BIOCHEMICAL STUDY ON THE ACTIVITIES OF GLUTATHIONE PEROXIDASE, SUPEROXIDE DISMUTASE AND CATALASE IN WOMEN BREAST CANCER

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ABSTRACT

A total of 21 cases of breast cancer patients, aging from 35 to 65 years were selected from Benha University Hospital, Department of Surgery, including 7 cases with non metastatic breast cancer, 7 cases with metastaic breasat cancer and 7 cases with beinign breast tumors. The activities of glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), and catalase (CAT) were detected in breast tissues, in addition, selenium and zinc were also measured in serum samples collected from the same different cases. The obtained results revealed that, the activities of GSH -PX and CAT showed a highly significant increase in breast cancer tissue with and without metastasis compared to the control (benign tissue) group. Also a highly significant increase in the activity of SOD was observed in tissues of non metastatic and metastatic breast cancer compared to the control. On the other hand, the mean values of serum zinc concentration of breast cancer patients with or without metastasis was significantly decreased. Morevoer, serum selenium levels showed a significant decrease in breast cancer patients with or without metastasis compared to control group. From the obtained results, it can be concluded that, the deficiency of the above antioxidant enzymes and their cofactors (selenium for GSH-PX and zinc for SOD) may be increase the risk of cancer incidence and the presence of antioxidant supplementation in diet may be helpful to decrease the risk of breast carcinogenesis.

INTRODUCTION

In Egypt, data reported by Mokhtar (32), indicate that breast cancer ranked as number one (27.3%) among females. Breast cancer incidence rates as suggested by Soew *et.al.* (45) are likely to continue to increase more sharply in the future as women born after the mid -20^{th} century reach the high-risk age groups.

Considerable evidence suggested that low intake of vegetables modestly increases the risk of breast caner. Lu *et.al.* (27) reported that soybean consumption is associated with reduced rates of breast, prostate and colon cancers, which is possibly related to the presence of isoflavones that are weakly estrogenic and anti – carcinogenic compounds.

Cellular antioxidants include scavenger enzymes e.g SOD and GSH-PX; some fat – soluble vitamins e.g vit A and E and some water soluble low molecular weight substances e.g ascorbic acid, uric acid and glutathione. During normal oxygen metbolism SOD enzyme rapidly promotes the dismutation of superoxide into hydrogen peroxide and oxygen (9)the resultant hydrogen peroxide can be destroyed by either of two enzymes, catalase which removes H_2O_2 when present at high concentration and GSH – PX which removes H_2O_2 When present at steady state.

Superoxide dismutase, catalase and enzyme of glutathione redox cycle are the primary intracellular antioxidants and are considered to be preventive as primary antioxidants as they prevent free radical chain reaction by decreasing the available concentration of free radical to initiate the process (21).

In several different species of animals dietary copper deficiency leads to low Cu-Zn SOD activity in most tissues, including lungs, aorta, liver and erythrocytes. The activity in erythrocytes tends to parallel the copper concentration and activities of various copper – containing enzymes in tissues (4). These observations in animals led several investigators to use Cu - Zn SOD activity as an index of copper status in studies with humans. Some studies of experimental copper deprivation of adult humans have demonstrated that Cu - Zn SOD activity is decreased during copper deprivation and is restored when copper is added to the diet (31). Erythrocyte Cu - Zn SOD activity does not seem to be affected by age, gender or hormones (30).

Some studies, however, have suggested that some conditions that produce an oxidative stress tend to increase Cu - Zn SOD activity, even during periods of low copper intake (34).

Oxidative stress, resulting from the imbalance between pro-oxidant and anti-oxidant states, damages DNA proteins, cell membranes, and mitochondria seems to play a role in human breast carcinogenesis. Dietary sources of antioxidants (chemical) and endogenous antioxidants (enzymatic), including the polymorphic manganese superoxide dismutase (Mn – SOD), can act to reduce the load of oxidative stress (1). Catalase has been found in the cytosol and subcellular organelles such as the peroxisomes, which contain many of the enzymes that generate H_2O_2 in aerobic cells (24). However Catalase activity is inhibited in the presence of high concentration of Na cl (10).

