Australian Journal of Basic and Applied Sciences, 7(7): 234-239, 2013
ISSN 1991-8178

"Preliminary studies related to oxidant and antioxidant status and Lactate dehydrogenase (LDH) activity in Libyan breast cancer patients"

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Abstract: Aim: The present study was carried out to examine the status of the antioxidants, superoxide dismutase (SOD) and glutathione (GSH) in the plasma of patients with breast cancer. Serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were also measured to establish their diagnostic value in breast cancer with and without lymph node (LN) metastases. **Materials and Methods.** This study was conducted on 90 newly diagnosed breast cancer patients from Surgical Department, 7th October Hospital Benghazi, with a mean age of 47.81 ± 3.75 years. Controls consisted of 50 healthy members of the public with no previous history of breast cancer and other cancer-related diseases with mean age 46.4±3.6 years. Patients were divided into two groups; 44 patients with lymph node metastases and 46 patients without lymph node metastases. Blood samples taken before surgery and a month after surgery were analyzed for glutathione (G.SH), Super oxide dismutase (SOD) (Randox Laboratories). Serum ALP and LDH activities were measured using standard kits supplied bu Bio-Meriex Company, France) Statistical analyses were done using Student's t-test using the Statistical Package for Social Sciences version 17.0. **Results and Discussion.** The patients were clinically categorized as stage II (46 patients) and stage III (44 patients) infiltrative ductal carcinoma of the breast. The patients were divided into 46 breast cancer cases without LN metastases and 44 cases with LN metastases. 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 a careful combination of antioxidant G.SH and SOD activity along with enzymes like LDH, ALP could be used as important biochemical parameter for breast cancer patients.

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

INTRODUCTION

Breast cancer is the third most common cancer in women worldwide and accounts for the highest morbidity and mortality (Parkin *et al*. 2005). Based on epidemiological studies, it has been established that risk factors for breast cancer include age, reproductive events (menarche, menopause, pregnancy), exogenous hormones (oral contraceptive and hormone replacement therapy), lifestyle risk factors (alcohol,diet, obesity) as well as genetic factors (high- and low-penetrance breast cancer susceptibility genes) among others (Levil *et al*. 2007; Stotter *et al*. 2000). The increasing global incidence of breast cancer emphasizes the need to understand the various mechanisms involved in breast tumorigenesis. Increased anaerobic glycolysis and activity of antioxidant enzymes were shown to be characteristics of cancer cell metabolism (Yeh *et al*.2005; Seth, Kharb, Kharb. 2003 Anderson, 2008)

Lactate dehydrogenase (LDH) is released into the surrounding medium at an increased rate when cells replicate more rapidly (Mehreen and Khanam 2005). It was shown that LDH levels were higher in malignant than in normal tissue (10). LDH activity correlates with tumor mass or prognosis in human breast cancer. Metastatic breast cancer frequently exhibits elevated level of LDH as compared to normal breast tissue, but this observation is not consistent and still remains controversial (Mehreen and Khanam, 2005).

Reactive oxygen species (ROS)-induced cell damage has been implicated in malignant transformation (Gonzalez ,1999). The formation of free radicals is physiologically prevented or scavenged by host antioxidative defense mechanisms.

Low levels of essential antioxidants in the circulation have been associated with an increased risk of cancer (Diplock, 1991). Previously, impairment in antioxidant defense mechanisms has been demonstrated in a wide variety of malignancies including breast cancer (Arivazhagan, Kavitha Nagini 1997; Nagini and Saroja 2001;

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Ray *et al*. 2000). Basic and clinical studies showed that over-expression of manganese containing superoxide dismutase (MnSOD) may lead to changes of superoxide anion radical/ hydrogen peroxide (O2.- /H2O2) balance causing changes in the cell redox state. Changes in cell redox state affects signal transduction pathways that modulate cell proliferation (Weydert *et al* 200, 2006). Over-expression of Cu,ZnSOD also significantly decreases growth and survival of breast cancer cells (Nagini and Saroja, 2001).

Therefore, it is suggested that both MnSOD and Cu, ZnSOD can act as a tumor suppressors (Nagini and Saroja 2001;Chung *et al*. 2007) On the other hand, some studies have reported correlation between high MnSOD level and invasiveness of breast cancer (Kattan *et al*. 2008; Tsanou *et al*. 2004; Zhongkui *et al*. 2001).

