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RESEARCH ARTICLE

BIOCHEMISTRY

ENZYMATIC AND NON-ENZYMATIC ANTI-OXIDANT STATUS OF BREAST CANCER PATIENTS IN TAMILNADU

S. SIVAKUMAR*[1,2] AND S. NIRANJALI DEVARAJ[2]

[1]Department of Biochemistry, Sri Sankara Arts and Science College, Enathur 631 561.
[2]Department of Biochemistry, University of Madras, Guindy Campus, Chennai - 600 025, Tamilnadu, India.

*Corresponding author

S. SIVAKUMAR
Department of Biochemistry, Sri Sankara Arts and Science College, Enathur 631 561.
Department of Biochemistry, University of Madras, Guindy Campus, Chennai - 600 025, Tamilnadu, India.

ABSTRACT

Breast cancer is a global public health problem as it is the third most common cancer leading to the death of women worldwide. The aim of the study was to find out anti-oxidant status of breast cancer patients in Tamilnadu. This study has been conducted on breast cancer patients admitted to Government Cancer Hospital, Kanchipuram, Tamilnadu. Fifty breast cancer patients and normal persons of an equal number of age and sex matched disease-free healthy subjects were used for data analysis. The levels of antioxidants' status in breast cancer patients and healthy subjects were assayed using colorimetric methods. Enzymatic antioxidants like Superoxide Dismutase, Catalase, Glutathione Peroxidase, Glutathione Transferase and Non-enzymatic antioxidants like Vitamin C and E were decreased significantly. Reduced Glutathione levels also decreased. The low availability of peroxidizable substrates and antioxidant capacity concluded that the oxidative stress induced in breast cancer patients confer a selective growth of breast cancer cells.

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KEYWORDS

Breast Cancer, Anti-oxidants, Tamilnadu, Reduced Glutathione

INTRODUCTION

Breast cancer is one of the leading causes of cancer related death in women[1]. Breast tumors are classified as noninvasive or invasive, the majority (76%) belonging to the group of invasive ductal breast cancers. Patients can be staged using the clinical characteristics of tumor diameter (T), lymph node involvement (N) and the presence of distant metastases (M), hence the nomenclature TNM. A number of risk factors have been associated with development of the disease, including the dose and duration of estrogen exposure, early menarche, late menopause, age at first childbirth, nulliparity, fat in the diet, postmenopausal hormone replacement therapy, alcohol consumption, cigarette smoking and ionizing radiation staging[2].

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. Oxidative stress is the steady state level of oxidative damage in a cell, tissues or organ caused by the reactive oxygen species[3]. Oxidative modification of nucleic acids by reactive oxygen species is of remarkable biological importance, as it results in the transformation of nonmalignant cells into malignant ones[4]. Reactive oxygen species formed in various biochemical reactions are however scavenged by enzymatic and nonenzymatic antioxidants[5].

The objective of the present study is to investigate the levels of antioxidants status in normal human volunteers in comparision with breast cancer patients.

MATERIALS AND METHODS

Blood samples were obtained from fifty breast cancer patients admitted in Government Arginar Anna Memorial Cancer Hospital, Karapettai, Kanchipuram, Tamilnadu. Health and Family Welfare Department approved the sample collection after the recommendation of ethical committee of Government Arginar Anna Memorial Cancer Hospital, Karapettai, Kanchipuram, Tamilnadu. The breast cancers were histopathologically confirmed and the clinical stages of breast cancers were classified according to TNM (tumor, node and metastasis) system[6]. Blood samples were taken from disease free healthy subjects. The female patients within age group of 25-65 years were selected. After obtaining prior consent, venous blood was collected from the subjects under aseptic condition by venipuncture using 5ml sterile disposable syringe and needle. About 5ml of blood was collected with Ethylene diamine tetra aceticacid (EDTA). Plasma was separated by centrifugation at 3000rpm for 10minutes at room temperature. Packed cells remaining after the removal of plasma were washed with isotonic saline to remove the buffy coat. Then four milliliters of packed cells were washed thrice with isotonic Tris-HCl buffer 0.31M pH 7.4. Haemolysis was performed by pipetting out the washed red blood cell suspension into polypropylene centrifuge tubes, which contained hypotonic buffer (Tris–HCl buffer 0.015M pH 7.2). Erythrocyte ghosts were sedimented in a high speed refrigerated centrifuge at 2,000xg for 40 minutes. The supernatant haemolysate was decanted carefully and used for further analysis. The samples were stored at 4^0C before analysis

and all the samples were analyzed on the same day of collection[7].

