



Assessment of Tissue Oxidative Stress, Antioxidant Parameters, and Zinc and Copper Levels in Patients with Breast Cancer

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Abstract

Breast cancer is a multifactorial disease, and among the many factors which are involved in the onset, progression, and invasion of the disease, oxidative stress plays a significant role. The concentration and activity of enzymatic antioxidants are proportional to the concentration of trace elements, and the concentration of trace elements is often deficient in malignancies. Therefore, in the present study, we studied the tissue levels of oxidative stress, antioxidant status, zinc (Zn), and copper (Cu) in breast cancer patients. Tissue samples were collected from 40 patients with breast cancer and 40 tumor margin tissue as a control group. All subjects gave their informed consent. The tissue samples were measured for superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), total antioxidants capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), malondialdehyde (MDA), Zn, and Cu. Data of all biochemical parameters of two groups were statistically analyzed by SPSS software, *t* test, and GraphPad Prism. Concentrations of MDA, TOS, and OSI in tumor tissue were significantly higher than tumor margin tissue, but the level of TAC and CAT, SOD, and GPX activities was significantly reduced in tumor tissue ($p < 0.05$). It was found that the concentrations of Zn and Cu in breast cancer patients were higher than tumor margin tissue. Patients with breast cancer have a rise in oxidative stress indicators and a decrease in antioxidant stress markers. Since oxidative stress is a significant contributor to the development and progression of breast cancer, more research might lead to a more effective method of breast cancer treatment. Considering the dual role of oxidative stress in cancer, which can both cause survival and adaptation, and the death of cancer cells, and with more information, it can be used to manage the treatment and destruction of cancer cells.

Keywords Breast cancer · Oxidative stress · Antioxidant markers · Zinc · Copper

Introduction

Cancer is a major health problem and the cause of 13% of deaths worldwide, and its incidence and prevalence are increasing [1]. The most common cancer in women is breast cancer [2]. Breast cancer is an intricate and heterogeneous

disease with different histological, molecular, and clinical phenotypes. Breast cancer accounts for 23% of all cancers and occurs in Iranian women at least a decade earlier and in more advanced stages than women in Western countries [3]. The results of a 2020 study show that one in four women will develop breast cancer [4]. The 5-year relative survival rate for breast cancer is reported to be 90% [2]. Developing the breast cancer is related to the interaction between tumor cells and tumor microenvironment. The effect of oxidative stress on how the microenvironment of the tumor changes can affect the prognosis of breast cancer [5].

In eukaryotic cells, small concentrations of reactive oxygen species (ROS) are continuously produced as metabolic byproducts by mitochondria, which play important roles in several cellular processes at physiological concentrations [6, 7]. By upsetting the balance between cell damage by free radicals and the body's antioxidant defenses,

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oxidative stress is created, which damages important macromolecules, such as deoxyribonucleic acid (DNA), proteins, and lipids [8]. By disrupting the layers of membrane lipids, inactivating receptors, and membrane enzymes, free radical assault on lipids results in peroxidation of membrane lipids and increases membrane permeability. As a result of the peroxidation of membrane androgens by free radicals, the production of toxic products that act as secondary messengers in the cell, such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA), is toxic to cell types [9, 10]. Numerous studies have confirmed the increase in these compounds in breast cancer [11].

Antioxidants are regenerative substances found inside and outside the cell which has the ability to react with free radical species and control the production of free radicals. In fact, antioxidants are the body's defense mechanisms against free radicals. Antioxidants play an important role to eliminate free radicals and balancing redox reactions [12]. Antioxidants are divided into several types, including enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX); water-soluble vitamins and fats, such as vitamins C and E; minerals such as selenium (Se), manganese (Mn), copper (Cu), and zinc (Zn); and proteins such as albumin, ceruloplasmin, transferrin, haptoglobin, flavonoids, and phytochemicals [13]. SOD is a metalloprotein that is the first enzyme active in antioxidant defense to reduce oxygen species, so this enzyme removes the superoxide radical to form two less dangerous oxidative compounds, hydrogen peroxide and hydrogen peroxide [8]. CAT is the most abundant antioxidant enzyme containing iron, which converts hydrogen peroxide to water and molecular oxygen in two steps [14]. GPX reduces hydrogen peroxide and lipid hydro peroxides to water and alcohol. During this stage, the glutathione of this enzyme is oxidized to glutathione disulfide, and in the last stage, it is reduced by glutathione reductase [15, 16]. In many types of cancer, we see a decrease in the effectiveness of the antioxidant system and a continuous increase in oxidative stress [2].

Trace elements play an important role in metabolism [17]. Cu and Zn are required for antioxidant enzymes to function optimally. The process of oxidative stress is more prevalent when the quantity of trace elements in the structure of these enzymes is out of balance [18]. Concentrations of trace elements (Zn and Cu) are different in patients with various cancers compared to normal people [19].

This study aimed to evaluate the activity of antioxidant enzymes, such as SOD, CAT, and GPX, and concentrations of total antioxidants capacity (TAC), total oxidant status (TOS), oxidative stress index (OSI), MDA, Cu, and Zn in tumor tissue and tumor margins tissue of breast cancer patients.

Materials and Methods

Patients and Samples

This study was approved by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1400.088). This case-control study was performed in Khatam Al-Anbia Hospital, Tehran, Iran, from June 2021 to January 2022. Breast cancer patients included 40 women with a histologically confirmed diagnosis of invasive ductal carcinoma — the most common type of breast cancer — who had not received any treatment such as chemotherapy surgery and/or radiation therapy. The conditions for entering and exiting the study are as follows: inclusion criteria, women with breast cancer whose disease has been confirmed by pathology and mammography and is scheduled to undergo surgery. Exclusion criteria were presence of any breast removal surgery, undergoing any treatment (chemotherapy, radiation therapy, and hormone therapy), and taking mineral supplements and antioxidants. After the approval of the Ethics Committee of Hamadan University of Medical Sciences, informed consent was obtained from all participants in this study. Social demographic characteristics, such as age and gender, were collected using a questionnaire. Clinicopathologic data were obtained from patients' pathology reports. Tumor tissue and tumor margin tissue were evaluated independently by the pathologist after removal in terms of clinical parameters, such as histopathological type, pathological stage of the tumor, and degree of differentiation.

Preparation of Tissues

Tumor tissue and tumor margin tissue were obtained at the time of surgical resection. Each sample was placed in a separate tube, stored at -80°C and transferred to Hamadan University of Medical Sciences for testing. Tissue samples were cut into small pieces; in order to prepare lysates for the analysis of MDA, we washed a small amount of tissue (5 to 10 mg) from the tissue with PBS buffer. Then combine 300 μl of the MDA lysis buffer with 3 μl of the BTH 100X solution and homogenize the sample on ice. The resulting homogeneity was centrifuged at 6000 g for 10 min, and the supernatant was separated and placed at -80°C until the test was performed. To prepare a tissue sample for SOD, CAT, and GPX activities and TOS, TAC, and trace element (Zn, Cu) levels, a certain volume of tissue was lysed by a homogenizer in a PBS buffer containing a protease inhibitor cocktail and then in centrifuge 12,000 RPM at 15°C for 15 min. Freeze the supernatant in a new tube at -80°C .

Determination of Oxidative Stress Markers

MDA concentration was measured by calorimetric method according to the instructions of the Kiazist Lipid Peroxidation Kit. Calorimetric readings were performed at 560 nm. Concentration of MDA was calculated by using a standard curve. Data are expressed in nanomols per milligram of protein (nmol/mg of protein).