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). The following preliminary study was carried out in Libyan patients with breast cancer patients to find out the antioxidant status as well as the activity of LDH as a marker of metabolic changes having their unique dietary habits, genetic predisposition and reproductive events.

MATERIALS AND METHODS

This study was conducted on 90 newly diagnosed breast cancer patients from Surgical Department, 7th October Hospital Benghazi, with a mean age of 47.81 ± 3.75 years.

The patients included in the study had a detailed history regarding the tumor type, vascular invasion, stage, number of excised lymph nodes, number of positive lymph nodes, type of surgery and estrogen receptor status.. The patients were not on hormones or oral contraceptives.

Patients with diabetes mellitus, hypertension, rheumatoid arthritis and cardiac diseases or any liver or kidney impairments were excluded from the study. Controls consisted of 50 healthy members of the public with no previous history of breast cancer and other cancer-related diseases with mean age 46.4±3.6 years. Patients were divided into two groups; 44 patients with lymph node metastases and 46 patients without lymph node metastases.

All patients and control groups were subjected to standard evaluation -clinical examination, blood chemistry, chest x- ray, CT scan whenever needed, Ultrasonographic examination (mammography) and histopathological typing. Tumors were staged according to the TNM classification (Sobin and Fleming, 1997) and graded World Health Organization guidelines (WHO, 1982).

Blood samples were taken before surgery and a month after surgery. These blood samples were analyzed for glutathione (G.SH), Super oxide dismutase (SOD) (Randox Laboratories), serum ALP and LDH activities were measured using standard kits supplied bu Bio-Meriex Company, France) Statistical analyses were done using Student's t-test using the Statistical Package for Social Sciences version 17.0.

Results:

Patients' profile regarding age, educational level, menopausal status and obesity were recorded. Pathological assessment of the tissue removed was done to determine the type of the tumor and its grade (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 categorized as stage II (46 patients) and stage III (44 patients) infiltrative ductal carcinoma of the breast. Blood samples of 46 breast cancer cases without LN metastases and 44 cases with LN metastases were analyzed before and after mastectomy.

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). 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), 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 the patients taken for the study

S.No	General Charactreristics	Breast Cancer Patients
1	Total number of subjects	90
2	Age (years)	35-60
3	Age at Menarche (Years)	12-14
4	Premenopausal	42
5	Postmenopausal	48
6	Left Breast	60
7	Right Breast	30
8	Pathological State Infiltrative ductal carcinoma	90
9	Clinical Stage Stage II	46
	Stage III	44

Table 2: Biochemical Parameters analyzed the patients

Parameters	SOD Unit/mg.protein	G.SH mg%	ALP (U/L)	LDH (U/L)
Controls	255.60± 31.06	30.05±4.12	144.60±21.45	315.00±42.45
LN-Free				
Preoperative	335.80±32.10*	53.50±6.45*	155.45±25.50	340.90±71.05*
Postoperative	285.80±30.10*	48.50±5.05*	135.45±21.45	278.85±62.05*
LN- Plus				
Preoperative	365.80±29.10*	65.50±4.05*	155.45±20.40	410.85±54.05*
Postoperative	275.80±24.10*	41.50±5.05*	145.45±19.40	280.85±54.05*

LN: Lymph Node *p value <0.05 when compared to controls
SOD: superoxide dismutase G.SH- glutathione ALP: alkaline phosphatase
LDH: lactate dehydrogenase

Discussion:

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 O2 and H2O2, 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).

In our preliminary studies a significant increase in antioxidant SOD and glutathione levels were observed in cancer patients with and without lymph node (LN) metastases and decreased after operation.

Glutathione (GSH), an important substrate for glutathione peroxidase (GPx) and glutathione S transferase (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 tumors by other workers (Balasenthil *et al*., 2000 and Yang *et al*., 1997). Over expression of antioxidants has been documented in a wide variety of malignant tumors, 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 (Danyelle *et al* 2003).

The role of GSH in tumors is not clearly understood. Its level was reported to be increased in malignant lesions (ElSharabasy *et al*., 1993) like the present study and in some other study it was reported to be reduced (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 preliminary study it appears that the levels of G.SH, SOD and LDH activities could be measured as possible biochemical parameters in laboratories that do not have sophisticated technology to measure tumor markers in breast cancer patients.

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