The controls and patients were divided into two groups, namely group 1: Fifty healthy age matched controls; group 2: Fifty patients with histologically proven breast cancer. The venous blood samples obtained from these subjects were used for the estimation of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), and Glutathione-S-transferase (GST) in Erythrocytes and Vitamin C, Vitamin E and Reduced glutathione (GSH) in plasma.

The activity of superoxide dismutase (SOD) was determined by the method of Marklund and Marklund (1974) [8]. The activity of catalase (CAT) was assayed according to Sinha (1972) [9]. Glutathione peroxidase (GPx) levels were estimated in the hemolysate by the method described by Rotruck (1973) [10]. Glutathione-S-transferase activity was determined by the method of Habig and Jackoy (1981) [11]. The level of Vitamin C (ascorbic acid) was estimated by the method followed by Omaye (1979) [12]. Vitamin E concentration was estimated colorimetrically by the method of Baker and Frank (1968) [13]. The level of reduced glutathione (GSH) was measured according to the method described by Moron *et al.* (1979) [14].

STATISTICAL ANALYSIS

Statistical analyses were carried out on a personal computer with the use of the Statistical Package for the Social Sciences (SPSS) software version 15.0 (SPSS Inc. Chicago, IL, USA). The results were expressed as mean ± standard deviation (SD). Data were analyzed using student independent "t" test by applying mean comparison method. Significant differences were defined as two tailed $p < 0.001$.

RESULTS

Table 1 showed the levels of antioxidants. Variations in the levels were statistically significant. Antioxidant GST showed greater variation of elevation compared to others. GST levels found to have 82% fluctuation compared to control. It might clearly indicate its prime role in the prevention of development of cancer. SOD, GPx, VitC and VitE showed around 72% change in their levels. CAT activity provided 50% variation, which might be the least percentage of variation among antioxidants.

Table – 1
Anti-oxidant Levels

Parameters	Group 1 (Controls) n=50	Group 2 (Patients) n=50
SOD (units/min/mg of protein)	1.43 ± 0.005†	0.40 ± 0.03 *
CAT (mg/min/mg of protein)	1.54 ± 0.10	0.76 ± 0.03 *
GPx (mg/min/mg of protein)	17.96 ± 1.86	4.07 ± 0.675 *
GST (mg/min/mg of protein)	0.922 ± 0.159	0.159 ± 0.241 *
Vitamin C (mg/100ml)	1.008 ± 0.130	0.249 ± 0.008 *
Vitamin E (mg/1000ml)	32.50 ± 2.5	8.40 ± 0.821 *

† denotes data were expressed as Mean ± SD. * denotes significantly different from normal subjects, $p < 0.001$.

Table 2 showed level of reduced glutathione. The decrease in the level of reduced glutathione level was around 58%. Reduced glutathione level decreased because of its usage in replenishing the antioxidants like Vitamin C and Vitamin E, whose levels have decreased due to the increase in lipid peroxidation process.