The TOS was determined using a Kiazist Kit. In this experiment, ferrous (Fe^{+2}) metal is oxidized to ferric (Fe^{+3}) in the presence of oxidants and produces dye in the presence of chromogen. The color produced was read at 560 nm. The amount of adsorption is directly related to the amount of oxidant, and the standard curve was drawn in the presence of H_2O_2 . Based on the adsorption of standards, we drew the standard curve and calculated the concentration of the samples using the following formula:

$$\text{H}_2\text{O}_2 \text{ Equivalent} \left(\mu\text{M or } \frac{\text{nmol}}{\text{ml}} \right) = A * \text{Dilution coefficient}$$

A = Values calculated from the standard curve

In order to determine the concentration of TAC, cupric (Cu^{+2}) is reduced to copper (Cu^{+1}) in the presence of antioxidants, and dye is produced in the presence of chromogen. TAC was measured by a Kiazist Kit, and the adsorption was read at 450 nm. Examples are expressed in terms of nmol of Trolox equivalent/mg protein.

The ratio of TOS to TAC is known as the oxidative stress index (OSI), which is calculated using Earl method [20]:

$$\text{OSI subjective unit} = \frac{\text{TOS } \mu \text{ molH}_2\text{O}}{\text{TAC mmol TroloxEq/l}} * 100$$

Determination of Enzymatic Markers of Oxidative Stress

The activity of SOD (both forms of Cu-Zn SOD and Mn SOD) was measured by calorimetric method according to the instructions of the Kiazist Kit. A superoxide anion radical (O_2^-) produced in a reaction catalyzed by xanthine oxidase produces a color product by performing several reactions. The concentration of this color product is proportional to the SOD activity in the samples, and finally, the adsorption was read at 560 nm. The activity was expressed based on the percentage of inhibition (inhibition rate %):

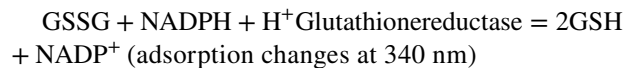
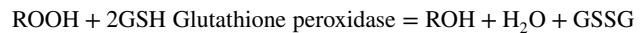
$$1\text{U SOD Activity} = \text{Inhibition rate } 50\%$$

CAT activity in the supernatant of breast homogenates was measured using a Kiazist Catalase Activity Measuring Kit, and the adsorbance was read at 560 nm. To determine

the enzymatic activity, first, the standard curve was drawn, and then the concentration of the samples was calculated based on the standard μM of formaldehyde. Finally, to calculate the enzymatic activity of μM , we put each sample in the following formula. The unit of enzyme activity is in terms of mU/mL or nmol/min/mL:

$$\text{CAT Activity} = \frac{\mu\text{M of sample}}{20\mu\text{M}} * \frac{0.24}{0.02} * \text{Sample dilution coefficient}$$

To measure the activity of GPX, a kit for measuring the activity of glutathione peroxide enzyme from Kiazist Company was used.



GPX enzyme activity was calculated according to the following formula:

$$\text{GPX Activity} = \frac{\Delta 340/\text{min}}{0.00216\mu\text{M}^{-1}} * \frac{110}{\text{Sample value}(\mu\text{l})} * \text{Sample dilution coefficient}$$

The unit of enzyme activity is mU/mL or nmol/min/mL.

Determination of Zn and Cu Concentration

The tissue levels of Cu and Zn were measured by an atomic adsorption spectrophotometer equipped with a graphite furnace (Varian SpectrAA220). The glassware used in this test was first completely washed with water and detergent and then rinsed with distilled water (three times). After rinsing, put the glassware in 10% nitric acid solution for 24 h and then wash them with deionized water at least 4 times. Standard solutions for determining the concentration of Cu and Zn were prepared using 0.1% nitric acid. The standard solutions were, respectively, diluted in different concentrations based on ppb. Ten ml of standard solution with different concentrations (10, 20, 40, 80 ppb) was injected into the atomic absorption spectrometer, and the standard curve was drawn. The desired samples were acid digested using nitric acid. The concentration of the samples was measured by the device, and the results were reported based on ppm.

Statistical Analysis

In order to analyze the data, SPSS statistical software (version 23) and descriptive and inferential statistics were used. The normality of the result distribution was confirmed. At the level of descriptive statistics, indicators such as mean, standard deviation, and frequency

distribution tables were used, and in inferential statistics for data analysis, *t* test or Mann–Whitney's test was performed, and analysis of variance was used. Statistical significance was set at a *p* value <0.5, and GraphPad Prism software was used to draw shapes and graphs.

Result

Demographic and Clinical Findings of Patients

Forty patients with breast cancer participated in this study. All participants were female, and their mean age was 54.2 ± 12.04 years with a minimum age of 29 to a maximum of 77 years. Demographic and clinical findings of all breast cancer patients were studied and tabulated in Table 1.

Table 1 Characteristics of breast cancer patients (*n* = 40)

Variable	Category	Patient number (N%)
Age (years)	<50	15 (37.5)
	>50	25 (80.9)
Marital status	Single	8 (20)
	Married	32 (80)
Abortion	Yes	12 (30)
	No	28 (70)
Breast feeding	Yes	34 (85)
	No	6 (15)
Use of contraception	Yes	14 (35)
	No	26 (65)
Family history	Yes	13 (32.5)
	No	27 (67.5)
Location of tumor	Left	24 (60)
	Right	16 (40)
Metastasis	Yes	6 (15)
	No	34 (85)
Histopathology	Ductal	38 (95)
	Lobular	2 (5)
Lymph node	Yes	20 (50)
	No	20 (50)
Grade	I	2 (5)
	II, III	38 (95)
Tumor size (cm)	<2.5	11 (27.5)
	≥2.5	29 (72.5)
Estrogen receptor	Positive	33 (82.5)
	Negative	7 (17.5)
Progesterone receptor	Positive	31 (77.5)
	Negative	9 (22.5)
HER2	Positive	7 (17.5)
	Negative	33 (82.5)

Tissue Level of Oxidative Stress Markers

Examination of the levels of oxidative stress markers including MDA, TOS, and TAC showed that tissue levels of MDA ($p=0.0095$), TOS ($p=0.016$), and OSI ($p=0.001$) were significantly higher in patients with breast cancer compared to tumor margin tissue. Tissue TAC levels are reduced in women with breast cancer compared to tumor margin tissue ($p=0.04$) (Fig. 1).

Tissue Level Enzymatic Markers of Oxidative Stress

Examination of the levels of oxidative stress enzymatic markers including SOD, CAT, and GPX showed that the enzymatic activity of SOD ($p=0.005$), CAT ($p=0.0001$), and GPX ($p=0.003$) in the tumor tissue of breast cancer patients was significantly reduced compared to the tumor margin tissue (Fig. 2).

Tissue Level of Zn and Cu

Examination of trace element levels including Zn and Cu showed that tissue levels of Zn ($p=0.0001$) and Cu ($p=0.0001$) were significantly higher in patients with breast cancer compared to tumor margin tissue (Fig. 3).

