Clinical Significance of Antioxidants, Lactate Dehydrogenase and Alkaline Phosphatase in Breast Cancer Patients with and without Lymph Node Metastases

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ABSTRACT

Carcinoma of the breast is the most prevalent cause of mortality from cancer in women aged 40-69 years. It is assumed that the development of this cancer is a result of interactions between genetic factors and environmental factors. The aim of the present study was to examine the status of the antioxidants, superoxide dismutase (SOD) and glutathione (GSH) in the plasma of patients with breast cancer, also evaluated serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) to establish their diagnostic value in breast cancer with and without lymph node (LN) metastases. This study was conducted on 80 newly diagnosed breast cancer patients from National Cancer Institute, Cairo University with a mean age of 48.51 ± 3.75 years and who had not undergone any previous treatment for their tumors. The patients were clinically categorised as stage II (36 patients) and stage III (44 patients) infiltrative ductal carcinoma of the breast. The patients were divided into 36 breast cancer cases without LN metastases and 44 cases with LN metastases. Blood samples of all patients were collected before and after one month of mastectomy, besides 30 healthy subjects. There were significant increase in LDH, GSH and SOD and no significant increase in ALP levels preoperatively in cancer patients with and without metastasis in comparison to normal control subjects. Individual patient's data revealed that 50% of patients without lymph node (LN) metastasis had LDH above normal while 65% and 62% of patients had GSH and SOD above normal level, respectively. When these biochemical parameters were again analyzed one month after surgery, they showed a decreasing trend in most of cases. There were a significant decrease in levels of LDH, GSH and SOD when compared to preoperative values. From the results of the present study, we suggest that combination of antioxidant, SOD and Glutathione along with enzymes LDH, ALP could be used as important biochemical parameter for breast cancer patients, these are cost effective and can be easily assayed in smaller laboratories not yet exposed to any sophisticated technology for more reliable cancer marker.

Key Words: Breast cancer, glutathione, superoxide dismutase, lactate dehydrogenase, alkaline phosphatase

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INTRODUCTION

Carcinoma of the breast is the most prevalent cause of mortality from cancer in women aged 40-69 years. It is also the second leading cause of cancer death in women, after lung cancer (*Parkin et al., 1999, WHO, 1997 and Jemal et al., 2007*). The etiology of breast cancer is multifactorial. Hormonal, genetic, environmental factors and life style are implicated in the pathogenesis of breast cancer. Much remains to be understood, however, about the underlying mechanism for the observed associations with these risk factors. One unifying pathway that has been suggested is that of oxidative stress. Epidemiological evidence indicates that breast cancer risk is strongly associated with prolonged lifetime exposure to estrogens. Indeed, early age at menarche and late age at menopause both increase breast cancer risk, presumably by extending duration of estrogen exposure. Although an extensive body of the literature lends strong support to the involvement of estrogens in breast carcinogenesis, the exact mechanisms by which estrogens exert their carcinogenic effects remain ill defined. Estrogens are suggested to induce mammary carcinogenesis via two distinct mechanisms:

The estrogen receptor (ER)-dependent mechanism and the ER independent mechanism. The ER-dependent pathway of estrogen action encompasses cell growth and proliferation secondary to the binding of estrogen to the ER. In addition to stimulation of cell growth in normal cells, estrogens can promote the expansion of estrogen-sensitive neoplastic cells by way of ER-mediated processes. The ER-independent mechanism of estrogen-induced carcinogenesis relies on metabolic activation of endogenous estrogens by cytochrome P450 enzymes to generate highly reactive genotoxic metabolites. Recent data also suggest mitochondrial involvement in the generation of estrogen-associated reactive oxygen species (ROS). The ER-dependent and ER-independent pathways of estrogen action are suspected to act synergistically to exacerbate DNA damage, produce gene mutation and promote aberrant regulation of gene expression (*Singh et al., 2009*).

