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Metal-Induced Oxidative Stress in Egyptian Women with Breast Cancer

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Abstract

Some trace metals are toxic and claimed to be carcinogenic. The present work aimed to determine the levels of some trace metals in breast tissues (healthy and tumor specimens) and to evaluate their concentrations in relation to the oxidative stress status in breast cancer which is a major health problem among Egyptian women. This study included 127 female patients with breast swelling. Analysis of trace metals in breast tissue was done using Atomic Absorption Spectrophotometry (AAS). Lipid peroxidation and oxidative status were assessed. There were statistically high levels of iron, zinc and copper in the benign and malignant breast tissues in comparison to the control group. Higher Malondialdehyde (MDA) levels were detected in patients with breast tumors while Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSH), Glutathione-S-transferase (GST) and Nitric oxide (NO) were low in comparison to the healthy group. In conclusion, the alteration of the elemental content in cancerous breast tissues and the disruption of oxidant/antioxidant balance highlight the role of trace metals in cancer development.

Keywords: Trace metals; Breast cancer; Oxidative stress; Antioxidants

Introduction

Studying the effects of trace metals on human health has been attracting a great deal of attention in recent decades**.** Some trace metals are essential in a wide variety of biological processes of living systems through activating or inhibiting enzymes or by competing with other elements and metalloproteins for binding sites, and by affecting the permeability of cell membranes or by other mechanisms. Therefore, homeostasis of metal ions is critical for life and is maintained within strict limits [1,2].

Oxidative stress is defined simply as a disturbance in the oxidant-antioxidant balance, favoring the oxidant environment. The human body is equipped with certain antioxidants (scavenging enzymes) such as glutathione, superoxide dismutase (SOD) and catalase (CAT) that can counteract the deleterious effects of reactive oxygen species and protect against cellular and molecular damages. Disruption of the balance between free radicals and antioxidants may cause a cellular damage and trigger carcinogenesis [3,4].

Interestingly, some trace metals are claimed to be carcinogenic and capable of inducing a toxic effect through the formation of ROS and acting as cofactors in the oxidative damage of biological macromolecules and DNA [5]. However, their exact role in carcinogenesis is still unclear [6,7]. On the other hand, breast cancer is one of the major health problems. It is the most common type of cancer among Egyptian women representing 26% of all cancer cases and it is considered the leading cause of cancer related deaths in women throughout the world [8,9].

The aim of the work

The present work is carried out to determine the levels of some trace metals in breast tissue and to evaluate the oxidative status in relation to these elements in patients with breast tumors.

Subjects and methods

The present study was conducted on female patients having breast tumors and admitted to the Oncology Center, Mansoura University-Egypt, in the period between January 2009 and June 2009.

Inclusion criteria: All study patients were non-smokers and did not receive either hormonal therapy or any treatment for the tumor.

Exclusion criteria: Patients who had any concomitant disease that would affect the results of the study were excluded e.g., diabetes mellitus, liver dysfunction, rheumatoid arthritis or any other prolonged illness.

Study groups:

a. Control group (Group I): It comprised 20 non-smokers women in the same age range of the tumor patients and they were selected on a voluntary basis from the same localities. They were admitted to the Oncology Centre for huge breast or chronic breast abscess, etc.

b. Patients groups: Breast tumor patients were divided into 2 groups:

- Group II: it included patients who had benign breast tumors.
- Group III: it comprised women who had breast cancer. This group was subdivided into two subgroups:
- Group III a: patients who had non-metastatic breast lesion.
- Group III b: patients who had metastatic breast lesion.

Ethical consideration: A written informed consent was taken from all studied patients to carry out the study**.**

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Sample collection:

a. Blood samples: 5 ml fasting blood samples were taken from all study groups and collected in EDTA containing tubes. 2 ml was taken for a quantitative analysis of the reduced glutathione concentration. The other 3 ml was centrifuged and the clear non-haemolyzed plasma was separated and stored in small vials at -20°C until further measurement of malondialdehyde (MDA) levels, the antioxidant activity (superoxide dismutase and catalase), glutathione-s-transferase and the nitric oxide concentration. After the separation of plasma, the buffy coat was removed and the packed cells were washed thrice with cold physiological saline to determine the MDA level in RBCs.

b. Surgical management: Control patients underwent either reduction mammoplasty or excision of chronic breast abscess. Excisional biopsy was performed for patients who had benign breast swelling. Modified radical mastectomy or wide local excision with at least 1 cm safety margin had been done in women diagnosed as breast cancer.

c. Tissue samples: Samples from the fresh normal and neoplastic breast tissues (benign and malignant) and adjacent histologically healthy tissue 6 cm away from the tumor were obtained from the patients during the surgical procedures. Tissues were washed with ice-cold normal saline for three times and the surrounded fat was trimmed carefully. A part of the tissue sample was fixed in 10% formalin and kept in refrigerator until trace metal analysis.