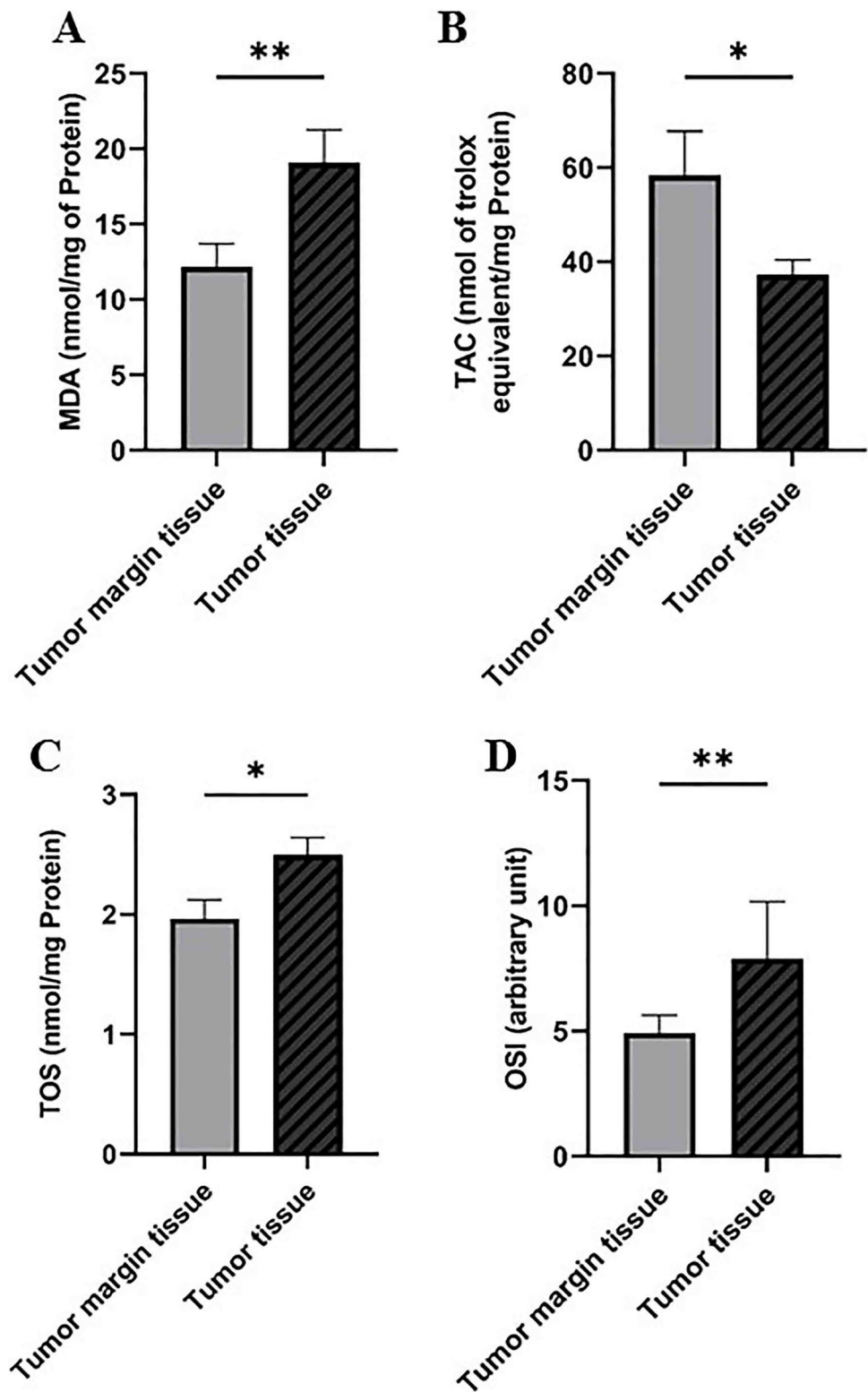
Relationship Between MDA, TAC, TOS, and OSI Levels and Clinicopathological Features in Breast Cancer Patients

The rate of OSI in the tissues of breast cancer patients was related to tumor location, metastasis, and tumor size. The level of OSI in patients' tissues in right breast tumors was significantly higher than left ($p=0.03$). Also, the level of OSI in the tissues of patients who had metastasized significantly increased compared to those who did not ($p=0.001$) and in breast cancer patients with tumor size <2.5 cm compared to tumor size ≥ 2.5 cm ($p=0.02$). Tissue concentration of MDA in breast cancer patients was associated with metastasis, the level of which was higher in patients who metastasized than in other patients ($p=0.006$). No significant relationship was observed between the concentration of MDA, TAC, TOS, and OSI in tissues with other clinical pathological features (age, menopausal status, pathological degree, tumor size, etc.) (all $p>0.05$) (Table 2).

Relationship Between CAT, SOD, and GPX Enzyme Activity and Clinicopathological Features in Breast Cancer Patients

The level of CAT enzyme activity in the tissues of breast cancer patients was associated with lymph node involvement. Patients with lymph node involvement had

Fig. 1 Tissue levels of oxidative stress markers. **A** Malondialdehyde (MDA), **B** total antioxidant capacity (TAC), **C** total oxidant status (TOS), and **D** oxidative stress index (OSI) in breast cancer patients compared to tumor margin tissue. * $p < 0.05$ and ** $p < 0.01$ compared to control



a significant increase in CAT activity compared to other patients ($p=0.03$). The relationship between SOD enzyme activity and tumor size showed that the activity of this enzyme increased significantly in people whose tumor size was less than 2.5 cm compared to patients whose tumor

size was more than 2.5 cm ($p=0.03$). No significant relationship was observed between the activity of CAT, SOD, and GPX in tissues with other clinical pathological features (age, menopausal status, pathological degree, tumor size, etc.) (all $p > 0.05$) (Table 3).

