

# Estimated some Antioxidant Enzymes and Trace Elements in some Iraqi Women Infected with Breast Cancer

Dr. Rasha Zuhair Jasim

Department of Chemistry , College of Education for Pure Sciences  
(Ibn – Al – Haitham), Baghdad University

## Abstract

Erythrocytes glutathione peroxidase (GP<sub>x</sub>) , catalase (CAT) and serum malondialdehyde (MDA), in addition to three trace elements including selenium, iron and zinc were determined in two groups of Iraqi women with breast cancer ( A and B) and compared with healthy control group ( group C) . Results have revealed that (GP<sub>x</sub>) activity was highly significance and significance increased in erythrocytes of groups A (37.2 ±6.5) U / gm Hb and B (30.5±5.1) U / gm Hb respectively compared with group C (28.5±4.5) U /gm Hb , while (CAT) activity was significant and highly significance increase in erythrocytes of groups A (2.55±0.45) U / gm Hb and B (2.16±0.25) U / gm Hb respectively compared with group C (1.65±0.25) U / gm Hb. Moreover , MDA level was highly significance increase in sera of groups A ( 12.2±4.3) mmol / L and B (18.6±5.6) mmol / L compared with group C (3.6±1.2) mmol / L. Results have also reported that selenium level was significantly increased in sera of groups A (65±9) ng/mL and B (59±10) ng/mL compared with group C ( 50±6) ng/mL. As well , iron level was non-significantly increased and significantly decreased in sera of group A (1.4±0.9) µg/ mL and B (0.9±0.1) µg /mL respectively compared with group C (1.2±0.5) µg/mL. Zinc level was significant and highly significance decreased in sera of groups A (0.77±0.21) µg/mL and B (0.98±0.14) µg/mL respectively compared with group C (0.66±0.22) µg / mL. . From the present study we concluded that MDA and Zn levels are good biochemical markers for breast cancer regardless the period of time for suffering from this disease and the duration of treatment, while GP<sub>x</sub> , CAT , Se and Fe could be considered biochemical markers only for the first two years of the disease.

**Keywords** Glutathione peroxidase , Catalase , Malondialdehyde , trace elements , breast cancer.

## **Introduction:**

Breast cancer or breast carcinoma is the second common case of cancer death and one of the most common neoplasm in women. Many factors adjust the risk of breast cancer including late age of menopause , late age of first pregnancy , hormone therapy , diet , family history and obesity. [1;2;3;4] Breast cancer is often related to age, Approximately two thirds of cases of breast cancer occur during the postmenopausal period. [2] Estrogen is an important steroid hormone involved in regulating the differentiation and proliferation of normal breast epithelial cells. [6] Several studies have reported that estrogen level decline in postmenopausal women. Estrogen cause the production of proteases like pro – cathepsin D which can promote the invasiveness of tumor cells so it is a promoter of the disease. [2;3;4;5;6] Breast cancer patients are generally treated with chemotherapeutic agents such as cyclophosphamide and doxorubicin. [1] This type of treatment has been shown to increase lipid peroxidation level and generation of free radicals which are chemical species possess an unpaired electron and are very active. [1] Doxorubicin is an anthracycline derived from the *Streptomyces* yeast. It has multiple mechanisms of action. These include intercalation of DNA, which leads to inhibition of protein synthesis and free radical formation, and inhibition of topoisomerase enzymes. [7] Although chemotherapy leads to increase of lipid peroxidation activity but in the same time inhibit the growth of cancer cells. Free radicals are produced spontaneously in the cells either as by-products of metabolism or during phagocytosis in the extra – cellular as compartment by the mitochondrial respiratory chain and the mixed function oxidase system. [1] Free radicals and reactive oxygen species (ROS) play an important role in diseases such as breast cancer by their interaction with cellular macromolecules such as proteins , lipids , DNA and leads to various pathological condition including cancer diseases. [1;3;8] All aerobic organisms have mechanisms by which they can minimize free radicals toxicity , for example antioxidant enzymes like glutathione peroxidase (GP<sub>x</sub>) which is reported as the first and best characterized mammalian selenoprotein , capable of reducing equivalents from glutathione to detoxify hydrogen and lipid peroxides , furthermore breakdown of hydrogen peroxide ( H<sub>2</sub>O<sub>2</sub>) to water and oxygen is done by catalase (CAT) which plays an important role in the detoxification of these poisonous compounds and protect against free radicals and reactive oxygen species damage[1,8,9] catalases are essential components of the cellular equipment used to cope with oxidative stress. [10] Malondialdehyde (MDA) level is an important product resulting from (Lipid peroxidation ,