Techniques:

a. Analysis of trace metals in breast tissue: Digestion of samples was done at 120-150°C using a mixture (3:2) of conc. nitric acid (69%) and perchloric acid (70%) till complete digestion and white ash was obtained. The ash was then dissolved in 100 µl 5% nitric acid and make it with deionized water up to 5 ml. Measurement of metal levels in breast tissue samples was performed using single beam Perkin-Elmer Atomic Absorption Spectrophotometry at wavelengths of 248.3, 213.4, 324.8 and 279.5 Å for iron, zinc, copper and manganese respectively. Absorbance values were determined and a calibration curve was constructed by plotting the concentrations of metals (µg/ml) against their absorbencies. Metals levels were obtained directly from the standard curve of each metal [10].

b. Determination of lipid peroxidation: This was done through analysis of malondialdehyde in plasma according to Draper and Hadely [11] as well as in RBCs as described by Stocks and Donnandy [12].

c. Determination of antioxidant activity: Superoxide dismutase and Catalase activity were assayed by the procedure of Dechatelet et al. [13] and Aebi [14] respectively.

d. Determination of reduced glutathione in blood was done as stated by Beutler et al. [15].

e. Determination of glutathione-s-transferase and nitric oxide activity was done according to Habig et al. [16] and Green et al. [17] respectively.

Statistical Analysis

The statistical analysis of data was done by using excel program for figures and SPSS (SPSS, Inc, Chicago, IL) program statistical package for social science version 16. Kolmogrov-Smirnov test was used for analysis of normality of distribution of data and it was insignificant. Quantitative data were presented as mean ± standard Deviation (SD). Differences between groups were assessed by t-test analysis of variance. $P<0.05$ was considered statistically significant for comparison of the results. Correlation coefficient (r) was done by using GraphPad InStat program version 5.02 to measure the correlation between two numerical variables. The correlation is perfect at r=1.0, the two variables are said to be increased or decreased together at r=0-1.0, one variable increases as the other decreases when r=0-(-1.0).

Results

Only 127 female patients were enrolled in this work as they fulfilled the inclusion criteria. They included 50 women who had benign breast swelling (age ranged from 26-60 years). 77 patients had malignant breast disease (36 patients were non metastatic and 41 showed metastasis). They were in the age group of 32-60 years old. Twenty healthy subjects participated in the control group.

The levels of Copper (Cu), Zinc (Zn), Manganese (Mn) and Iron (Fe) in breast tissue are shown in Table 1.

Levels of different oxidative parameters measured in the studied groups in relation to the control group are presented in Table 2.

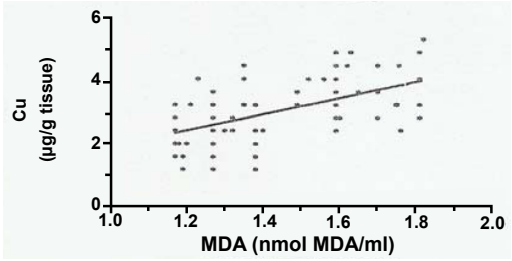

Figure 1: Significant direct correlation between plasma MDA level (nmol/ml) and tissue Cu level (µg/g tissue) in patients with breast cancer.

