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Determination of SDO Activity and LBSA in Serums of Benign and Malignant Breast Tumors

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Abstract

The present investigation was carried out to get it some model distinctions among the content of LBSA and the vigor of SOD in serums of normal donors (n=10) and patients with breast cancer (premenopausal n=20, postmenopausal n=20, and benign n=20). Data analysis show a lesser SOD activity in the serum of breast cancer patients (premenopausal 1.29 ± 0.14 and postmenopausal 1.05 ± 0.23) as compared to the benign patients 1.53 ± 0.17 and normal controls 1.65 ± 0.21, whereas, LBSA levels in sera of breast cancer patients show a significantly higher increase (premenopausal 29.32 ± 3.55 and postmenopausal 27.35 ± 5.29) than in benign patients 18.73 ± 2.29 and normal healthy 16.9 ± 2.13 respectively. The ratio of LBSA/SOD was higher in patients with breast cancer. On the other hand, a negative correlation (P > 0.001) exists between LBSA and superoxide dismutase in the serum of breast cancer patients. Results of this research show that SOD and LBSA to be delusory features of malignant with possible, usefulness in another cases connected with an raise hazard of neoplastic development in bonus to breast cancer.

***Keywords*:**LBSA, SOD, dismutase;premenopausal; postmenopausal; breast cancer

1. Introduction

SOD is an enzymewhich catalyzes the dissociation of superoxide free radical (O2-•) according to the reaction: O2-• + O2-• + 2H+ → O2 + H2O2.

It has been filtered from bovine erythrocytes by simple aprocess [1]. SOD, contains two equivalent of Cu per mole of it. Cu is required for activity, and may be reversibly taken away. SOD has been appeared to be congruent with the formerly illustrated Cu which have erythrocuprein (human) and hemocuprein (bovine)[1]. The enzyme has since been discover in a senior number of tissues and living creature , and it is believed that it is safeguard the cell from spoilage by the highly reactivity of superoxide free radical[2].

Superoxide is formed by the one electron reduction of oxygen and has been identified as a product in a number of biological reactions [2]. It is particularly likely to be produced when oxyhemoglobine is outoxidized to methemoglobine [3].

$$Hb\text{-}\ Fe^{2+} + O_2 \longrightarrow Hb - Fe^{3+}O_2^{-\bullet}$$

Other likely sources include reactions initiated by ionizing radiation.

The superoxide radical stable solutions are created by the reduction of O2 electrolytically in a non-protonated solvent, DMF. Allowed slow leaking of these solutions into buffered aqueous media, a proof that O2-• can minimize ferricytochrome C and tetra-nitromethane, and that SOD by competing for the superoxide radicals can notically damp these reactions.

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The purpose for use SOD enzyme to explain epinephrine oxidation to adrenochrome by milk xanthine oxidase is mediated by the O2- radical[1].

Furthermore, there are numerous indications in human that prolonged increased cell proliferation is necessary for the development of tumors [4]. On the other hand, the changes in the cell surface and the serum during malignant have been established[5].

The content and composition of glycoproteins and glycolipids are affected, with an increase in sialic acid on membrane of the cell surface & in the serum[6,7]. Decreased activity of the enzyme superoxide dismutase (SOD) has also been found in all malignant tumors investigated so far [8]. Such changes in the content of glycolipids (LBSA) and the activity of SOD are not found in patients with benign tumors [6,9]. The present investigation was carried out to compare the content of LBSA and activity of SOD in sera of patients with breast cancer as compared to the normal healthy individuals.

2. Materials and Methods

Patients and collected of specimens

Ten samples of blood were taken from healthy volunteers, these samples were used as a control groups (female volunteers aged between 25-35 years), who gave no history of previous diseases.

Two groups of breast cancer patients and one group of patients with benign breast tumors were included in this study.

Group 1 contained 20 premenopausal patients with breast cancer.
Group 2 consisted 20 postmenopausal patients with breast cancer.
Group 3 comprised 20 cases of benign breast tumors.

All patients were admitted for treatment to Al-Karama Teaching Hospital, patients suffered from any diseases that may interfere with our study were excluded.

The blood samples were collected from these patients. Left for 30 min. At room temperature; blood clots were separated at 3.000 r.p.m. for 10 min. by using centrifuge. Sera were aspirated and stored in sterilized tubes at -20 ^{o}C until time of analysis.

Appreciation*of serum SOD activity*

Theactivity of SOD was appreciated spectrophotometery by the manner of Winterbourn et al. [10], with our modification for serum. The manner is depending on the ability of the enzyme to dampen the reduction of NBT by superoxide produced through the reaction of photoreduction riboflavin and oxygen.

