\ Value\ (2.5\frac{mg}{dl})$$

Statistical analysis:

The statistical analysis was performed by utilizing the statistical program (MINI TAP-17) and the analysis of variance test (T-test) was used to clarify the difference between the arithmetic means of the groups included in the study and to determine the significant differences between them at a probability level of $P \geq 0.01$ or $P \geq 0.05$.

Ethical Approval

The College of Science at the Tikrit University ethical committee approved this study's ethical approval, obtaining verbal consent from each patient and control. A local ethics committee reviewed and approved the subject information and consent form.

Results

This study including measuring the level of superoxide dismutase (SOD), levels of liver function (alkaline phosphatase (ALP), glutamate -oxaloacetate aminotransferase (GOT) and Glutamate-pyruvate aminotransferase (GPT), prolactin (PRL) and the level of magnesium in the blood of women with breast cancer compared with control women. as recording in the following Table:

Table 1: SD ± Mean and P-Value of (ALP, GOT, GPT) and magnesium in women with breast cancer and the control group

Groups (NO.)	SD±Mean					
	SOD U/L	ALP U/L	G.P.T U/L	G.O.T U/L	Prolactin PRL (ng/mL)	Magnesium mg/dl
Patients (60)	1.15±6.82	100.8±16.9	18.51±3.0	22.30±4.71	27.4± 5.71	1.98±0.33
Control (30)	10.13±1.25	77.8±13.0	9.80±2.73	5.52±1.652	75.0± 10.3	2.13±0.50
P-Value	0.0005	0.002	0.0002	0.782	0.019	0.157

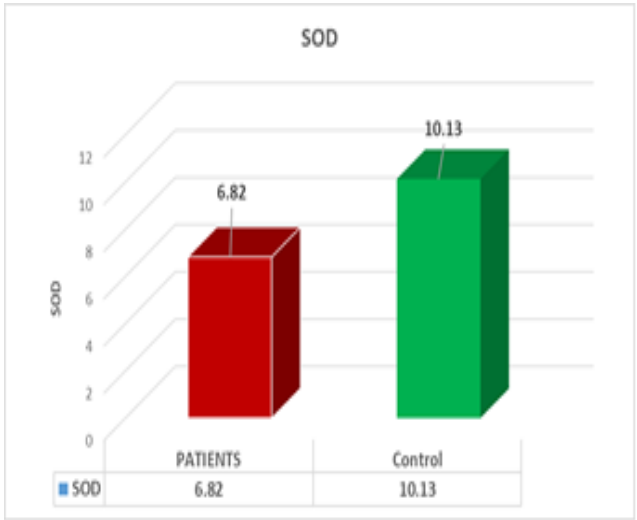


Figure 1: Superoxide dismutase (SOD) enzyme level (ng/mL) ± standard deviation in the studied groups

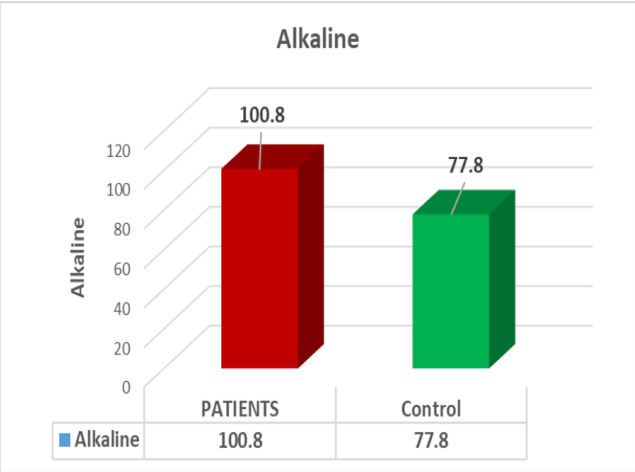


Figure 2: alkaline phosphate (ALP) level (U/L) ± standard deviation in the studied groups