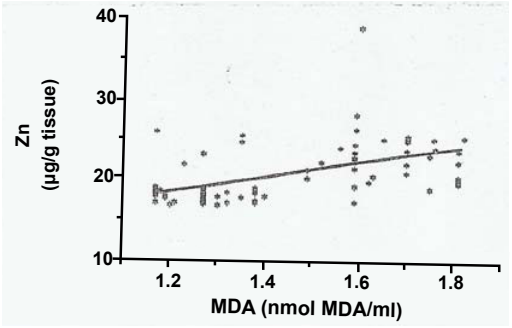

Figure 2: Significant direct correlation between plasma MDA level (nmol/ml) and tissue Zn level (µg/g tissue) in patients with breast cancer

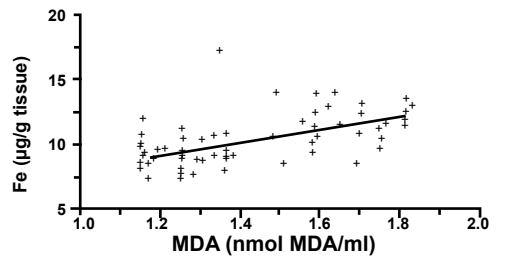

Figure 3: Significant direct positive correlation between plasma MDA level (nmol/ml) and tissue Fe level (µg/g tissue) in patients with breast cancer.

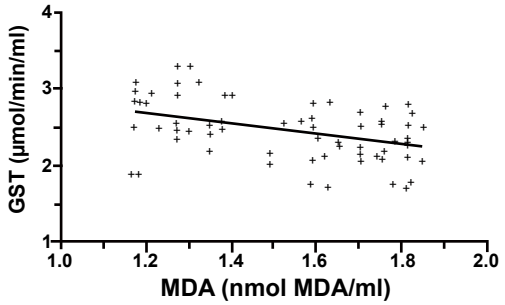

Figure 4: Significant negative indirect correlation between plasma MDA level (nmol/ml) and plasma GST level (µmol/min/ml) in patients with breast cancer.

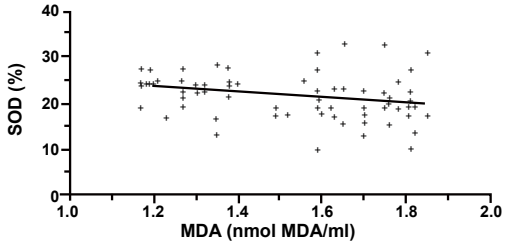

Figure 5: Significant negative indirect correlation between plasma MDA level (nmol/ml) and plasma SOD level (%) in patients with breast cancer.

Figures 1, 2 and 3 illustrated the positive (direct) correlation between tissue copper levels and plasma MDA in patients with breast cancer (r=0.5707, p<0.0001), zinc (r=0.5397, p<0.0001) and iron (r=0.6075, p<0.0001) respectively.

Figure 4 showed a significant negative (indirect) correlation between plasma MDA level (nmol/ml) and plasma GST level (µmol/min/ml) in patients with breast cancer (r=-0.3692, p=0.003).

Figure 5 demonstrated the significant negative (indirect) correlation between plasma MDA level (nmol/ml) and plasma SOD level (%) in patients with breast cancer (r=-0.3239, p=0.0015).

Discussion

Farquharson et al. [18] mapped the distribution of elements in breast cancer in comparison to normal tissues. The levels of Fe, Cu and Zn were found to be increased significantly in neoplastic cells. This indicates a strong correlation between the location of cancer cells and areas of high content of these metals. In the present work, a statistically significant elevation of iron, copper and zinc concentrations was detected in breast tissue of women having breast tumors (benign and malignant) in comparison to healthy control group. They also significantly increased with the progression of malignancy and presence of metastasis.

The present results are nearly similar to other findings reported by da Silva et al. [2], Pasha et al. [19] and Magalhães et al. [20] irrespective of the samples examined in different types of cancers. The increased

Table 1: Levels of different trace metals in breast tissue of the study groups (µg/g tissue).

Trace metal (µg/g tissue)	Control group (Group I) (n=20)	Group II (50 benign breast tumor patients)	Group III (77 malignant breast tumor patients)	
			Non-metastatic (Group III a)	Metastatic (Group III b)
Copper	0.65 ± 0.2	0.84 ± 0.28	2.35 ± 0.62 (a)	3.79 ± 0.69 (a, c)
			3.07 ± 0.99 (a, b)	
Zinc	13.85 ± 4.54	14.86 ± 2.36	18 ± 0.67 (a)	23.22 ± 3.6 (a, c)
			20.71 ± 3.71 (a, b)	
Manganese	0.25 ± 0.09	0.25 ± 0.1	0.26 ± 0.096	0.31 ± 0.122
			0.3 ± 0.11	
Iron	3.67 ± 0.95	6.56 ± 1.16	8.89 ± 1.03 (a)	11.65 ± 1.83 (a, c)
			10.32 ± 2.04 (a, b)	

(a) Significant against control group; (b) Significant against benign group; (c) Significant against non-metastatic cancer group; **Significant at P<0.05**

Table 2: Levels of different oxidative parameters measured in the study groups (mean ± SD) in relation to the control group.