Table – 2
Reduced Glutathione Level

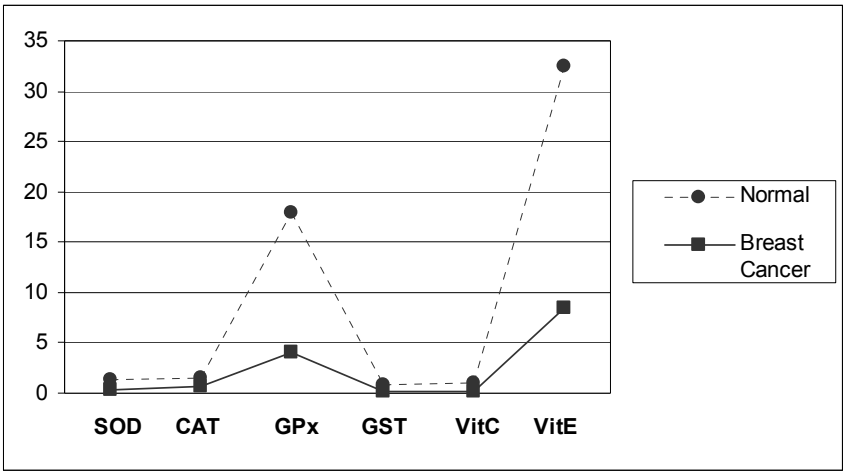
Parameters	Group 1 (Controls) n=50	Group 2 (Patients) n=50
GSH (mg/100ml)	9.37 ± 0.529†	3.86 ± 0.476 *

† denotes data were expressed as Mean ± SD. * denotes significantly different from normal subjects, p < 0.001.

DISCUSSION

Free radicals are highly reactive compounds that activate procarcinogens and alter the cellular antioxidant defense system, which includes enzymatic and non-enzymatic antioxidants such as SOD, CAT, GPx, GST, vitamin-C and vitamin-E, respectively. Due to excessive oxidative stress, cellular antioxidants are generally depleted. Enzymes such as SOD, CAT and GPx are considered to be the primary antioxidant enzymes, as they are involved in the direct elimination of active oxygen species. Secondary antioxidant enzymes (e.g., GST) help in the detoxification of ROS by decreasing peroxide levels (GST) or by maintaining a steady supply of metabolic intermediates (GR) for the primary antioxidant enzymes. Antioxidants have been shown to inhibit initiation and promotion in carcinogenesis, and counteract cell immortalization and transformation[15].

Graph 1
Levels of antioxidants in breast cancer patients and controls



Antioxidants levels were depicted in Graph 1. Superoxide dismutase activity found to have decreased around 70% in cancer patients when compared to control groups, corroborating with previous findings[16,17]. These antioxidant enzymes protect the erythrocytes against $O2^{\cdot -}$ and H_2O_2 mediated lipid peroxidation. The increase in erythrocyte lipid

peroxidation in breast cancer patients in the present study correlates with the decline in SOD activity. The SOD activity is decreased due to the increase in circulating lipid peroxides[17].

Catalase is also one of the primary antioxidant enzymes since it is involved in the elimination of O_2° and H_2O_2. Catalase activity reduced about 50% compared to control in cancer patients. The findings of Petit[18] and Kumaraguruparan[19] are in accordance with the results obtained in the present study. Catalase controls the hydrogen peroxide catabolism, which limits the action of peroxide. It was subsequently shown that the reduction of catalase activity enhanced the malignant progression[18]. The deficiency of catalase in the circulation of breast cancer patients may be due to increased utilization to scavenge lipid peroxides as well as sequestration by tumor cells. Thus determination of lipid peroxidation and antioxidants in circulartion may be useful in evaluating patients with benign and malignant tumors of the breast[19].