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LPO) , it is a good marker or indicator to LPO because there is a positive relationship between MDA and oxidative stress. [11] Trace elements have been strongly studied in recent years because they essentially act as cofactors for antioxidant enzymes. Selenium (Se) has a protective effect against oxidative damage by increasing of (GP<sub>x</sub>) synthesis. Catalase is a hemoprotein activated by iron . [8;11;12;13] Zinc is a component of superoxide dismutase (SOD) which removes free radicals and is necessary for vitamin A , lipid metabolism and DNA repair [14;15] zinc appeared to scavenge free hydroxyl (OH<sup>•</sup>) and superoxide (O<sub>2</sub><sup>-•</sup>) radicals produced by the xanthine/xanthine oxidase reaction. [16] The aim of this study is to link between the measured biochemical parameters and the duration of the treatment.

### **Materials and methods :**

Blood samples were collected from 50 women (with breast cancer with age in range 48-45 years) and This conducted on two groups :- the first (group A) included 25 women suffering from breast cancer (from 1 to 2) years, second (group B) also included 25 women who suffer from this disease (between 2 to 4 years) . The two groups from the same disease were studied to determine the relationship between the measured parameters and the duration of the disease and treatment . Data resulted from patients of groups (A and B) were compared with 25 healthy women in the same range of age as a control group ( group C) . All patients have a histopathological reports from different laboratories revealed that they are suffering from this disease. Blood samples were collected from patients in (Al- Benok) laboratory in Baghdad and the work was conducted cooperatively with the center of chemical researches in Ministry of Science and Technology. Every patient included in this study had undergone treatment with chemotherapy drugs ( both cyclophosphamide and doxorubicin) and they took the same doses of treatment per each year of the disease. No treatment was given to the control group. All patients and control groups were married , postmenopausal , none of them was infertile , some of them have a familiar history with gynecological diseases and the others have none. Six parameters were determined in the present study :- glutathione peroxidase (GP<sub>x</sub>) (EC 1.11.1.9) , catalase (CAT) (EC 1.11.1.6), [17] , Malondialdehyde (MDA) in addition to three trace elements :- Se , Fe and Zn. Glutathione peroxidase (GP<sub>x</sub>) activity was measured in erythrocytes using glutathione as a substrate. The enzyme uses glutathione as the ultimate electron donor to regenerate the reduced form of the selenocysteine. [18] Philips spectrophotometer was used in this study. The

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spectrophotometric detection (flame) was recorded at 340 nm . Catalase activity was also determined in erythrocytes using hydrogen peroxide as a substrate, The assay is based on the disappearance of  $H_2O_2$  in the presence of the enzyme source at  $26^\circ C$ . [19] the detection was spectrophotometrically(flame) recorded at 240 nm. Activity in samples of GPx and CAT was normalized for hemoglobin content and expressed as U/gm Hb. Malondialdehyde (MDA) (mmole / mL) level was determined in serum of patients by a colorimetric test basing on formation of colored complex upon the reaction with thiobarbutyric acid , i.e. concentration of malondialdehyde was measured by the thiobarbituric acid test. This method evaluates the oxidative stress assayed for malondialdehyde, the last product of lipid breakdown caused by oxidative stress. Determination of malondialdehyde level in proteins is used as an index of the extent of lipid peroxidation. [20] The detection was recorded by flame (spectrophotometer) at 500 nm. Se level was estimated by (flameless) atomic absorption spectrophotometer while Zn and Fe levels were determined by Atomic Absorption spectrophotometer (flame) .[21] Students t.test was used as a statistical analysis, data have been expressed in tables as mean  $\pm$  S.D, meanwhile values of ( $p < 0.01$ ) were considered to be significant , values of ( $p < 0.001$ ) were considered to be high significant , while values of ( $p > 0.05$ ) were considered to be non-significant.