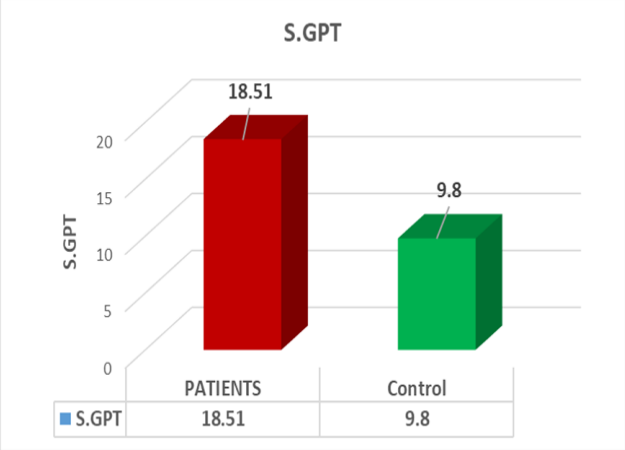


Figure 3: Level Alanine aminotransferase GPT (U/L) ± standard deviation in the studied groups.

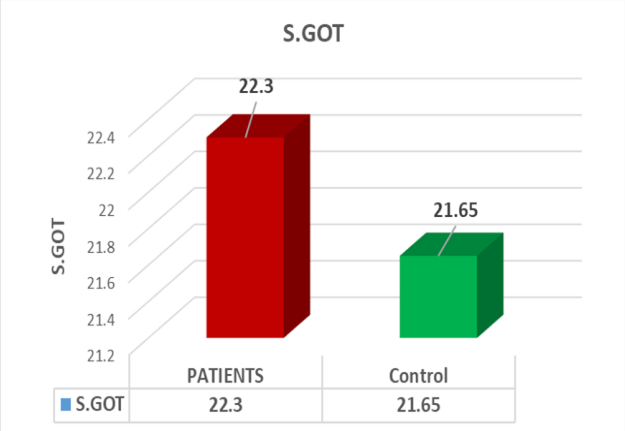


Figure 4: Level aspartate aminotransferase GOT (U/L) ± standard deviation in the studied groups

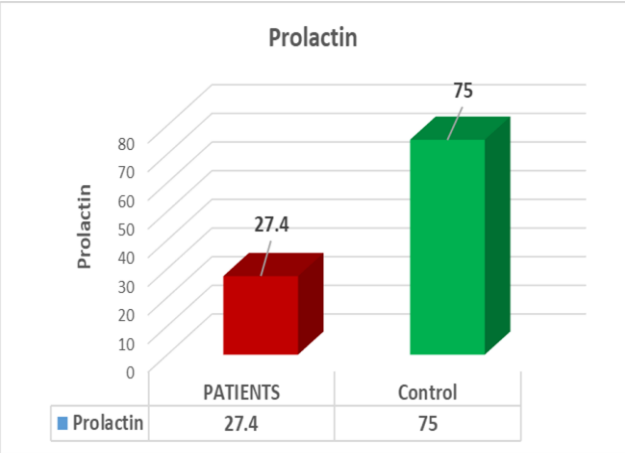


Figure (5): Prolactin hormone level (ng/mL) ± standard deviation in the studied groups

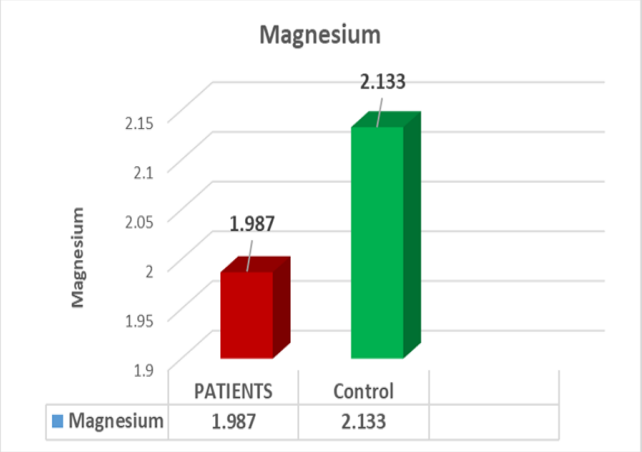


Figure 6: Magnesium level ± mg/dl standard deviation in the studied groups

Discussion

The results revealed a decrease in the level of SOD table-1, fig (1) in women with breast cancer compared with control group. This result approved with some studies that confirmed that the imbalance between the production of free radicals and the strategic defense that scavenging of these free radicals is an essential factor that leads to the sophisticated of the tumor, and the depression of SOD level makes the cells produces free radical species. [28,29] Over-expression of SOD may serve as a compensatory mechanism to protect cells from oxidative stress. Hence, the results of decreased SOD activity refer to a higher consumption of antioxidant