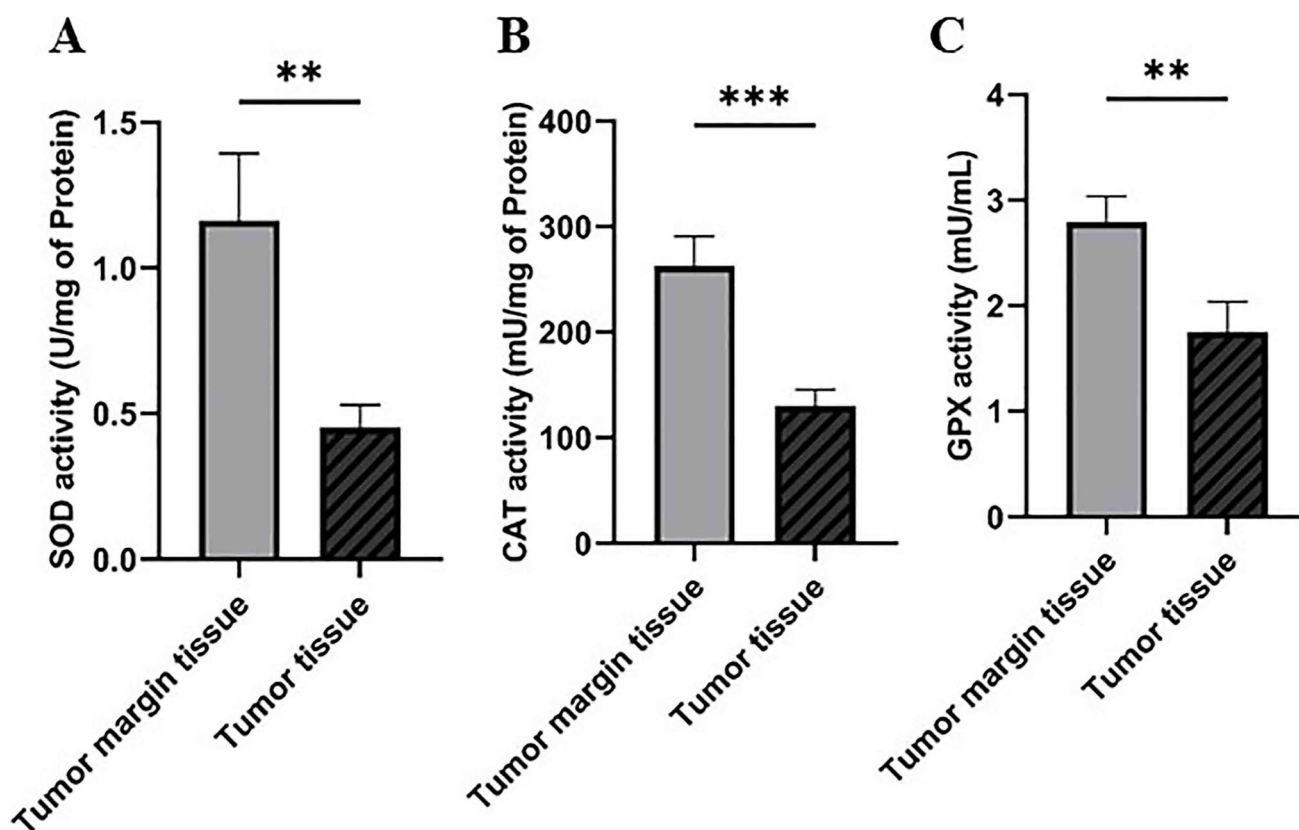


Fig. 2 Tissue levels enzymatic markers of oxidative stress. **A** Superoxide dismutase (SOD) activity, **B** catalase (CAT) activity, and **C** glutathione peroxidase (GPX) activity in breast cancer patients compared to tumor margin tissue. ** $p < 0.01$ and *** $p < 0.001$ compared to control

Relationship Between Zn and Cu Levels and Clinicopathological Features in Breast Cancer Patients

Based on the results, a correlation was found between tissue concentration of Zn and tumor location, lymph node involvement, and HER2 receptor. Tissue concentration of Zn in patients with tumors on the right side of the breast showed a significant increase compared to the left set ($p = 0.01$). We also observed an increase in Zn concentration in patients with lymph node involvement ($p = 0.03$). An increase in Zn concentration was observed in HER2⁺ patients compared to HER2⁻ ($p = 0.01$). No significant relationship was observed between the concentration of Zn and Cu in tissues with other clinical pathological features (age, menopausal status, pathological degree, tumor size, etc.) (all $p > 0.05$) (Table 4).

Correlation of Zn Concentration and Cu Concentration with Oxidative Stress Markers in Breast Cancer Patients

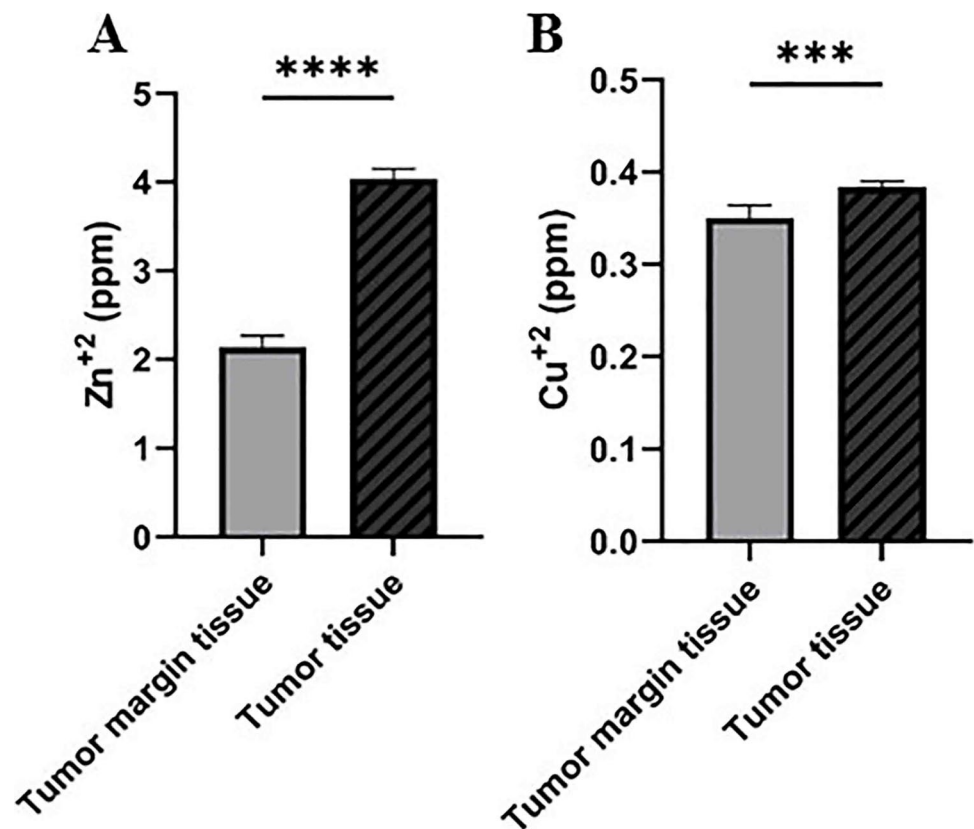
As shown in Table 5, Pearson correlation coefficients were used to calculate the correlation of Zn and Cu tissue concentration with oxidative stress markers. No correlation

was found between tissue concentration of Zn and oxidative stress parameters ($p > 0.05$), and the results of the study showed no correlation between tissue concentration of Cu and oxidative stress parameters ($p > 0.05$).

Discussion

Cancer is one of the leading causes of death and public concern worldwide [21]. According to the International Agency for Research on Cancer, the most common types of cancer among women are breast (33%), cervical (17%), and ovarian (6%) cancers [22]. Oxidative stress is a significant factor in the spread of cancer and a key factor in developing the disease [23]. Previous studies have confirmed an increment in oxidative stress in various types of cancer, including breast cancer [18]. Predicted mechanisms for enhanced oxidative stress in breast cancer have the potential to cause genetic changes in antioxidant enzymes, estrogen therapy, neglected production of reactive oxygen species, and disruption of the antioxidant system [24]. Oxidative stress biomarkers are clinically significant and can be used to determine cancers from breast cancer by determining their levels in tissue and blood [25, 26].

Fig. 3 Tissue levels of zinc (Zn) and copper (Cu). Tissue level of Zn (A) and Cu (B) in breast cancer patients compared to tumor margin tissue *** $p < 0.001$ and **** $p < 0.0001$ compared to control



This research paper is designed to determine the tissue content of MDA, TAC, TOS, OSI, antioxidant enzymes (CAT, GPX, and SOD), and trace metals (Zn and Cu). The results of the present study showed that the concentrations of MDA, TOS, and OSI in tumor tissue were significantly higher than tumor margin tissue, but the level of TAC and enzyme activities of CAT, SOD, and GPX were significantly reduced in tumor tissue. It was found that the concentrations of Zn and Cu in breast cancer patients were higher than tumor margin tissue. There was also a significant relationship between MDA and metastasis ($p=0.006$) and between OSI with tumor location ($p=0.03$), metastasis ($p=0.001$), and tumor size ($p=0.02$). Besides, there is a significant relationship between CAT and lymph node involvement ($p=0.03$), SOD, and tumor size ($p=0.03$). Our studies on the relationship between tissue concentrations of Cu and Zn with the clinicopathological parameters of breast cancer patients show the relationship between Zn and tumor location ($p=0.01$), lymph node involvement ($p=0.03$), and HER2 receptor ($p=0.01$).