### **Results and Discussion:**

Results have reported that (GPx) was highly significant increase ( $p < 0.001$ ) in erythrocytes of group A ( $37.2 \pm 6.5$ ) U / gm Hb compared with group C ( $28.5 \pm 4.5$ ) U / gm Hb , while a significant increase ( $p < 0.01$ ) was suggested in group B ( $30.5 \pm 5.1$ ) U / gm Hb compared with group C ( $28.5 \pm 4.5$ ) U / gm Hb , ( table 1) . Also , these results have revealed that (CAT) activity was significantly increase ( $p < 0.01$ ) in erythrocytes of group A ( $2.55 \pm 0.45$ ) U / gm Hb compared with group C ( $1.65 \pm 0.25$ ) U / gm Hb , while highly significant increase ( $p < 0.001$ ) was suggested in group B ( $2.16 \pm 0.25$ ) U / gm Hb compared with group C ( $1.65 \pm 0.25$ ) U / gm Hb ,( table 1). Moreover , results of (table 1) have shown that MDA was highly significant increase in sera of group A ( $12.2 \pm 4.2$ ) mmol/L and B ( $18.6 \pm 5.6$ ) mmol/L compared with group C ( $3.6 \pm 1.2$ ) mmol/L. Results related to the trace elements were represented in (table 2) , these results have suggested that selenium level was significantly increase in sera of groups A ( $65 \pm 9$ ) ng / mL and B ( $59 \pm 10$ ) ng/mL compared with group C ( $50 \pm 6$ ) ng/mL. As well , iron level was non significantly increase ( $p > 0.05$ ) in sera of group A ( $1.4 \pm 0.9$ )  $\mu g$  / mL compared with group C ( $1.2 \pm 0.5$ )  $\mu g$  / mL , while it was significantly decrease ( $p < 0.01$ ) in group B ( $0.9 \pm 0.1$ )  $\mu g$  / mL compared

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with group C ( $1.2 \pm 0.5$ )  $\mu\text{g} / \text{mL}$ . On the other hand , zinc level was significantly ( $p < 0.01$ ) and highly significant decrease ( $p < 0.001$ ) in sera of group A and group B ( $0.66 \pm 0.22$ )  $\mu\text{g} / \text{mL}$  respectively compared with group C ( $0.98 \pm 0.14$ )  $\mu\text{g} / \text{mL}$ .

**Table (1) : Activities of glutathione peroxidase , catalase and level of malondialdehyde in breast cancer Patients ( groups A and B) compared with healthy control ( group C).**

Parameter	group C mean $\pm$ S.D	group A mean $\pm$ S.D	group B mean $\pm$ S.D	P A/C	P B/C
GPx(U/gm Hb)	28.5 $\pm$ 4.5	37.2 $\pm$ 6.5	30.5 $\pm$ 5.1	P<0.001	P<0.01
CAT(U/gmHb)	1.65 $\pm$ 0.25	2.55 $\pm$ 0.45	2.16 $\pm$ 0.25	P<0.01	P<0.001
MDA(mmol/L)	3.6 $\pm$ 1.2	12.2 $\pm$ 4.2	18.6 $\pm$ 5.6	P<0.001	P<0.001

**Table (2) : Levels of selenium , iron and zinc in breast cancer patients (groups A and B) compared with Healthy control ( group C).**

Parameter	group C mean $\pm$ S.D	group A mean $\pm$ S.D	group B mean $\pm$ S.D	P A/C	P B/C
Se (ng/mL)	50 $\pm$ 6	65 $\pm$ 9	59 $\pm$ 10	P<0.01	P<0.01
Fe ( $\mu\text{g}/\text{mL}$ )	1.2 $\pm$ 0.5	1.4 $\pm$ 0.9	0.9 $\pm$ 0.1	P>0.05	P<0.01
Zn ( $\mu\text{g}/\text{mL}$ )	0.98 $\pm$ 0.14	0.77 $\pm$ 0.21	0.66 $\pm$ 0.22	P<0.01	P<0.001

Two studies have shown that chemotherapy routinely used for treatment of postmenopausal women with breast cancer increases free radicals and reactive oxygen species that cause a damage of normal tissues. [8;9] These drugs including (cyclophosphamide and doxorubicin) have multiple mechanisms of action including intercalation of DNA, which leads to inhibition of protein synthesis and free radical formation, and inhibition of topoisomerase enzymes. [7] The antioxidant enzymes are the backbone of the cellular antioxidant defense system. [3] Generally, there are several enzyme systems that catalyzes reactions to neutralize free radicals and reactive oxygen species including peroxidase and catalase. [10] Glutathione peroxidase (GPx) which is a key enzyme in the defense system against oxidative stress, was increased in group A compared with group C because this antioxidant enzyme reduce hydrogen peroxides, lipids and phospholipids hydroperoxides thereby damping the propagation of free radicals and reactive oxygen species that confirm lipid peroxidation [8;12], (table1). Conversely, the variation was less in group B compared with group C. A study has proven that several antioxidant enzymes including glutathione peroxidase (GPx) , were observed to decrease after long period of treatment with chemotherapy. A previous study has reported that taking high doses of these drugs causes an increase in peroxidation of unsaturated fatty acids of membrane phospholipids which leads to a decrease in the level of antioxidant enzymes like (GPx) and generates a