Parameter	Control group (Group I) (n=20)	Group II (50 benign breast tumor patients)	Group III (77 malignant breast tumor patients)	
			Non-metastatic (Group III a)	Metastatic (Group III b)
Erythrocyte MDA (µmol/ml packed RBCs)	7.11 ± 1.07	10.8 ± 2.49 (a)	14.70 ±0.885 (a)	21 ± 2.72 (a, c)
			18.00 ± 3.81 (a, b)	
Plasma MDA (nmol/ml)	0.59 ± 0.11	0.88 ± 0.22 (a)	1.26 ± 0.08 (a)	1.59 ± 0.19 (a, c)
			1.44 ± 0.22 (a, b)	
SOD (%)	58.86 ± 3.44	49.79 ± 4.71 (a)	24.36 ± 1.8 (a)	19.61 ± 4.68 (a, c)
			21.41 ± 4.52 (a, b)	
CAT (µmol/Sec/ml)	0.73 ± 0.09	0.58 ± 0.09 (a)	0.41 ± 0.05 (a)	0.31 ± 0.06 (a, c)
			0.36 ± 0.07 (a, b)	
GSH (mmol/L)	1667.8 ± 176.3	1526.8 ± 141.1 (a)	1150.6 ± 242.4 (a)	956.4 ± 206.3 (a, c)
			1053.62 ± 240.61 (a,b)	
GST (µmol/min/ml)	3.59 ± 0.22	2.94 ± 0.81 (a)	2.71 ± 0.42 (a)	2.39 ± 0.27 (a, c)
			2.53 ± 0.38 (a,b)	
Nitric oxide (µmol/L)	11.85 ± 2.2	15.51 ± 1.85 (a)	18.78 ± 1.31 (a)	20.59 ± 2.38 (a, c)
			19.94 ± 2.42 (a, b)	

MDA (malondialdehyde), SOD (superoxide dismutase), CAT (catalase), GSH (reduced glutathione), GST (glutathione-S-transferase)
a) Significant against control group; b) Significant against benign group; c) Significant against non-metastatic cancer group; **(Significant at P ≤ 0.05)**

levels of Zn, Cu and Fe in plasma and cancerous breast tissue samples and their suggested role in tumor development could be related to their action as enzymatic co-factors involved in carcinogenesis. In addition, copper and zinc belong to the group of oxidant metals causing disruption of the oxidative balance. Iron may be implicated through its role as a regulatory factor for angiogenesis. Moreover, the demand for increased blood supply for a growing tumor provides a basis for the accumulation of many elements [7,21].

Iron can also promote carcinogenesis by causing tissue damage as it acts as a catalyst in the conversion of hydrogen peroxide to free radical ions that attack cellular membranes, breaks DNA strands, inactivate enzymes and initiate lipid peroxidation [22]. Enhancement of these trace metals in cancerous tissues suggested that these elements compete for the binding sites in the cell, change its enzymatic activity and exert direct or indirect action on the carcinogenic progress accelerating the growth of tumors [23].

The present research clarified that there is a significant rise of plasma nitric oxide (NO) level in breast cancer patients when compared to benign tumor cases and control subjects. Likewise, Gonenc et al. [24] detected increased levels of nitrate and nitrite in serum and tissue of breast carcinoma in comparison to benign breast lesions. NO is one of the reactive species and serves as an important messenger molecule in carcinogenesis. It may have several effects such as the induction of DNA damage, inhibition of DNA repair enzymes, and the modulation of apoptosis and metastasis [25,26].

Metals such as iron and copper, exhibit the ability to produce ROS, thus resulting in molecular damage and alterations of cell homeostasis. For instance, excess Cu is a potent oxidant and promotes oxidative stress as it catalyzes the formation of ROS that oxidize important biomolecules, such as lipids, proteins, and DNA [27,28]. Thus, increased copper levels could play a role in the development and progression of various cancers [3,29].