Catalase has reductive activity only for small molecules such as H_2O_2 and methyl or ethyl hydroperoxide and does not metabolize large molecular peroxides such as lipid hydroperoxide products of lipid peroxidation (49). Catalase function is assumed to be the destruction of hydrogen peroxide formed by the action of aerobic dehydrogenases. Microbodies or peroxisomes are found in many tissues, including liver. They are rich in aerobic dehydrogenase and in catalase, which suggests that there may be a biologic advantage in grouping the enzymes which produce H_2O_2 with the enzyme that destroys it.

The glutathione redox cycle is a central mechanism for reduction of intracellular hydroperoxides. It complements catalase as a reducing system for hydrogen peroxide but exceeds catalase in its capacity to eliminate additional varieties of hydroperoxides (41).

GPX activity of various tissues have been conducted in relation to the effects of selenium deficiency on GPX, In studies of long term selenium deficiency, some decrease in GPX activity was observed in all body components, but the magnitude of decrease varies greatly for different tissues. There was rapid depletion in plasma and liver but little or no depletion in tissue, such as, lens and erythrocytes (6).

It was found that colon cancer caused by a decreased activity in glutathione peroxidase in erythrocytes which is caused by decreased absorption of selenium from the diseased colon(23).

Data on GSH-PX in human tumor tissues may be of relevance in cancer chemotherapy as many of anticancer drugs seem to destroy cancer cells by producing reactive oxygen species including hydrogen peroxide. Thus reactive

oxygen species destroy cancer cells via anticancer drugs which raise GSH-PX due to the oxidative stress caused (12).

Yucel et.al.(51) found that the mean serum zinc level in patients with breast cancer was significantly lower than the control group. They also found that neither serum copper and zinc levels nor the Cu/Zn ratio were of value in discriminating the disease activity and severity. They suggested that the further combined biological and epidemiological studies are necessary to investigate the roles of copper and zinc in breast cancer. Garfinkel (16) suggested that intracellular zinc deficiency may be a primary cause of aging process. Zinc appears to modify cell membranes. Zinc has been shown to inhibit collagen included platelet aggregation (11). Ma and Jiang (28) stated that it was shown that Cu and Zn and Cu / Zn ratio was unfortunately of no help in detecting early stage cancers and in differentiating them from benign diseases but was useful in predicting stages of cancers before operation for a better choice of therapy. Dorgan et.al.(14) stated that serum zinc levels were lowered in advanced colorectal cancer compared to controls, however, the serum copper and Cu / Zn ratio are of value in estimating the extent of the carcinoma as well as in determining the prognosis of these patients.

Besides the role of selenium in the maintenance of an optimal immune response in human (38), selenium also influences growth, muscular function, and the integrity of the liver (26). Selenium has been shown to have an antiproliferative, anti-inflammatory, antiviral and immune altering effects. Serum selenium levels in patients with seropositive rheumatoid arthritis were found to be significantly lower than those of healthy controls. It is speculated that selenium may modulate the effect of viral or other infections and in this way enhance the development or progression of rheumatoid arthritis (35) Also, the reactive oxygen radicals were trapped by the antioxidant selenium containing glutathione peroxidase which also inhibits the oxygenation of arachidinic acid to pro-inflammatory prostaglanding and leukotriens (22). Selenium is emerging as a dietary factor that proves to be of a major significance as prophylactic against cancer.

Among the suggestions proposed is the direct role of selenium in the liver mixed function oxidase (13). Selenium is a constituent part of the enzyme glutathione peroxidase which catalyses the conversion of hydrogen peroxide and organic hydroperoxides into water and corresponding alcohols, so, it serves as an antioxidant by protecting cells from radical damage (8-36).

MATERIAL AND METHODS

The present study was performed on 21 female patients aging 35 to 65 years ,divided into 3 equally groups:

Group I : Benign breast cancer

Group II : Non - metastatic breast cancer

Group III : Metastatic breast cancer

All the previous female patients were selected from the University Hospital at Benha. Small specimen of breast tissue was collected after surgery and the tissue was directly freezed in the deep freezer $(-20^{\circ}c)$ until the biochemical analysis were performed. For the determination of enzymatic activity in breast, 0.5 gm of tissues were homogenized in 4.5 ml buffer (potassium phosphate buffer PH 7.4, 0.1 mM EDTA) Then centrifugated at 3000 rpm and the supernatant was used freshly for determination of various enzyme activites (42). The breast tissue homogenates was used for determination of Cu/Zn superoxide dismutase activity, catalase activity and glutathione peroxidase activity (3,33,44).