Normally, reactive oxygen derivatives are formed as by-products of normal metabolism and are eliminated

by antioxidant enzymes (SOD, catalase (CAT), glutathione peroxidases). Antioxidant defenses protect against free radicals, but these defenses are not completely adequate and systems that repair damage by ROS are also necessary (*Kostrykina et al., 2009*). While some ROS are necessary and play important physiological roles, ROS can also cause harm. Excess oxidative species can directly damage DNA, proteins and lipids. Furthermore, reactive oxygen species (ROS), such as superoxide anions and hydrogen peroxide induced lipid peroxidation, play a major role in malignant transformation and tumor cell proliferation and invasion (*Russo et al., 2000*). It has been hypothesized that the production of ROS in combination with a decrease in the activity of antioxidant enzymes may be characteristic of tumor cells. Antioxidants, both endogenous and exogenous, help to counteract the impact of ROS. Under normal conditions, a careful balance between oxidants and antioxidants exists in the body. Oxidative stress occurs when this balance is disrupted in favor of an excess of oxidative species. Oxidative stress is believed to play an important role in the development and progression of breast cancer. Three central enzymes in oxidative stress pathways are manganese superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX).

SOD enzymes are a class of oxidoreductase enzymes, which, in mammals, contain either Cu or Mn at the active site and catalyse the dismutation of superoxide, the one-electron reduction product of molecular oxygen. Superoxide dismutase (SOD) is a family of ubiquitous antioxidant metalloproteinases that catalyze the conversion of superoxide anion radicals to hydrogen peroxide and molecular oxygen (*Alvarez-Gonzalez, 1999*).

Glutathione is the major intracellular low-molecular-weight thiol that plays a critical role in cellular defense against oxidative stress in tissues and cells and may consequently play a role in the development of cancer (*Antrup, 2004*).

Lactate dehydrogenase (LDH) (EC 1.1.1.27), one of a group of enzymes that found in the blood and other body tissues and involved in energy production in cells. An increased amount in the blood may be a sign of tissue damage and some types of cancer or other diseases. LDH is a protein that normally appears throughout the body in small amounts, it is involved in anaerobic glycolysis. Many cancers can raise LDH levels, so it is not useful in identifying a specific kind of cancer. Measuring LDH levels can be helpful in monitoring treatment for cancer. Noncancerous conditions that can raise LDH levels include heart failure, hypothyroidism, anemia and lung or liver disease. Serum and tissue LDH is up-regulated in gynaecologic and breast malignancies and in a subset of benign conditions such as fibro- and cystadenomas. The release of LDH, however, in the bloodstream is partly related to the LDH gene up-regulation and is linked to poor prognosis. (*Koukourakis et al. 2009*)

Alkaline phosphatase (ALP) (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. The optimal pH for the activity of the E. coli enzyme is 8.0 while the bovine enzyme optimum pH is slightly higher at 8.5. It is sometimes used synonymously as basic phosphatase (*Harada et al., 1986*). In humans, alkaline phosphatase is a serum enzyme present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone and the placenta. The normal range is 20 to 140 IU/L. The skeletal isoenzyme originates in osteoblasts that release large amounts of the enzyme when bone repair activity occurs, for example with bone metastases. In cancer patients, ALP is a sensitive indicator of mild biliary obstruction, thus being a very sensitive indicator of liver progression. In a study conducted by the International Breast Cancer Study Group (IBCSG), ALP, aspartate transaminase (AST) and γ-glutamyltransferase (GGT) were examined for their sensitivity in detecting breast cancer recurrence. ALP alone was abnormal in a high proportion of breast cancer patients with bone metastases and/or liver metastases and was more effective than AST and GGT in distinguishing patients with relapse from those without (*Crivellari et al., 1995*).

In this study, the aim was to examine the status of the antioxidants SOD and glutathione in the plasma of patients with breast cancer, also evaluated serum lactate dehydrogenase and alkaline phosphatase to establish their diagnostic value in breast cancer with and without lymph node (LN) metastases.

PATIENTS AND METHODS

This study was conducted on 80 newly diagnosed breast cancer patients from Surgical Department, National Cancer Institute, Cairo University with a mean age of 48.51 ± 3.75 years. All patients had histollogically confirmed breast cancer. Also, it was documented the details of the disease including the histological grade, tumor type, vascular invasion, stage, number of excised lymph nodes, number of positive lymph nodes, type of surgery and estrogen receptor status were recorded as a routine for each patient. The patients were not using hormones, oral contraceptives and were non-smokers. All cases having co- morbid conditions in the form of diabetes mellitus, hypertension, rheumatoid arthritis and cardiac diseases or any liver or kidney impairments were excluded from the study. Controls consisted of 30 healthy members of the public with no previous history of breast cancer and other cancer-related diseases with mean age 47.7±4.6 years. Patients were divided into two groups; 44 patients with lymph node metastases and 36 patients without lymph node metastases.