MDA is the end product of lipid peroxidation which can be used to estimate the severity of oxidative stress or damage caused by lipid peroxidation [2]. It was shown that its level is higher in cancer patients than in controls [27–30]. The tissue concentration of MDA was greater

in breast cancer patients than in the tumor margin tissue group ($p = 0.0095$). Several studies have shown that MDA levels are considerably elevated in breast cancer patients [31, 32]. Moreover, the results of group study showed an increase in the concentration of MDA without a statistically significant difference in the serum of the patients [27]. The results of these studies confirm the findings of our study. The concentration of MDA seems to depend on many factors, including measurement time, age of patients, and stage of the disease. The results of the study by Didžiapetriene et al. showed that the changes in MDA levels depend on the age of the patients and the stage of the disease, and the highest concentration of MDA was seen in patients over 50 years and cancer patients who were in stage III [33]. Our finding did not show a relationship between the level of MDA and the age of the subjects ($p=0.16$). However, in patients in stages II and III, MDA levels were increased, but this difference was not significant ($p=0.13$). Our study showed a significant relationship between the level of MDA and metastasis, which significantly increased the level of MDA in patients who had metastasized ($p=0.006$). Patients with breast cancer have significantly lower concentrations of TAC in the tumor tissue than in the tumor margin tissue ($p=0.04$). The results of a study by Hewala et al. show a reduction in serum TAC levels in cancer patients compared to controls [32].

Table 2 Association between tissue concentration of stress oxidative marker with clinicopathological features in the breast cancer patients

Variable	MDA (nmol/mg of protein)		TAC (nmol of Trolox equivalent/mg protein)		TOS (nmol/mg protein)		OSI (arbitrary unit)	
	Mean \pm SD	<i>p</i> value	Mean \pm SD	<i>p</i> value	Mean \pm SD	<i>p</i> value	Mean \pm SD	<i>p</i> value
Age (years)								
<50	21.02 \pm 9.42	0.16	35.29 \pm 16.68	0.25	2.25 \pm 0.66	0.07	11.49 \pm 4.82	0.09
>50	20.10 \pm 15.16		40.12 \pm 18.17		2.49 \pm 1.02		7.43 \pm 6.19	
Location of tumor								
Left	20.48 \pm 15.15	0.41	41.39 \pm 19.42	0.07	2.52 \pm 0.93	0.98	7.13 \pm 0.91	0.03
Right	20.39 \pm 9.97		33.69 \pm 13.66		2.22 \pm 0.84		11.61 \pm 4.69	
Metastasis								
Yes	27.45 \pm 9.82	0.006	40.03 \pm 18.71	0.66	2.53 \pm 0.96	0.71	19.35 \pm 11.72	0.001
No	19.21 \pm 1.77		38.00 \pm 17.64		2.38 \pm 0.90		7.08 \pm 0.97	
Histopathology								
Ductal	20.44 \pm 2.16	0.99	38.92 \pm 17.64	0.65	2.39 \pm 0.92	0.07	8.78 \pm 2.03	0.82
Lobular	20.64 \pm 10.18		26.68 \pm 15.67		2.65 \pm 0.00		11.60 \pm 7.55	
Lymph node								
Yes	22.88 \pm 15.49	0.22	41.04 \pm 17.84	0.89	2.39 \pm 1.00	0.32	10.67 \pm 3.80	0.07
No	18.01 \pm 10.19		35.57 \pm 17.31		2.41 \pm 0.80		7.18 \pm 4.21	
Grade								
I	13.74 \pm 1.77	0.13	35.86 \pm 11.12	0.37	2.06 \pm 0.32	0.22	5.17 \pm 1.63	0.53
II, III	20.80 \pm 13.43		38.44 \pm 17.93		2.42 \pm 0.91		9.12 \pm 2.05	
Tumor size (cm)								
< 2.5	22.96 \pm 19.17	0.11	34.95 \pm 13.53	0.30	2.40 \pm 1.15	0.21	14.04 \pm 6.43	0.02
\geq 2.5	19.49 \pm 10.35		39.58 \pm 18.93		2.40 \pm 0.81		6.98 \pm 1.11	
Estrogen receptor								
Positive	20.94 \pm 13.87	0.56	37.56 \pm 18.77	0.07	2.47 \pm 0.94	0.16	9.75 \pm 2.34	0.29
Negative	18.11 \pm 9.74		41.85 \pm 10.48		2.05 \pm 0.56		5.01 \pm 0.96	
Progesterone receptor								
Positive	20.87 \pm 14.20	0.43	36.59 \pm 18.79	0.10	2.52 \pm 0.95	0.12	10.15 \pm 2.47	0.20
Negative	18.98 \pm 9.31		44.23 \pm 11.37		2.00 \pm 0.55		4.71 \pm 2.30	
HER2								
Positive	19.52 \pm 11.01	0.57	43.90 \pm 15.33	0.53	2.12 \pm 0.67	0.28	5.00 \pm 2.51	0.28
Negative	20.64 \pm 13.73		37.12 \pm 17.99		2.46 \pm 0.93		9.76 \pm 2.34	

Bold entries in the tables indicate statistical significance (*p* value less than 0.05)

Mehdi et al. examined the serum and tissue levels of TAC in cancer patients, and the results of this group showed a decrease in serum and tissue concentration of TAC [22], which confirmed the results of our group. The results of a study by Erten Şener et al. showed that TAC in the patients with breast cancer was 2.01 ± 0.01 mmol Trolox Eq/L and 2.07 ± 0.03 mmol Trolox Eq/L in the control group ($p < 0.05$) [34]. Zowczak Drabarczyk [35] et al. obtained results almost identical to Erten Şener which confirms our results. Conversely, the results showed that TOS was significantly increased in tumor tissue of breast cancer patients compared to tumor margin tissue of breast cancer patients ($p < 0.01$). Tahmasebpour and his colleagues found that TOS was increased in breast cancer patients ($p < 0.05$) [36]. The results of Mehdi et al. [22] and Tahmasebpour et al. [36] confirm our results. The examination of the ratio of TOS to TAC in this study showed that the OSI of tumor

tissue was significantly higher than the tumor margin tissue ($p = 0.001$). The results of a study by Feng et al. [37] confirm our findings. The results of Zarini et al.'s study show the relationship between OSI and the stage of the disease, which was lower in the lower stage than in the upper stage [38]. However, this relationship was not seen ($p = 0.53$), but a significant relationship was seen between OSI with tumor location ($p = 0.03$), metastasis ($p = 0.001$), and tumor size ($p = 0.02$).