Also venous blood samples were collected and the serum used for determination of zinc and selenium by Atomic Absorption Spectrophotometry (7-50).

Statistical analysis of the obtained results using t-test was performed according to Lewis (25).

RESULTS

Table (1) showed a highly significant increase (p < 0.01) in the mean values of SOD, CAT and GSH-PX in femal patients with non-metastatic and metastatic breast cancer as compared to benign (control).

Table 2 showed highly significant decrease in mean values of serum selenium and zinc in femal palients with non-metastatic and metastatic breast cancer when compared with benign (control).

DISCUSSION

The present data revealed a highly significant increase in the activity of SOD in non-metastatic and metaststic breast cancer. Similar results were recorded by Portakal *et.al.* (40) who observed that the activities of SOD, GSH-PX and catalase were significantly increased in tumor tissues. Also, Gharieb *et.al.*(2) reported that, the SOD activity observed in malignant mammary tissues

was significantly higher than that observed in benign tumours. Moreover, Sulayman et.al. (46) studied a total of 70 premenopansal females with breast cancer (stage I, II, III) and recorded a significant increase in the levels of SOD, GPX activities in cancer tissue .The increasing activity of SOD in tumor tissues may be due to the reactive oxygen species which increased in malignant cells and cause over expression of antioxidant enzymes (40). In malignant and metastatic breast cancer tissue catalase (CAT) was found to be significantly higher than that of the control (benign) group. The recorded result agreed with Portakal et.al.(40) and disagreed with Sulayman et.al (46) who found significant decrease in CAT activity in breast cancer tissue. The present study showed that the mean value of GSH - PX activity was significantly higher in both breast cancer patients with and without metastasis. This result agreed with the result of Perquin et.al.(39) who found that GSH activity was increased significantly in tumors. Furthermore Gromadzinska (18) found that the activities of GSH-PX in patients with breast cancer was increased significantly. Also Gharieb (2) found the same result in malignant breast cancer. This increase in the activity of GSH-PX may be due to reactive oxygen species which increased in malignant cells. Dissimilar results were recorded by Gorodzanskays et.al.(17) found that low SOD activity and high activity of GSH - dependent enzymes in tumors suggest that GSH-PX play a major role in peroxide utilization in malignant tumors (uterus, breast and ovaries) taking normal tissue instead of bening tumor as control.

The results of the present study may be attributed to the action of antioxidant enzymes which scavenging biologically reactive oxygen species by preventing their formation or reparing the damage they do. Antioxidants can act by removing oxygen on decreasing local oxygen concentration, removing catalytic ions, removing key reactive oxygen species such as superoxide, hydroxyl and peroxyl species, breaking the chain of an initial sequence and quenching and scavenging single oxygen (20).

Regarding to the level of serum zinc in malignant and metastatic breast cancer. It was found that the mean level of zinc significantly decreased when compared with control (benign) subjects. This result came in accordance to those reported by Yucel *et.al.* (51) who found that the mean serum zinc level in patients with breast cancer was significantly decreased. Also,Gaber (15), reported that serum zinc was insignificantly lowered in cancer breast group. This

decrease in serum zinc concentration may be due to the action of zinc as a cellular growth protector, including growth of neoplastic cells and its deficiency was demonstrated to be involved in several stages of malignant transformations (52).

Serum selenium levels showed significant decrease in breast cancer patients without and with metastasis. The results of the present study agreed with Mcconnell (29) who found that the difference note between the mean serum selemium concentration of breast cancer patients were found to be significant. Also (37) recorded significant decrease in serum selenium concentration in all investigated breast cancer patients. Moreover Borrela et.al. (5) found that there was a decrease in the selenium levels in women affected by breast cancer and that selenium and zinc have protective effect against breast cancer. On the other hand, Dorgan et.al.(14) did not observe any evidence for protective effects of selenium in breast cancer. This results could be attributed to the anti-neoplastic effect of selenium on breast cancer and supplementation of selenium enhanced chemotherapeutic effect of drugs used in cancer chemotherapy (47). Selenium has antioxidant as well as anti-proliferative properties, both of which may be related to its anti-carcinogenic properties (43,48). Also glutathione peroxidase is one of the most abundant antioxidant selenium containing protein in mammals.From the obtained results of the present work. it can be concluded that the of the above antioxidant enzymes and their cofactors (Selenium for GSH-PX and zinc for SOD) may increase the risk factor of cancer incidence in women.