All patients and control groups were subjected to standard evaluation included full history and clinical examination, blood chemistry, chest x- ray, CT scan whenever needed, Ultra sonographic examination (mammography)

and histopathological typing. Tumors staged according to the TNM classification (*Sobin and Fleming, 1997*) and graded using criteria recommended by the World Health Organization (*WHO, 1982*).

Blood samples were taken before surgery and a month after surgery. Blood was collected by venous arm puncture for patients and controls. Five ml of collected blood was injected into EDTA vaccutainets and the plasma was separated by centrifuging at 1,000 g for15 minutes, also five ml of fasting venous blood samples were left to clot at room temperature to separate sera after centrifuging for 10 minutes at 3000 r.p.m,

Laboratory investigations:

* Determination of Glutathione and SOD using the Randox kit (Randox Laboratories, San Francisco, CA, USA) (*Winterbourn et al., 1975*), (*Saydam et al., 1997*).

* Total alkaline phosphatase (ALP) activity measurement in serum according to the Recommended method of the Committee on the enzymes of the Scandinavian Society of Clinical Chemistry and clinical *Physiology* (*1974*). Total ALP activity was determined using a kinetic test at 37 °C with absorbance readings at 405 nm taken at 10-s intervals for 3 min, using p-nitrophenyl phosphate substrate

* Measurement of lactate dehydrogenase (LDH) activity in serum according to the Recommended method of the Committee on the enzymes of the Scandinavian Society of Clinical Chemistry and clinical *Physiology* (*1974*) using pyruvate as substrate at 37°C. the reaction was monitored at 340nm. The test was performed using the commercially available kit from Bio-Merieux Company, France.

Statistical analysis:

The data for biochemical analyses are expressed as mean and standard deviation (SD). Statistical comparisons were performed by Student's t-test using the Statistical Package for Social Sciences version 10.0

RESULTS

This study was conducted on 80 newly diagnosed breast cancer patients admitted to the Surgical Department, National Cancer Institute, Cairo University and who had not undergone any previous treatment for their tumors. Patients' profile regarding age, educational level, menopausal status and obesity were recorded. Pathological assessement of the tissue removed was done to determine the type of the tumor and its grade in the Pathology Depatrment according to *Bloom and Richardson* (*1957*). The size of the tumor and the number of lymph nodes were also determined. according to American Joint Committee of Cancer (*Beahrs et al., 1992*). The patients were clinically categorised as stage II (36 patients) and stage III (44 patients) infiltrative ductal carcinoma of the breast. Blood samples of 36 breast cancer cases without LN metastases and 44 cases with LN metastases were analyzed before and after mastectomy.

The general characteristics of breast cancer patients are presented in (Table 1).

The results of the study showed a significant increase in LDH, GSH and SOD and no significant increase in ALP levels preoperatively in cancer patients with and without lymph node metastasis in comparison to normal control subjects. Individual patient's data revealed that 50% of patients without LN metastasis had LDH above normal while 65% and 62% patients had GSH and SOD above normal level, respectively (Table 2, Figure 1). When these biochemical parameters were again analyzed a month after surgery, they showed a decreasing trend in most of cases. There was a significant decrease in level of LDH, GSH and SOD when compared to preoperative values (Table 2, Figure 2), however, although a month after surgery seems to be too short period and a longer follow up is needed to establish prognostic importance of these investigations.

Table 1: General characteristics of studied group.