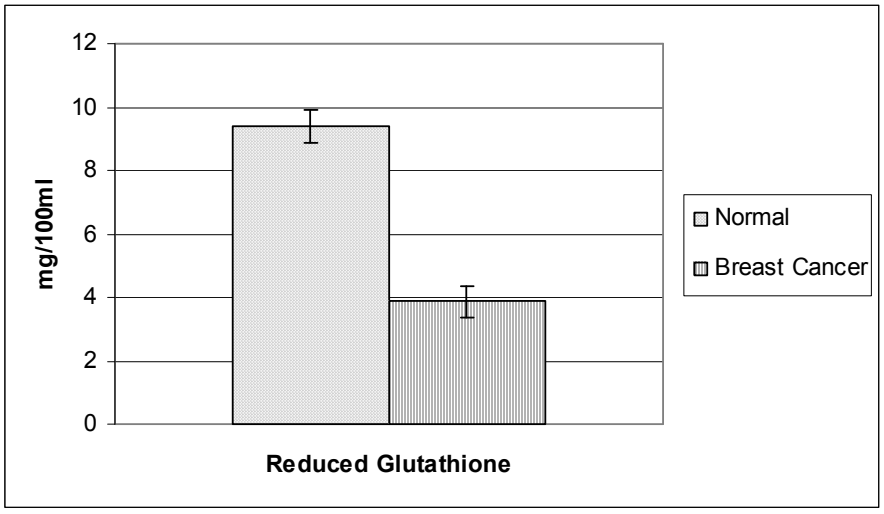
Glutathione peroxidase reacts with hydrogen peroxide thus preventing intracellular damage. Glutathione peroxidase use reduced glutathione as a substrate, plays a crucial role in protection against the deleterious effects of xenobiotics. Several studies have reported the decreased activities of Glutathione peroxidase in various cancerous conditions[20,21]. The depletion of Glutathione may be responsible for the decreased activity of Glutathione peroxidase due to low expression in fibroadenoma and breast cancer patients.

The activity of Glutathione-S-transferase decreased about 82% in hemolysate of breast cancer patients with respect to control. Glutathione-S-transferase also uses reduced glutathione as a substrate and plays an important role in protection against xenobiotic compounds. Glutathione-S-transferase involved in the detoxification of electrophilic toxins and carcinogens. Glutathione-S-transferase is the main enzyme involved in the glutathione redox cycle. Decreased Glutathione-S-transferase activity also reported in hepatic carcinoma[22].

Ascorbic acid, the major circulating water-soluble antioxidant, which acts as a free radical scavenger, can react with vitamin E radical to yield a vitamin C radical while regenerating vitamin E. A vitamin C radical is converted back to vitamin C by glutathione. The decrease in glutathione level might be responsible for the decreased level of vitamin C because of the unavailability of gluatathione for replerrishment of vitamin C radical. The level of vitamin C is decreased in the breast cancer patients[23,24,25,26].

Plasma Vitamin E levels significantly decreased in breast cancer patients compared with control groups. Vitamin E is the major lipid-soluble antioxidant and probably the most important non-enzymatic protective defense against lipid peroxidation. Vitamin E acts as powerful quencher of singlet oxygen and scavenger of free radicals[27]. The average plasma concentrations of α-tocopherol of breast cancer patients were significantly lower than those of control subjects[28].

Graph 2
shows Reduced Glutathione levels in breast cancer patients and controls



Low levels of glutathione (Graph 2) were observed in breast cancer patients compared to control. Reduced glutathione, an important substrate for glutathione peroxidase and glutathione-S-transferase, has been documented to have regulatory effects on cell proliferation. Glutathione synthesis is generally decreased in the breast cancer patients as previously reported[29]. Glutathione is very important in maintaining the stability of erythrocyte membranes. A decrease in blood glutathione in circulation has been reported in several diseases including malignancies. The lower reduced glutathione levels seen in breast cancer patients support the hypothesis that the glutathione status is inversely related to malignant transformation[30]. The regeneration of both vitamins E and C requires reduced glutathione. Deficiency of reduced glutathione may be responsible for low levels of these antioxidants in breast cancer patients. Several researchers have reported decrease in the levels of reduced glutathione in the breast cancer patients[31].

CONCLUSION

Breast cancer is the most common malignancy in women, affecting approximately one in eight women over their lifetime. As with most cancers, the incidence is much higher in older women than younger. For example, seventy-seven percent of new cases and eighty-four percent of deaths from breast cancer occurred in women with 50 and older. Breast cancer is by far the most common cancer in women, accounting for 18% of all cancers in the world among women. In conclusion, erythrocyte antioxidants activities are more closely related to breast cancer. So that it could be considered as biological markers of breast cancer. Present study results clearly showed reduction in the antioxidant levels so that dietary supplementation with natural antioxidant might be recommended to breast cancer patients in addition to normal diet and also for normal women as preventive measure. The extension of this work might play an important role in early and timely diagnosis of breast cancer and can result in good prognosis with effective treatment.

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