Table 1. Mean values \pm S.E. of supeoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX) in femal patients with benign (control), malignant and metastalic breast cancer (u / mg protein).

Parameters	Control benign	Malignant	Metastaticr
SOD	2.99 <u>+</u> 0.3	7.45 <u>+</u> 0.88**	13.4 <u>+</u> 1.74**
CAT	29.62 <u>+</u> 3.89	58.87 <u>+</u> 7.28**	116.64 <u>+</u> 14.62**
SH-PX	0.72 <u>+</u> 0.06	1.1 <u>+</u> 0.09**	1.73 <u>+</u> 0.14**

** *Highly significant* (*P* < 0.01)

Mohamed R.R. et al., 2004 **Table 2**. Mean values \pm S.E. of serum selenium mg% and zinc mg % in control (benign) malignant and metastatic breast cancer.

Groups Parameters	Control benign	Malignant breast cancer	Metastatic breast cancer
Selenium Mg %	11.98 <u>+</u> 0.49	7.16 <u>+</u> 0.47**	6.74 <u>+</u> 0.42**
Zinc mg %	91.81 <u>+</u> 5.28	71.71 <u>+</u> 1.88**	52.5 <u>+</u> 1.29**

** Highly significant at $(P \leq 0.01)$

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دراسة بيوكيميائية على نشاط الجلوتاثيون بيروكسيديز والسوبر أوكسيد ديزميوتاز والكتاليز لسرطان الثدى فى النساء

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اجريت هذه الدراسة لقياس التغيرات البيوكيميائية لبعض مضادات الأكسدة فى سرطان الثدى ونشاط كلا من الجلوتاثيون بيروكسيداز والسوبر أكسيد ديزميوتاز والكتاليز فى نسيج الثدى وعواملهم المساعدة (الزنك والسيلنيوم) فى مصل الدم. وقد تمت الدراسة على سبع حالات مريضة بسرطان الثدى الغير منتشر وسبع حالات مريضة بسرطان الثدى المنتشر مع استخدام سبع حالات من أورام الثدى الحميدة كمجموعة ضابطة. تم اخذ العينات من مستشفى جامعة بنها ، قسم الجراحة وتراوحت أعمار المريضات ما بين الخمسة والثلاثون والخمسة وستون عاما وكانت نتائج هذه الدراسة كالآتى :

ارتفاع فى مستوى نشاط السوبر أكسيد ديزميوتاز فى نسيج مرضى سرطان الثدى المنتشر والغير منتشر ذو دلاله إحصائية مقارنة بالمجموعة الضابطة. كما أوضحت نتائج هذه الدراسة وجود ارتفاع ذو دلالة إحصائية فى نشاط الكتاليز فى كلا من مجموعتى المريضات مقارنة بالمجموعة الضابطة وكذلك وجد ارتفاع ذو دلالة إحصائية فى نشاط الجلوتاثيون بيروكسيداز فى كلا من مجموعتى المريضات مقارنة بالمجموعة الضابطة. وأظهرت النتائج انخفاض فى مستوى الزنك والسيلينوم فى مصل المريضات انخفاضا ذو دلالة إحصائية مقارنة بالمجموعة الضابطة.

يتضح من النتائج السابقة أن الارتفاع فى الإنزيمات المضادة للأكسدة فى نسيج الثدى المصاب بالسرطان المنتشر أو الغير منتشر يكون نتيجة زيادة عمليات الأكسدة فى نسيج الثدى المصاب كما أن النقص فى العوامل المساعدة لهذه الإنزيمات (السيليتوم والزنك) فى المصل من الممكن أن تزيد من خطر حدوث السرطان.