Cancers have an altered metabolism with an increase in glucose uptake and lactate production in the presence of oxygen. This increased energy supplied by aerobic glycolysis is called "the Warburg effect" [39]. Tumor cells produce more of the angiogenic factors interleukin 8 (IL-8) and vascular endothelial growth factor (VEGF) when exposed to oxygen radicals. Oxidative stress in tumor cells may trigger angiogenesis in breast cancer by producing matrix

Table 3 Association between tissue concentration of enzymatic markers of stress oxidative with clinicopathological features in the breast cancer patients

Variable	CAT (mU/mg of protein)		SOD (U/mg of protein)		GPX (mU/mL)	
	Mean \pm SD	<i>p</i> value	Mean \pm SD	<i>p</i> value	Mean \pm SD	<i>p</i> value
Age (years)						
<50	117.62 \pm 68.15	0.98	0.42 \pm 0.18	0.29	1.48 \pm 0.86	0.17
>50	115.68 \pm 97.84		0.42 \pm 0.56		1.81 \pm 0.41	
Location of tumor						
Left	119.18 \pm 98.55	0.92	0.44 \pm 0.11	0.29	1.84 \pm 0.40	0.84
Right	112.26 \pm 68.79		0.38 \pm 0.04		1.46 \pm 0.28	
Metastasis						
Yes	111.32 \pm 36.53	0.56	0.38 \pm 0.17	0.55	1.72 \pm 0.44	0.78
No	117.31 \pm 93.41		0.43 \pm 0.08		1.68 \pm 0.30	
Histopathology						
Ductal	111.68 \pm 83.43	0.26	0.42 \pm 0.07	0.45	1.71 \pm 0.28	0.33
Lobular	206.23 \pm 142.33		0.46 \pm 0.02		1.18 \pm 0.25	
Lymph node						
Yes	131.52 \pm 118.26	0.03	0.52 \pm 0.13	0.21	1.99 \pm 0.48	0.23
No	101.30 \pm 32.42		0.32 \pm 0.03		1.38 \pm 0.23	
Grade						
I	96.14 \pm 59.18	0.91	0.36 \pm 0.29	0.95	2.84 \pm 0.68	0.56
II, III	117.48 \pm 88.60		0.42 \pm 0.07		1.63 \pm 0.27	
Tumor size (cm)						
< 2.5	153.66 \pm 137.190	0.12	0.54 \pm 0.24	0.03	2.19 \pm 0.84	0.06
\geq 2.5	102.28 \pm 55.27		0.37 \pm 0.19		1.49 \pm 0.19	
Estrogen receptor						
Positive	112.47 \pm 87.03	0.38	0.44 \pm 0.08	0.62	1.72 \pm 0.32	0.16
Negative	134.98 \pm 90.80		0.30 \pm 0.18		1.55 \pm 0.56	
Progesterone receptor						
Positive	115.10 \pm 88.58	0.55	0.45 \pm 0.09	0.44	1.77 \pm 0.34	0.12
Negative	120.93 \pm 85.99		0.30 \pm 0.16		1.40 \pm 0.58	
HER2						
Positive	101.73 \pm 37.40	0.37	0.26 \pm 0.09	0.30	1.12 \pm 0.79	0.34
Negative	119.52 \pm 94.30		0.45 \pm 0.08		1.81 \pm 0.31	

Bold entries in the tables indicate statistical significance (*p* value less than 0.05)

metalloproteinase-1 and enhance blood supply to breast cancer by promoting blood vessel dilation [40]. Alterations in metabolic pathways can provide advantages for tumor proliferation and growth by securing energy and substrates. These changes can create favorable conditions in tumor microenvironment (TME) that are optimal for cancer survival [39]. As you know, the body has an advanced antioxidant defense system to fight oxidants, which includes GPX, SOD, and CAT [11]. In our study, SOD enzyme activity in breast cancer tumor tissue was significantly lower than tumor margin tissue ($p=0.005$). The decrease in SOD enzyme activity may be in terms of the production of free radicals, which can lead to mutation and damage to the enzyme through cross-linking and damage to the nuclear DNA [41]. Moreover, under the circumstances of oxidative stress, in breast cancer patients, the rise in the concentration of free radicals and oxidants, as well as the ensuing damage, leads to a decrease in the amount of this beneficial antioxidant [27]. The results of a group study in

India in 2020 showed that SOD enzyme activity in people with breast cancer was significantly lower than in the control group [27], which confirms our result. The findings of Kilic et al. show a reduction in SOD enzyme activity in the patients with breast cancer [42]. In a study by Kangari et al., SOD enzyme activity in breast cancer patients was significantly increased compared to healthy individuals [43], which results are inconsistent with our results. This increase in enzymatic activity may be in terms of the inflammation of oxidants and activation of gene expression via antioxidant-responsive elements (AREs) [26]. Contradictory results can be seen from the activity of CAT in breast cancer. Some studies show an increase in the activity of this enzyme [29, 44], and others show a decrease in the activity of CAT in cancer patients relative to control [11, 41]. Our findings show a decrease in the activity of CAT in tumor tissue compared to tumor margin tissue ($p=0.0001$). And the decrease in CAT activity can be caused by the toxicity produced by H_2O_2 [41].

Table 4 Association between tissue concentration of trace element with clinicopathological features in the breast cancer patients

Variable	Zn (ppm)		Cu (ppm)	
	Mean ± SD	<i>p</i> value	Mean ± SD	<i>p</i> value
Age (years)	3.92 ± 0.44	0.45	0.38 ± 0.01	0.13
<50				
>50	4.00 ± 0.72		0.51 ± 0.14	
Location of tumor	3.88 ± 0.38	0.01	0.52 ± 0.15	0.12
Left				
Right	4.11 ± 0.87		0.37 ± 0.00	
Metastasis	3.80 ± 0.14	0.13	0.39 ± 0.04	0.47
Yes				
No	4.00 ± 0.67		0.47 ± 0.10	
Histopathology	3.96 ± 0.63	0.75	0.46 ± 0.09	0.69
Ductal				
Lobular	4.17 ± 0.40		0.40 ± 0.02	
Lymph node	3.77 ± 0.33	0.03	0.38 ± 0.02	0.05
Yes				
No	4.18 ± 0.78		0.54 ± 0.18	
Grade	4.93 ± 0.54	0.91	0.34 ± 0.04	0.72
I				
II, III	3.92 ± 0.59		0.47 ± 0.09	
Tumor size (cm)	3.86 ± 0.31	0.14	0.34 ± 0.03	0.36
< 2.5				
≥ 2.5	4.01 ± 0.71		0.50 ± 0.12	
Estrogen receptor	3.96 ± 0.66	0.60	0.47 ± 0.11	0.41
Positive				
Negative	4.03 ± 0.42		0.39 ± 0.01	
Progesterone receptor	3.95 ± 0.68	0.49	0.48 ± 0.11	0.32
Positive				
Negative	4.05 ± 0.38		0.39 ± 0.01	
HER2	4.07 ± 1.20	0.01	0.37 ± 0.01	0.37
Positive				
Negative	3.95 ± 0.45		0.48 ± 0.11	

Bold entries in the tables indicate statistical significance (*p* value less than 0.05)

GPX is a glycoprotein containing selenocysteine that catalyzes the conversion of hydrogen peroxide and lipid hydroperoxides to water and alcohol [45]. The results of Wang [46] and Kangari [43] study show an increase in GPX activity in breast tumor tissue, while the findings of Gupta et al. [47] indicate a decrease in GPX activity in cancer tissue. Our findings show a decrease in GPX enzyme activity (*p*=0.003).