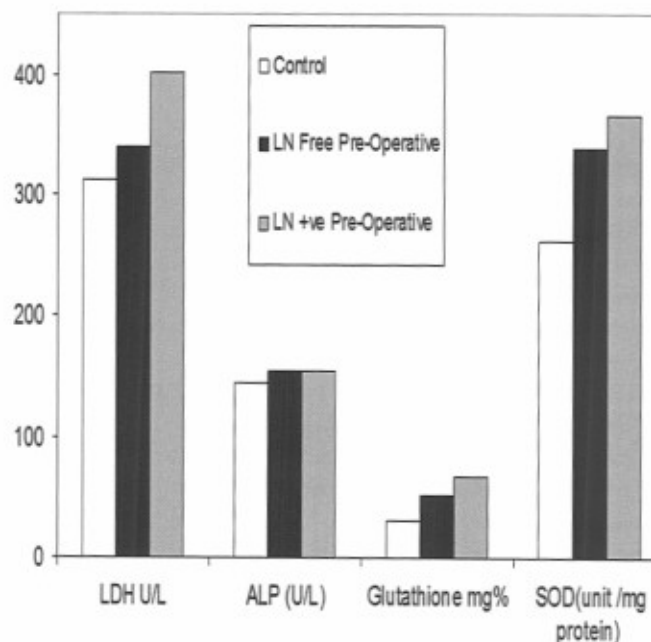| General characteristics	Breast cancer patients
Total number of subjects	80
Age range (years)	35-59
Age at menarche (years)	12–15
Menopausal status:	
Premenopausal	32
Postmenopausal	48
Cancer site:	
Left breast	56
Right breast	24
Pathological status:	
Infiltrative ductal carcinoma	80
Clinical stage:	
Stage II	36
Stage III	44

Figure 1: Comparison of different biochemical parameters in controls and in pre- operative groups of breast cancer.

Table 2: The Biochemical findings of the studied groups.

Clinicopathological parameters	LDH U/L M ± SD	ALP (U/L) M ± SD	Glutathione mg% M ± SD	SOD (unit/mg protein) M ± SD
Controls	312± 42.41	143.6 ± 21.47	30.04 ± 4.12	261.93 ± 31.07
LN Free Pre-Operative	339.92± 71.01*	154.32 ±26.27	52.52 ± 6.42*	338.8 ± 32.92*
LN free Post-operative	281.76 ± 41.18 *	138.24 ± 18.79	30.28 ± 4.66*	276.73 ± 22.49*
LN +ve Pre-Operative	401.41 ± 46.38*	154.88 ± 26.03	68.11 ± 9.77*	366.08 ± 41.17*
LN +ve Post-Operative	285.71 ± 30.34*	146 ± 28.27	40.85 ± 4.56*	271.71 ± 22.20*

LN : Lymph node. *P value showed significant increase when compared to controls, *P value showed significant decrease when compared to pre-operative group.
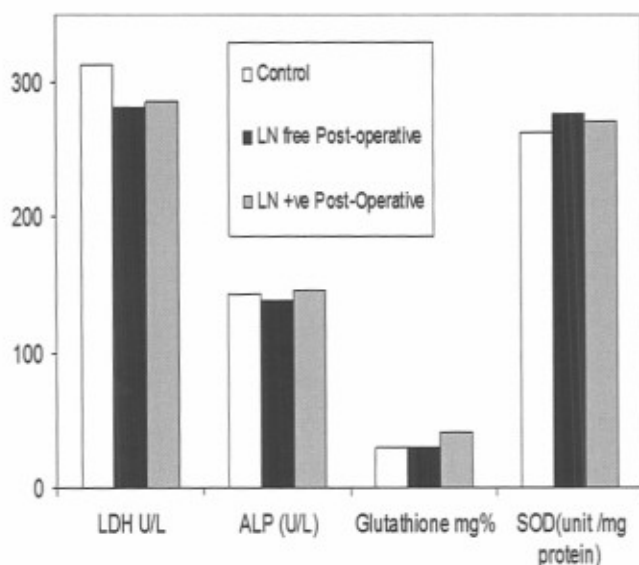

Figure 2: Comparison of different biochemical parameters in controls and in post- operative groups of breast cancer.

DISCUSSION

Breast cancer is the leading malignancy and also the leading cause of death from cancer in women (*Jemal et al., 2007*). The vast majority of these deaths are owing to metastasis. Although a number of significant advances have been made, the molecular mechanisms contributing to progression of breast cancer are poorly understood.

Glutathione transferases (GST) and glutathione peroxidases (GPx) are essential components of cellular detoxification systems that defend cells against reactive oxygen species (ROSs) (*Qian et al., 2009*).