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high level of oxidative stress. [1] Selenium is an integral part of (GPx) and so it followed the same pattern of changes as GPx [22] (table 2). On the other hand , the increase of catalase activity in group A may be explained by the fact that Free radicals generate the reactions of lipid peroxidation related to breast cancer , free radicals are highly reactive and toxic. Biochemical systems in the first years of breast cancer have evolved an array of enzymatic antioxidant system mechanism like catalase (CAT) to combat the deleterious effect of free radicals, therefore catalase (CAT) activity must be increased because this enzyme play a key role in the detoxification of superoxide anion and hydrogen peroxide. [12; 13] In this regard , a present study has revealed that (CAT) activity was decreased after long period of treatment with chemotherapy indicating enhanced free radicals activity in breast cancer patients while the the antioxidant defense is weakened with the increase of the duration of the disease under taking chemotherapy. [22] Two studies suggested the increase of lipid peroxidation in solid tumors. [2] This study reflects the status of catalase activity in group B, ( table 1). Oxidative stress is a result of enhanced hydrogen peroxide production as well as other reactive oxygen species are accepted as causative factors of breast cancer. [2;3] Generally, cancer cells generate ROS and biochemically have low levels of antioxidant enzymes in most animal and human cancer. [22] In other words , the low activity of any antioxidant enzyme like catalase ( CAT) after long period of treatment with chemotherapy might be due to depletion of antioxidant defense system. [2,3] Recently, it has been shown that cyclophosphamide caused a decrease in (CAT) activity in female rats. [23] Data in (table 1) have also confirmed that malodialdehyde( MDA) level was highly significant increase in sera of group A compared with group C and this increase was continued with increasing the duration of this disease (group B) , (table 1). Malodialdehyde( MDA) level is found to be useful as a tumor marker in cancer patients because it is a reactive product for lipid peroxidation. [24;25;26;27]

Many trace elements play important roles in a number of biological processes through their action as activators or inhibitors of enzymatic reactions by competing with other elements and protein for binding sites by influencing the permeability of cell membrane or through other mechanisms. [28] A previous study has demonstrated the ability of selenium compounds to inhibit growth and induce tumor cell apoptosis has been suggested to be a potential mechanism for cancer chemoprevention [29]. This study agrees with our results related to selenium level in groups A and B ( table 2) . Further , results in (table 2) suggested that iron level

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was non significantly increase in sera of group A compared with group C. However , hematological abnormalities are reported in breast cancer cases. These may be due to the release of iron from its store, thus iron level elevated relatively, [30] but in the progressive cases (group B) iron was significantly decrease compared with group C , these results explained as because free iron is used for initiation lipid peroxidation which is correlates positively with the progression of breast cancer because of chemotherapy effects. [12] Lastly, zinc level was significantly decrease in sera of group A compared with group C and this decrease highly significantly continued to group B , in other words , this decrease correlates positively with the duration of the disease . A recent study has revealed that common zinc was found as ligands within proteins. [31] Zinc generally binds to proteins via coordination with electronegative atoms in the protein such as nitrogen oxygen and sulfur. [32] At this point, increase of lipid peroxidation associated with breast cancer causes a defect to the immune system and damage of metalloproteins, the result is the loss of a unique capability to retain high levels of zinc (Zn). [12, 14] Another recent study has reported that zinc deficiency results in immune dysfunction and promote inflammation. [33] Zinc deficiency can be associated with malignancy. [34]

### **Conclusions:**

- 1) Malondialdehyde level was increased in sera of Iraqi breast cancer women and this increasing directly correlated with the progression of the disease and the duration of the treatment with cyclophosphamide and doxorubicin ( four years).
- 2) Zinc level was decreased in sera in Iraqi breast cancer women and this decreasing directly correlated with the progression of the disease and the duration of the treatment with cyclophosphamide and doxorubicin ( four years).
- 3) From 1 and 2 malondialdehyde and zinc could be considered good biochemical markers for Iraqi breast cancer women through four years of the disease under treatment with chemotherapy (cyclophosphamide and doxorubicin).
- 4) Glutathione peroxidase, catalase , selenium and iron could be considered biochemical markers for Iraqi breast cancer women only in the first two years of the disease and under treatment with chemotherapy ( cyclophosphamide and doxorubicin).