Procedure:

a. Addition of 0.2 mL of EDTA/NaCN solution to 0.1 mL of serum sample was carried out, then 0.1 mL of NBT solution was added.
b. The assay tubes were brought to a standard temperature (20-22 ^{o}C), after that 0.05 mL of riboflavin solution were added to each tube. The final assay volume of 3 mL was made up with phosphate buffer 0.067 M, pH=7.8.
c. Subsequent exposure to bright lighting was controlled by placing the assay tubes in a white-light box whereabouts them recipient uniform lighting for 20 minutes with 18 W fluorescent pipe attached to the lid, then the absorbance was red at 560 nm against distilled water.

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d. To determine the control value, the absorbance for another set of tubes containing the same mixture was read at 560 nm against distilled water immediately after the addition of riboflavin (riboflavin was added after the addition of buffer).
e. To determine SOD unit, ten tubes containing (10, 20, 40, 60, 100, 200, 300, 400 and 500 µL) of normal serum samples and another tube containing no serum were treated as described in the steps a, b and c.

Calculations:

a. Percentage inhibition was calculated from each absorbance in the presence and absence of the enzyme:
Inhibition % = $(A_E - A_{NE})$ X 100
where:
A_E: the absorbance at 560 nm of the tubes containing different amounts of the enzyme.
A_{NE}: the absorbance at 560 nm in the absence of the enzyme.

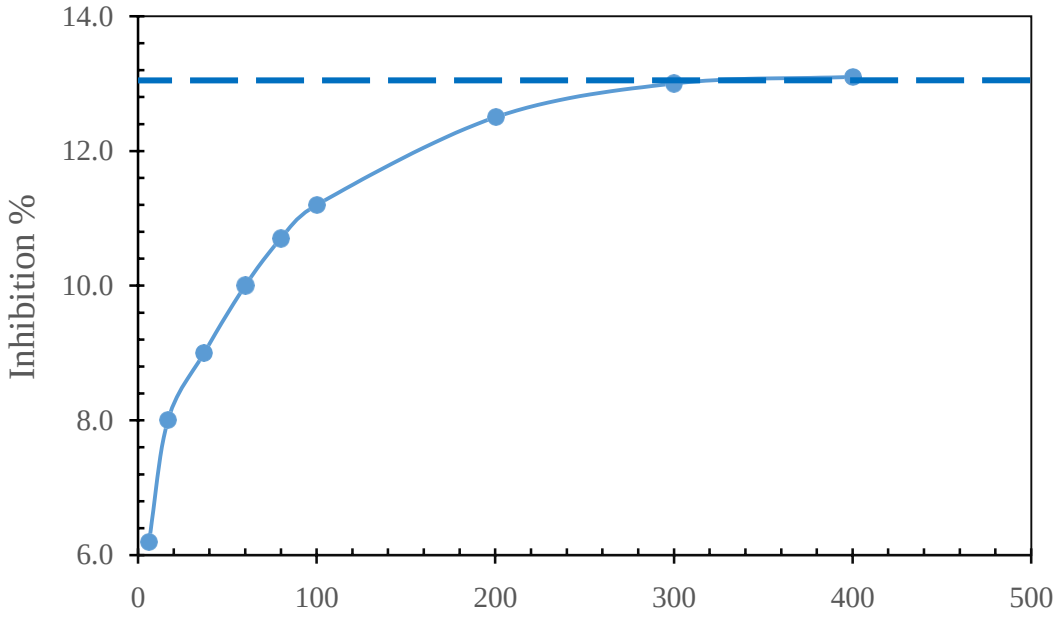

Figure 1. The standard curve for the determination of SOD unit

b. The percentages of inhibition were plotted against the corresponding amounts of serum Figure 1. SOD unit was calculated from Figure 1 according to the following: the amount of serum (V µL) which gives half the maximum inhibition of NBT reduction (1 unit = 10.0 µL). To calculate the SOD activity in sera of patients, the differences between absorbance before and after the light irradiation were multiplied by the SOD unit.

Determination of serum lipid-bound sialic acid:

Serum LBSA was determined by the method of Katopodis et al. [6].

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3. Results and discussion

The individual values of superoxide dismutase (SOD) activity and individual levels of LBSA for the normal healthy controls and patients with breast cancer are summarized in Table 1.

Figure 2 shows the distribution of the individual values of SOD activity in different types of breast cancer patients.