Cu and Zn play an important role in biological processes; one of the most important activities of these metals is their cofactor role in the functioning of antioxidant enzymes [48]. Regarding the numerous roles of Zn in the metabolism of macromolecules in the body, this metal can be considered an essential element in life [49]. Several studies have reported changes in serum and tissue Zn concentrations in the patients with breast cancer. The results of several studies show an increase in the concentration of Zn in tumor tissues compared to tumor margin tissue [49–51]. The findings of our study showed an increase in Zn concentration in tumor tissues compared to tumor margin tissues (*p*<0.0001). In addition, increasing the concentration of Zn in tumor tissue showed a significant relationship with tumor location (*p*=0.01), lymph node involvement (*p*=0.03), and HER2 receptor (*p*= 0.01). The results of several studies have shown that Zn levels in the serum are reduced in cancer patients [49]. Intracellular Zn levels are regulated via the coordinated expression of Zn transporters that modulate Zn expression and flux. Intracellular Zn activates Zn-dependent metalloproteinases, which catalyze the breakdown of extracellular matrix and are involved in tumor proliferation, invasion, angiogenesis, and metastasis [52].

Cu is essential to promote various biochemical processes, such as angiogenesis and its antioxidant role. Numerous studies have reported increased Cu concentrations in breast cancer tissue, which can induce breast cancer through angiogenesis [48]. Our findings also show an increase in Cu concentration in breast tumor tissue compared to tumor marginal tissue (*p*=0.0001). The reason for the increase in copper levels in tumor tissue is the tumor's need for copper to activate angiogenesis, growth, and metastasis [53].

Table 5 Correlation of zinc and copper concentrations with oxidative stress markers in breast cancer patients

	MDA (nmol/mg protein)		TOS (nmol/mg protein)		TAC (nmol of Trolox equivalent/mg protein)		OSI (arbitrary unit)		CAT (mU/mg of protein)		SOD (U/mg of protein)		GPX (mU/mL)	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
Concentration of Zn (ppm) in tumor tissues	−0.101	0.535	−0.143	0.377	−0.182	0.260	−0.106	0.513	−0.055	0.736	−0.072	0.661	−0.032	0.846
Concentration of Cu (ppm) in tumor tissues	0.161	0.322	0.300	0.06	−0.310	0.051	0.067	0.681	−0.017	0.919	0.038	0.816	−0.003	0.986

Bold entries in the tables indicate statistical significance (*p* value less than 0.05)

Conclusion

This study was designed to confirm the important role of oxidative stress in the development/progression of many chronic diseases, including cancer. For this purpose, we determined the tissue content of MDA, TAC, TOS, antioxidant enzymes (CAT, GPX, and SOD), and trace metals (Cu and Zn). Changes in oxidant and antioxidant levels were observed in tumor tissue of breast cancer patients relative to tumor margin tissue in our study. MDA, TOS, and OSI levels increased, while TAC level and SOD, GPX, and CAT activities decreased, and Cu and Zn concentrations increased in tumor tissue relative to tumor margin tissue. One of the primary reasons of breast cancer development is the presence of elevated oxidative stress markers.

Limitations of the Study

This study was limited in terms of time, geographical area, and sample number. Also, in this study, we used tumor peripheral tissue instead of healthy tissue, which may have been affected by the disease. It would have been better if these parameters were checked in my head at the same time.

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Author Contribution Z.B. did all the experiments and wrote the manuscript, N.M. confirmed patients and gave samples, R.A. analyzed and interpreted the data, S.A.M-N revised the manuscript, and N.Z. generated and developed the study hypothesis and design and completed the final version of the manuscript.

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Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Ethics Approval and Consent to Participate IR.UMSHA.REC.1400.088.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

References

- Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 69(3):562–573
- Kundaktepe BP, Sozer V, Durmus S, Kocael PC, Kundaktepe FO, Papila C et al (2021) The evaluation of oxidative stress parameters in breast and colon cancer. *Medicine* 100(11):e25104
- Manoochehri J, Abdollahi A, Tajik A (2018) Epidemiological study of breast tumors in Iranian patients. *Acad Res Int* 9(1):35–38
- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 68(6):394–424
- Pantelimon I, Gales LN, Zgura A, Serbanescu GL, Georgescu DE, Nita I et al (2021) Analysis of oxidative stress in patients with breast cancer and obesity. *Ann Med Health Sci Res* 11(6):1578–1585
- Nita M, Grzybowski A (2016) The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. *Oxidative Med Cell Longev* 3164734:23. <https://doi.org/10.1155/2016/3164734>
- Quinlan CL, Goncalves RL, Hey-Mogensen M, Yadava N, Bunik VI, Brand MD (2014) The 2-oxoacid dehydrogenase complexes in mitochondria can produce superoxide/hydrogen peroxide at much higher rates than complex I. *J Biol Chem* 289(12):8312–8325
- Zalewska-Ziob M, Adamek B, Kasperczyk J, Romuk E, Hudziec E, Chwalińska E et al (2019) Activity of antioxidant enzymes in the tumor and adjacent noncancerous tissues of non-small-cell lung cancer. *Oxidative Med Cell Longev* 2019:1–9. <https://doi.org/10.1155/2019/2901840>
- Lymperaki E, Makedou K, Iliadis S, Vagdatli E (2015) Effects of acute cigarette smoking on total blood count and markers of oxidative stress in active and passive smokers. *Hippokratia* 19(4):293–297
- Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Med Cell Longev* 2014:1–31. <https://doi.org/10.1155/2014/360438>
- Al-Mayali HM, Kadhem WM (2021) Evaluation of some antioxidants and malondialdehyde (MDA) in Iraqi women infected with breast cancer and toxoplasmosis in Al-Diwaniyah and Al-Najaf provinces. *Mater Today: Proc* 236(7):1–3. <https://doi.org/10.1016/j.matpr.2021.07.236>
- Aguilar TAF, Navarro BCH, Pérez JAM (2016) Endogenous antioxidants: a review of their role in oxidative stress. A master regulator of oxidative stress-the transcription factor nrf2 3-20
- Mak S, Newton GE (2001) The oxidative stress hypothesis of congestive heart failure: radical thoughts. *Chest* 120(6):2035–2046
- Gomes EC, Silva AN (2012) Oliveira MRd(2012) Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxidative Med Cell Longev*:1–12. <https://doi.org/10.1155/2012/756132>
- Aldini G, Yeum K-J, Niki E, Russell RM(2011) Biomarkers for antioxidant defense and oxidative damage: principles and practical applications: John Wiley & Sons
- Shrivastava A, Mishra SP, Pradhan S, Choudhary S, Singla S, Zahra K et al (2021) An assessment of serum oxidative stress and antioxidant parameters in patients undergoing treatment for cervical cancer. *Free Radic Biol Med* 167:29–35
- Kanafchian M, Mahjoub S, Esmaeilzadeh S, Rahsepar M, Mosapour A (2018) Status of serum selenium and zinc in patients with the polycystic ovary syndrome with and without insulin resistance. *Middle East Fertil Soc J* 23(3):241–245
- Omar MEA-S, Eman RY, Hafez FH (2011) The antioxidant status of the plasma in patients with breast cancer undergoing chemotherapy. *Open J Mol Integrative Physiol* 3:8342–8347
- Ahmadi N, Mahjoub S, Hosseini RH, Taherkhani M, Moslemi D (2018) Alterations in serum levels of trace element in patients with breast cancer before and after chemotherapy. *Caspian J Internal Med* 9(2):134–139

20. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 38(12):1103–1111
21. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 127(12):2893–2917
22. Mehdi M, Menon M, Seyoum N, Bekele M, Tigeneh W, Seifu D (2018) Blood and tissue enzymatic activities of GDH and LDH, index of glutathione, and oxidative stress among breast cancer patients attending Referral Hospitals of Addis Ababa, Ethiopia: hospital-based comparative cross-sectional study. *Oxidative Med Cell Longev*. <https://doi.org/10.1155/2018/6039453>
23. Tuma DJ (2002) Role of malondialdehyde-acetaldehyde adducts in liver injury. *Free Radic Biol Med* 32(4):303–308
24. Rao S, Kumari DS (2012) Changes in plasma lipid peroxidation and the antioxidant system in women with breast cancer. *Int J Basic Appl Sci* 1(4):429–438
25. Czernska M, Mikołajewska K, Zieliński M, Gromadzińska J, Wąsowicz W (2015) Today's Oxidative Stress Markers *Medycyna Pracy* 66(3):393–405
26. Hauck AK, Bernlohr DA (2016) Oxidative stress and lipotoxicity. *J Lipid Res* 57(11):1976–1986
27. Rajendra C, Kedari G (2020) Role of antioxidant levels and lipid peroxidation in patients with breast cancer. *Biomedicine* 40(2):170–174
28. Wang M, Dhingra K, Hittelman WN, Liehr JG, De Andrade M, Li D (1996) Lipid peroxidation-induced putative malondialdehyde-DNA adducts in human breast tissues. *Cancer Epidemiol, Biomarkers Prev: Public Am Assoc Cancer Res, Cosponsored Am Soc Prev Oncol* 5(9):705–710
29. Kumaraguruparan R, Subapriya R, Viswanathan P, Nagini S (2002) Tissue lipid peroxidation and antioxidant status in patients with adenocarcinoma of the breast. *Clin Chim Acta* 325(1–2):165–170
30. Upadhyaya S, Upadhyaya S, Mohan SK, Vanajakshamma K, Kunder M, Mathias S (2004) Oxidant-antioxidant status in colorectal cancer patients—before and after treatment. *Indian J Clin Biochem* 19(2):80–83
31. Jabir FA, Shaker AS (2020) Roles of Superoxide dismutase (SOD), Malondialdehyde (MDA), 8-iso-prostaglandinF2 α (8-iso-PGF2 α) as oxidative stress in development and progression of Breast cancer in Iraqi females patients. *Al-Qadisiyah J Pure Sci* 25(1):1–4
32. El-Soud MRA, Hewala T (2019) The clinical significance of serum oxidative stress biomarkers in breast cancer females. *Med Res J* 4:1–7
33. Didžiapetrienė J, Kazbarienė B, Tikuišis R, Dulskas A, Dabkevičienė D, Lukosevičienė V et al (2020) Oxidant/antioxidant status of breast cancer patients in pre-and post-operative periods. *Medicina* 56(2):70–79
34. Erten Şener D, Gönenç A, Akıncı M, Torun M (2007) Lipid peroxidation and total antioxidant status in patients with breast cancer. *Cell Biochem Function: Cell Biochem Modul Active Agents Dis* 25(4):377–382
35. Zowczak-Drabarczyk MM, Murawa D, Kaczmarek L, Połom K, Litwiniuk M (2013) Total antioxidant status in plasma of breast cancer women in relation to ER β expression. *Contemp Oncol/ Współczesna Onkologia* 17(6):499–503
36. Tahmasebpour N, Feizi MAH, Ziamajidi N, Pouladi N, Montazeri V, Farhadian M et al (2020) Association of omentin-1 with oxidative stress and clinical significances in patients with breast cancer. *Adv Pharmaceut Bull* 10(1):106–113
37. Feng J-F, Lu L, Zeng P, Yang Y-H, Luo J, Yang Y-W et al (2012) Serum total oxidant/antioxidant status and trace element levels in breast cancer patients. *Int J Clin Oncol* 17(6):575–583
38. Zarrini AS, Moslemi D, Parsian H, Vessal M, Mosapour A, Kelagari ZS (2016) The status of antioxidants, malondialdehyde and some trace elements in serum of patients with breast cancer. *Caspian J Int Med* 7(1):31–36
39. Mossenta M, Busato D, Dal Bo M, Toffoli G (2020) Glucose metabolism and oxidative stress in hepatocellular carcinoma: role and possible implications in novel therapeutic strategies. *Cancers* 12(6):1668–1698
40. Brown NS, Bicknell R (2001) Hypoxia and oxidative stress in breast cancer Oxidative stress-its effects on the growth, metastatic potential and response to therapy of breast cancer. *Breast Cancer Res* 3(5):1–5
41. Hussain S, Ashafaq M (2018) Oxidative stress and anti-oxidants in pre and post-operative cases of breast carcinoma. *Turkish J Pharmaceut Sci* 15(3):354–359
42. Kilic N, Yavuz Taslipinar M, Guney Y, Tekin E, Onuk E (2014) An investigation into the serum thioredoxin, superoxide dismutase, malondialdehyde, and advanced oxidation protein products in patients with breast cancer. *Ann Surg Oncol* 21(13):4139–4143
43. Kangari P, Farahany TZ, Golchin A, Ebadollahzadeh S, Salmaninejad A, Mahboob SA et al (2018) Enzymatic antioxidant and lipid peroxidation evaluation in the newly diagnosed breast cancer patients in Iran. *Asian Pacific J Cancer Prev: APJCP* 19(12):3511–3515
44. Woźniak B, Mila-Kierzenkowska C, Kedziora-Kornatowska K, Drewa T, Drewa G, Woźniak A et al (2007) Influence of the management of cervical carcinoma on the activity of catalase and glutathione peroxidase in erythrocytes. *Eur J Gynaecol Oncol* 28(6):461–463
45. Jomova K, Jenisoava Z, Feszterova M, Baros S, Liska J, Hudecova D et al (2011) Arsenic: toxicity, oxidative stress and human disease. *J Appl Toxicol* 31(2):95–107
46. Wang C, Yu J, Wang H, Zhang J, Wu N (2014) Lipid peroxidation and altered anti-oxidant status in breast adenocarcinoma patients. *Drug research* 64(12):690–692
47. Gupta RK, Patel AK, Kumari R, Chugh S, Shrivastav C, Mehra S et al (2012) Interactions between oxidative stress, lipid profile and antioxidants in breast cancer: a case control study. *Asian Pac J Cancer Prev* 13(12):6295–6298
48. Magalhaes T, Becker M, Carvalho M, Von Bohlen A (2008) Study of Br, Zn, Cu and Fe concentrations in healthy and cancer breast tissues by TXRF. *Spectrochim Acta B At Spectrosc* 63(12):1473–1479
49. Jouybari L, Kiani F, Akbari A, Sanagoo A, Sayehmiri F, Aaseth J et al (2019) A meta-analysis of zinc levels in breast cancer. *J Trace Elem Med Biol* 56:90–99
50. Geraki K, Farquharson M, Bradley D (2002) Concentrations of Fe, Cu and Zn in breast tissue: a synchrotron XRF study. *Phys Med Biol* 47(13):2327
51. Cui Y, Vogt S, Olson N, Glass AG, Rohan TE (2007) Levels of zinc, selenium, calcium, and iron in benign breast tissue and risk of subsequent breast cancer. *Cancer Epidemiol Biomark Prev* 16(8):1682–1685
52. Cabré N, Luciano-Mateo F, Arenas M, Nadal M, Baiges-Gaya G, Hernández-Aguilera A et al (2018) Trace element concentrations in breast cancer patients. *Breast* 42:142–149
53. Lossow K, Schwarz M, Kipp AP (2021) Are trace element concentrations suitable biomarkers for the diagnosis of cancer? *Redox Biol* 42:101900. <https://doi.org/10.1016/j.redox.2021.101900>

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