In our study there was significant increase in antioxidant SOD and glutathione in cancer patients group with and without lymph node (LN) metastases and decreased after operation, this results were in accordance with *Zieba, et al. (2001)* who found that damage to the breast epithelium by oxygen free radicals (OFR) can lead to fibroblast proliferation, epithelial hyperplasia, cellular atypia and breast cancer. Studies have shown increased lipid peroxidation in solid tumors (*Skrzydlewska et al., 2001*). Tamoxifen therapy

in postmenopausal women with breast cancer reduced the increase in lipid peroxidation. Damage to mammary epithelium by reactive oxygen species can lead to fibroblast proliferation epithelial hyperplasia, cellular atypia and breast cancer (*Thangaraju et al., 1994*). Increased generation of oxygen free radical (OFR), such as O_2 and H_2O_2, can induce SOD and catalase (CAT). An increase in total and mitochondrial SOD activities due to over expression has been reported. Increased SOD mRNA expression was observed in cancer samples from patients with carcinoma of the breast. Higher activity of CAT has been documented in tumor cell lines compared to controls (*Ripple et al., 1997*).

Glutathione (GSH), an important substrate for GPx and GST, has been documented to have regulatory effects on cell proliferation (*Obrador et al., 1997*). Over expression of GSH has been reported in both animal and human tumours by us as well as by other workers (*Balasenthil et al., 2000 and Yang et al., 1997*) the lipid peroxidation level over expression of antioxidants has been documented in a wide variety of malignant tumours, including breast cancer (*Iscan et al., 2002*). Cancer cells with increased activities of antioxidant enzymes are presumed to escape recognition by cytotoxic lymphocytes (*Zachara et al., 1993*)

Glutathione as a reductant is very important in maintaining stability of erythrocyte membranes, its sulfhydryl group reduces peroxides formed during oxygen transfer and thus it provides protection against free radical injury (*Devlin, 1997*). The role of GSH in tumors is not clearly understood. Similar to the present study its level was reported to be elevated in malignant lesions (*ElSharabasy et al., 1993*), however contrary report are also available (*Kumaraguruparan et al., 2002*). Carcinogenesis begins with the interaction of carcinogen with SH groups of an enzyme system directly associated with cell division and this results in high level of free (acid soluble) SH which has role in mitotic initiation and cell division. The SH materials involved in carcinogenesis may be found within one of the system in which glutathione is reduced, oxidized or synthesized. If GSH is considered as the principal free SH group compound in cells and tissues, an increase in the level could be due to imbalance between the reducing and oxidizing system of glutathione (*Vaid and Shastri,1974 and Sharma et al., 2001*).

In this study LDH was significantly increased in cancer patients group with and without LN metastases and decreased after operation, these results agreed with *Konjevic, et al. (2001)* who reported that LDH increases in case of breast cancer due to spontaneous release of LDH from peripheral blood lymphocytes due to membrane damage and it is stage dependent, increases with clinical stage of breast cancer aside from reflecting natural killer cell depression and subsequently facilitate progression of the malignant process. *Konjevic, et al. (2002)* denoted that LDH is a prognostic factor in different malignancies as its increase reflects tumor mass and response to therapy. Serum LDH is the consequence of the disruption of the cell membrane of a large fraction of dividing malignant cells whose metabolic hallmark is anaerobic glycolysis that leads to increased LDH enzyme activity (*Koukourakis et al., 2009*).

In this study ALP enzyme showed no statistical increase in cancer patient groups compared to controls. Similar to the present study *Van Hoof, et al. (1992) and Steiber, et al. (1992)* did not find any significant difference in ALP levels in non-metastatic breast cancer while others have revealed a significant rise in ALP and äGT in metastasis suggesting involvement of bone and liver. The extent of rise in the parameters can be one of the criteria to establish its diagnostic value (*Ramaswamy et al., 2000*). Lamerz, et al. *(1993)* showed that LDH and GSH are nonspecific for diagnosis of metastasis. Although these are non-specific parameters and it is difficult to ascertain their diagnostic importance in cancer patients, yet their prognostic importance cannot be undetermined. They denoted that combination of ALP and äGT along with GSH could be used as important biochemical parameter for differentiation of breast cancer with and without metastasis. *Keshaviah, et al. (2007)* revealed that in cancer patients ALP is a sensitive indicator of mild biliary obstruction, thus being a very sensitive indicator for liver progression, thus it can be used in detecting breast cancer recurrence as ALP alone was abnormal in high proportion of breast cancer patients with bone and /or liver metastases.

CONCLUSION

From the results of the present study, we suggest that combination of antioxidant SOD, Glutathione along with enzymes LDH, ALP could be used as important biochemical parameter for patients with breast cancer these are cost effective and can be easily assayed in smaller laboratories not yet exposed to any sophisticated technology for more reliable cancer marker.

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