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تقدير بعض الإنزيمات المضادة للأكسدة و العناصر النزرة في بعض النساء العراقيات

المصابات بسرطان الثدي .

د. رشا زهير جاسم

قسم الكيمياء ، كلية التربية للعلوم الصرفة (إبن الهيثم)، جامعة بغداد

### الخلاصة

قدرت فعالية انزيمي الكلوتاثايون بيروكسيديز و الكاتاليز و مركب المألون داي ألديهيد فضلا عن ثلاث من العناصر النزرة المتضمنة السلينيوم و الحديد و الزنك لدى مجموعتين من النساء العراقيات المصابات بسرطان الثدي A و B و تمت مقارنتها مع مجموعة من النساء الأصحاء (مجموعة ضابطة) (group C) . أشارت البيانات المستحصلة من هذه الدراسة إلى أن فعالية إنزيم الكلوتاثايون بيروكسيديز قد ارتفعت بشكل معنوي عالي و معنوي في كريات الدم الحمراء للمجموعتين A  $U / gm Hb (37.2 \pm 6.5)$  و B  $(30.5 \pm 5.1)$   $U / gm Hb$  على التوالي بالمقارنة مع المجموعة C  $(28.5 \pm 4.5) U / gm Hb$  ، بينما وجد أن فعالية إنزيم الكاتاليز قد ازدادت بشكل معنوي و معنوي عالي في كريات الدم الحمراء للمجموعة A  $(2.55 \pm 0.45) U / gm Hb$  و المجموعة B  $(2.16 \pm 0.25) U / gm Hb$  على التوالي بالمقارنة مع المجموعة C  $(1.65 \pm 0.25) U / gm Hb$  . فضلا عن ذلك أشارت هذه الدراسة إلى أن مستوى المألون داي ألديهيد قد ارتفع بشكل معنوي عالي في مصل دم المجموعتين A  $(12.2 \pm 4.2) mmol/L$  و B  $(18.6 \pm 5.6) mmol/L$  مقارنة مع المجموعة C  $(3.6 \pm 1.2) mmol/L$  . إن هذه النتائج أثبتت أيضا أن مستوى السلينيوم قد ارتفع بشكل معنوي في مصل دم المجموعتين A  $(65 \pm 9) ng/mL$  و B  $(59 \pm 10) ng/mL$  بالمقارنة مع المجموعة C  $(50 \pm 6) ng/mL$  ، أيضا لوحظ أن مستوى الحديد قد ارتفع بشكل غير معنوي في مصل دم المجموعة A  $(1.4 \pm 0.9) \mu g/mL$  و لكنه إنخفض معنويا لدى المجموعة B  $(0.9 \pm 0.1) \mu g/mL$  و بالمقارنة مع المجموعة C  $(1.2 \pm 0.5) \mu g/mL$  . كما أن مستوى الزنك إنخفض بشكل معنوي عالي و معنوي لدى المجموعتين A  $(0.77 \pm 0.21) \mu g/mL$  و B  $(0.66 \pm 0.22) \mu g/mL$  على التوالي بالمقارنة مع المجموعة C  $(0.98 \pm 0.14) \mu g/mL$  . من هذه الدراسة نستنتج أن مستويات المألون داي ألديهيد و الزنك تعد مؤشرات ( معايير ) كيموحيوية جيدة لسرطان الثدي بغض النظر عن فترة الإصابة بالمرض و فترة العلاج ، بينما فعاليت الكلوتاثايون بيروكسيديز و الكاتاليز و مستويات السلينيوم و الحديد يمكن أن تعتبر مؤشرات لهذا المرض فقط ضمن السنتين الأولى و الثانية من الإصابة بالمرض .

الكلمات المفتاحية كلوتاثايون بيروكسيديز ، كاتاليز ، مركب المألون داي ألديهيد ، العناصر النزرة ، سرطان الثدي