Table 1. Comparison of mean values for the superoxide dismutase (SOD) activity and lipid-bound sialic acid (LBSA) levels in sera from healthy and patients with breast cancer

Groups	No.	SOD activity (mean ± SD)	LBSA mg/dL (mean ± SD)	LBSA/SOD (mean ± SD)
Breast cancer:				
Premenopausal	20	1.29 ± 0.14	29.32 ± 3.55	22.74 ± 2.25
Postmenopausal	20	1.05 ± 0.23	27.35 ± 5.29	30.05 ± 5.87
Benige	20	1.53 ± 0.17	18.73 ± 2.29	12.34 ± 3.64
Healthy (normal)	10	1.65 ± 0.21	16.90 ± 2.13	10.15 ± 2.38

SOD levels in sera of patients with breast cancer differ significantly (P> 0.001) from those of healthy and patients with benign tumor, but the SOD levels in sera of benign patients shows no significant differences from those of healthy individuals.

It is clear from Table 1, patients with breast cancer had lower values of SOD activity compared with that of healthy individuals, (premenopausal 1.29 ± 0.14 and postmenopausal 1.05 ± 0.23). Very low SOD activity in comparison with healthy was observed in sera from patients with postmenopausal. However, a reliable decrease in the SOD activity was found in patients with breast cancer (premenopausal and postmenopausal) as compared to that of benign patients (P> 0.001), on the other hand, patients with breast cancer have a higher levels of LBSA (premenopausal 29.32 ± 3.55 and postmenopausal 27.35 ± 5.29), compared with healthy individuals and benign patients. No statistically significant difference was found between LBSA levels in patients with benign and healthy individuals, also there was no statistically significant difference among LBSA levels between the different subgroups of breast cancer (premenopausal and postmenopausal).

Furthermore, the ratio of LBSA/SOD was higher in patients with breast cancer compared with those with benign and normal healthy controls; the mean values of this index are shown in Table1.

Knee et al. [11] and Bolzan et al. [12] reported that lower levels of SOD (especially the manganese dependent enzyme) are found in various malignant tumors compared with normal control cells.

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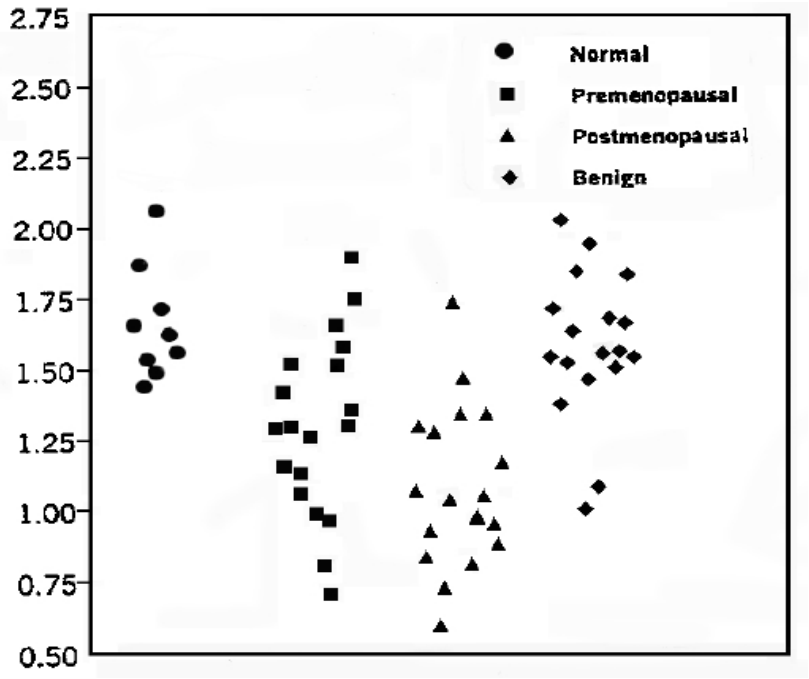

Figure 2. Distribution of the individual values of SOD activity in sera of patients with different types of breast cancer and normal healthy

High metastatic cell lines contain less SOD than low metastatic cell ones [11]. Prognosis for patients with low SOD levels in the leukaemic blasts is unfavourable [13] and a negative correlation has been established between SOD activities in the whole blood the chromosomal sensitivity after X-ray irradiation of lymphocytes from patients with cancer of the mammary gland [12]. The results show that lower SOD activity in patients with tumors compared to the healthy controls (Table 1), and the mean values for SOD activity in benign patients are near to those in healthy controls, these results are in agreement with the same results have been observed by Abella et al. [14].

Table 2. Specificity and sensitivity of LBSA and SOD activity in normal controls and in patients with breast cancer

Groups	No.	parameter	Negative results	Specificity % true negative	Positive results	Sensitivity % true positive
Healthy (normal)	10	LBSA	9	90	1	10
		SOD	8	80	2	20
Premenopausal	20	LBSA	1	5	19	95
		SOD	3	15	17	85
Postmenopausal	20	LBSA	0	0	20	100
		SOD	1	5	19	95
Benige	20	LBSA	12	60	8	40
		SOD	17	85	3	15

Note: Sensitivity and specificity of the SOD measuring as follows, sensitivity was calculated as % of patients with breast cancers who had positive results (lower SOD activity than healthy). Specificity was calculated as % of patients with breast cancers who had negative results (normal or higher SOD activity than healthy).