enzymes due to oxidative stress[30] (SOD) is a mainly tool of the cellular antioxidant defense system[31]. Decreased activity of detoxifying enzymes[32]. Our results conflict some studies that reported elevated superoxide dismutase in women with cancer. [33,34]

In this study also the effectiveness of alkaline phosphatase (ALP) was elevated (table-1, fig (2)) may be due to the presence in high levels within the cells. In the case of tumors, changes occur in the permeability of cell membranes which leads to the release of the enzyme and its influence on the circulatory system in the body [35]. One of the most important indicators of the presence of a malignant tumor in breast cancer patients is an increase in blood serum alkaline phosphatase activity that indicate to spread of breast cancer to the liver or bones. ALP levels in non-metastatic breast cancer insignificantly differ, according to certain studies. [36]

Regarding with alanine aminotransferase the test revealed significantly higher level in blood serum of breast cancer patients compared to the control group, and that there was a gradual increase in the level of alanine aminotransferase in the blood with the progression of the disease stage and malignancy [27]. Tumor invasion may be due to for elevated alanine aminotransferase levels, which indicates poor liver function [28]. Other studies also indicated that tumor invasion may be due to elevated of SGOT and SGPT, which indicates poor liver and kidney function [24]. The results showed a non-significant change in the level of aspartate aminotransferase in women with breast cancer compared to the control group.

During our study a decreasing of prolactin hormone level was observed in breast cancer patients(table-1, fig-5), where this results disagree with the studies that reported a high prolactin level attributed to changes in the amounts of luteinizing hormone (LH) in blood serum of breast cancer patients in addition to irregular secretion of the sex gland stimulant GnRH, which is secreted by the hypothalamus [37]. The concentration of the hormone prolactin increases in women with breast cancer for genetic reasons[38], It basically proved that by promoting cell proliferation and preventing apoptosis, one important factor in the early onset of breast cancer is prolactin stimulates cell migration and angiogenesis, both of which may be important factors in the spread of cancer. As a result, the fundamental processes of this association remain unclear. According to some studies women who have high levels of prolactin may be considering a vital biomarker to developing of tumors [39,40].

The level of magnesium in women with BC does not demonstrate any difference compared to the healthy group (table-1, fig-6). This result is not approved with other studies, that showed decreasing the level of magnesium in breast cancer patients because generation of free radical ls that alteration permeability of the plasma membrane to potassium through ATPase-Na pump, where this pump make to flow potassium ions from outside into inside the cell[21]. As magnesium is responsible for giving the tumor a solid consistency, it also works to inhibit tumor spread [22]. Studies have also indicated that increasing magnesium concentrations within breast cancer cells can lead to tumor development by regulating enzymes embedded in energy generation. Its presence is also necessary for cell adhesion and the spread of cancer [23][24]. These studies suggest that magnesium has a protective influence in the early stages of chemical carcinogenesis but promotes tumor growth [25].

Conclusion

The results indicated depression of superoxide dismutase level due to oxidative stress, negative changes in the hormone prolactin, as a result of a decrease in the level of prolactin in the blood of women with breast cancer compared to the control group, while positive changes occurred in the levels of alanine amino transferase and alkaline phosphatase, as a result of their increase in the serum of women with breast cancer compared to the control group. Elevation may be a strong indicator of the presence of a malignant tumor, as it was noted that there is no significant difference in the levels of magnesium and aspartate aminotransferase in the blood of women with breast cancer compared to healthy people. These findings can predict the presence of breast cancer.

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