Regarding the oxidative status, SOD and CAT are found to be low in all breast tumor patients of the current work in comparison to control group. More suppression of their activity is detected in the metastatic cancer patients. Similarly, Kasapović et al. [30] and Sinha et al. [4] detected suppressed SOD activity in breast cancer with increased toxic effect of $O_2^{\bullet}$ which could lead to severe cellular damage. SOD and CAT are considered the primary antioxidant enzymes, since they are involved in the direct elimination of reactive oxygen species [31].

The present work detected higher plasma and erythrocyte MDA levels in patients with benign and malignant breast tumors (especially metastatic lesions) in comparison to the healthy group. Huang et al. [32] reported a significant rise of MDA level in patients with breast tumor in relation to control subjects. In contrast, Khanzode et al. [33] found lower levels of MDA in different clinical stages of breast cancer when compared to controls.

One of the most important expressions of oxidative stress is lipid peroxidation as it enhances the production of free radicals which could lead to cellular damage [34]. MDA constitutes a highly cytotoxic major aldehyde final peroxyl radical product of lipid peroxidation. It is claimed to be an inhibitor to protective enzymes. Hence, it could have both mutagenic and carcinogenic effects. It is also implicated as a key molecule in DNA adducts formation (a piece of DNA covalently bonded to a cancer-causing chemical) by interacting with DNA bases inducing DNA interstrand cross-links [3,35].

This work demonstrated a negative indirect correlation between plasma MDA concentration and SOD and GST activities. The increased level of lipid peroxidation products plays a role in tumor growth. The high MDA level could be explained by defect in the antioxidant system with accumulation of lipid peroxides in cancer tissue as stated by Kumaraguruparan et al. [36]. Furthermore, Sener et al. [37] reported statistically significant lower total antioxidant capacity with significantly higher serum MDA levels in breast cancer patients compared to control subjects.

Additionally, the present work detected a significant direct correlation between levels of copper, zinc, iron in breast tissue and MDA in plasma. This may be explained by the fact that elevation of these metals could lead to formation of free radicals or other reactive oxygen species. Valko et al. [38] stated that disruption of metal homeostasis may lead to uncontrolled metal-mediated formation of deleterious free radicals participating in the modifications to DNA bases and enhanced lipid peroxidation with subsequent formation of MDA.

We also revealed a significant decrease of Glutathione- S-transferase (GST) and reduced glutathione (GSH) in both benign and malignant tumor cases in relation to the control group. In contrast, levels of reduced glutathione were found to be elevated in malignant lesions by Mishra et al. [39].

On the other hand, Kumaraguruparan et al. [36] reported similar significant reduction of the concentrations of plasma glutathione, as well as levels of antioxidant enzymes such as SOD, catalase, glutathione peroxidase, glutathione-transferase in both fibroadenoma and adenocarcinoma patients compared to control subjects with tendency of blood GST to decrease with cancer progression.

Depletion of GSH may be responsible for the lower activity of GST in breast tumor patients and this might explain the direct correlation observed between the level of GSH and GST activity. This supports the assumption that glutathione status is inversely related to malignant transformation. GSH is a powerful antioxidant and implicated in the cellular defense against deleterious compounds such as free radicals [40].

GST is an antioxidant enzyme which has a protective role against oxidative stress**.** Furthermore, it can metabolize a variety of environmental carcinogens as it can catalyze the conjugation of toxic and carcinogenic molecules with glutathione and thereby protect cellular macromolecules against toxic foreign chemicals and oxidative stress**.** GSH depletion in tumor cases may be related to increased oxidative stress and GSH consumption in scavenging free radicals and its conversion to inactive glutathione disulfide [41].

Conclusion

The alteration of the elemental content (excess Cu, Fe and Zn) in benign and cancer tissues in comparison to healthy breast tissues highlights the role for these trace elements in the initiation or promotion of breast cancer. One possible interpretation is that the increased levels of these elements could lead to formation of free radicals or other reactive oxygen species inducing oxidative stress which affects adversely DNA and thereby causing breast cancer. It is recommended to use trace elements and antioxidant activity as biomarkers for breast cancer and its progression.

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