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Table 2 shows the sensitivity and specificity of LBSA and SOD in normal controls and in patients with different types of breast cancer. Sensitivity and specificity of the determination of serum SOD activity in distinguishing patients with benign from premenopausal and postmenopausal patients are (85% and 95% respectively) for sensitivity, and it is possible that the lower specificity of this determination (15% and 5% respectively), while the sensitivity of LBSA for determination of benign from malignant is (95% premenopausal and 100% postmenopausal). Also, as with SOD activity measurement the specificity of the determination (premenopausal 5% and postmenopausal 0%) is lower than its sensitivity.

These results are due to the fact that patients with breast cancer have different diagnosis, also the differences in the serum activity of SOD in patients with malignant tumors and benign are probably due to the decreased enzyme level in the tumor cell [12]. Such deficiency appears, for example, when enhanced serum levels of gangliosides (e.g. LBSA) are released by the tumor cells. LBSA binds to the plasma membranes of the mononuclear cells and inhibits their functions, which may be an important mechanism for immunosuppression in malignant diseases [15].

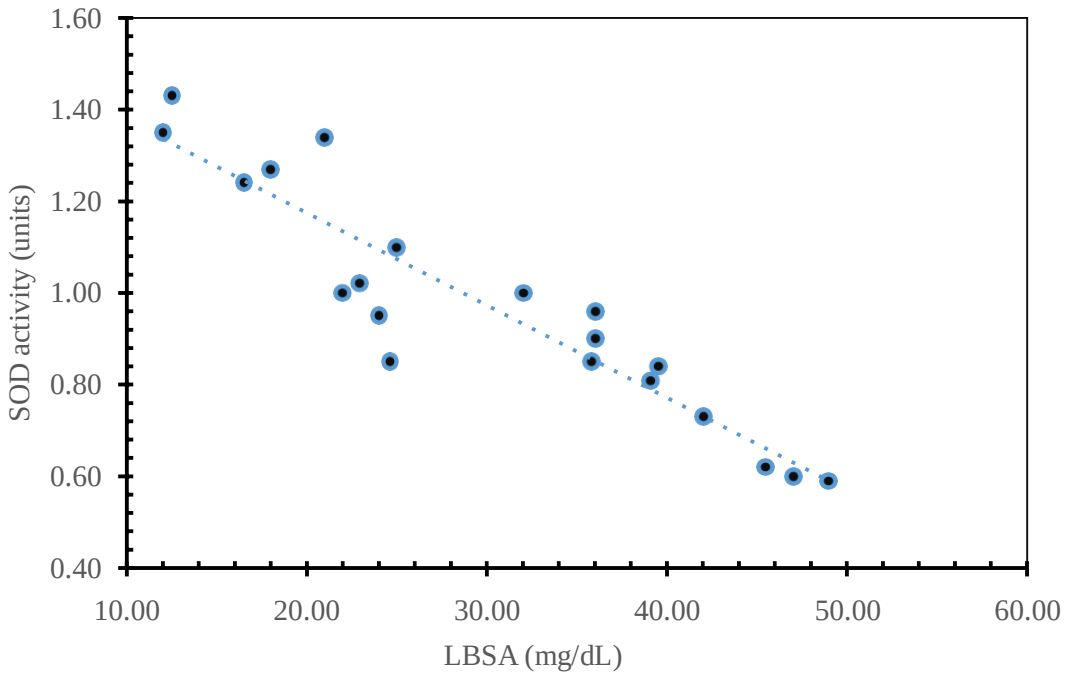

Figure 3. Correlation between superoxide dismutase (SOD) activity and the levels of lipid-bound sialic acid (LBSA) in sera from patients with breast cancers

However, from this result, it possible to conclude that the serum levels of SOD and LBSA reflect the changes in the content of membrane glycolipids and cellular activity of SOD.

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Some authors admit that these changes in the tumour cell are in closed connection the membrane of the malignant cell has an altered lipid content and structure organization, which leads to decreased antioxidant protection [16].

The loss of cell differentiation leads to an increase of the cell glycolipids, on the other hand, to decrease of the intracellular SOD [17]. Figure 3 shoes a significant negative correlation between the two parameters in patients with breast cancer, and it is also indicates that patients with levels of LBSA have low SOD content in the serum. Such negative correlation dose not appear in benign. This is in accordance with the observations that changes in membrane glycolipids and cellular antioxidants occur in malignant, but not benign tumors [18]. These findings suggest that SOD and LBSA are good tumor markers.

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