Lipid Peroxidation and Antioxidant Status in Breast Cancer Patients before and after Therapy

MOHAMAD A. EL-HEFNY, Ph.D.; SEVIL T. KARIMOVA, Ph.D. and ARIF M. AFANDIEV, Ph.D.

The Department of Biochemistry, Azerbaijan Medical University, Baku, Azerbaijan.

Abstract

Breast cancer, the third most common cancer worldwide, accounts for the highest morbidity and mortality. Oxidative stress has been implicated in playing a crucial role in the pathogenesis of a number of diseases, including breast cancer. Oxidative stress occurs due to an imbalance in prooxidant and antioxidant levels. The aim of this study is to examine oxidative stress and antioxidant status in patients with breast cancer and to investigate the changes of the lipid peroxidation and antioxidant status in the blood of breast cancer patients before and after therapy. The level of malondialdehyde (MDA), as an index of lipid peroxidation, was measured. Also, the activities of superoxide dismutase (SOD) and catalase (CAT) enzymes, as well as the level of reduced glutathione (GSH) was measured in groups of preoperative breast cancer patients (n=40), 3 weeks after surgery (n=30), 3 weeks after chemotherapy (n=30) and healthy female controls (n=35). The level of malondialdehyde (MDA) was significantly higher in all groups of breast cancer patients compared to controls (p<0.05). Similarly, antioxidant SOD in preoperative group significantly was increased (p<0.05). However, the levels of GSH and CAT activities were found significantly decreased in all groups of breast cancer patients compared to control subjects (p<0.05). In addition, there was no significant difference between preoperative, postoperative and after chemotherapy patients. In Conclusions, a poor antioxidant status and high oxidative stress are associated with breast cancer risk. There no difference in the lipid peroxidation and antioxidant status in the blood of breast cancer patients before and after therapy. Prospective studies in a larger population should be carried out to demonstrate our present findings.


Introduction

Breast cancer is one of the most common malignant tumors in women. Breast cancer has become one of the leading malignancies affecting women [1]. The etiology of breast cancer is still unknown. Reactive oxygen species (ROS) such as superoxide anion radical (O₂⁻), hydroxyl (OH⁻) and hydrogen peroxide (H₂O₂) are produced in aerobic metabolism [2]. The levels of free radical molecules are controlled by various cellular defense mechanisms, consisting of enzymatic (catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic (vit. E, vit. C, glutathione, carotenoids and flavonoids) components [3]. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This balance is essential for the survival of organisms and their health. An imbalance between the production and detoxification of ROS results in oxidative stress.

ROS has been implicated in the pathogenesis of certain diseases, including cancers [4,5]. ROS reacts with polyunsaturated fatty acids to induce the release of toxic and reactive aldehyde metabolites such as malondialdehyde (MDA), one of the end products of lipid peroxidation (LPO). MDA may be involved in tumor promotion because it can interact with the functional groups of a variety of cellular compounds [6]. To control the overproduction of ROS, the cells protect themselves against oxidative damage by antioxidant detoxifying mechanisms that help to lower ROS concentrations in the body.

Superoxide dismutase (SOD) catalyses the dismutation of the O₂⁻ into H₂O₂. While catalase (CAT) is responsible for the decomposition of hydrogen peroxide to oxygen and water [7]. GSH acts as a reducing agent that maintains enzymes in an active state and an antioxidant [8]. The main protective roles of glutathione against oxidative stress are: (i) glutathione is a cofactor of several detoxifying enzymes against oxidative stress; (ii) GSH participates in amino acid transport through the plasma membrane; (iii) GSH scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase; (iv)
glutathione is able to regenerate the most important antioxidants back to their active forms [8,9].

Lipid peroxidation level and antioxidant status in breast cancer patients after surgery and therapy remain unknown. To address this issue, we measured the circulating level of Superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA) in series of breast cancer patients and examined the effect of tumor removal and therapy on their preoperative levels.

**Material and Methods**

The present study has been based on 100 women with diagnosed breast cancer classified into three groups and 35 healthy volunteers. In the first group 40 women with preoperative breast cancer ranging in age from 31 to 65 years with the mean age of 48.6±7.2 years. In the second group 30 women with postoperative breast cancer three weeks after mastectomy ranging in age from 38 to 66 years with the mean age of 49.6±8.3 years. In the third group 30 women with breast cancer three weeks after chemotherapy ranging in age from 36 to 65 years with the mean age of 49.2±9.2 years. The patients and control subjects were non-smokers and they used no hormones or oral contraceptives. The patients were clinically classified as stage I (17, 14, 15 patients), stage II (20, 12, 12 patients) and stage III (3, 4, 3 patients) according to the TNM staging system (Table 1).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Preoperative</th>
<th>3 weeks postoperative</th>
<th>3 weeks after chemotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Total number of subjects</td>
<td>40</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>2- Age in years (mean±SD)</td>
<td>48.6±7.2</td>
<td>49.6±8.3</td>
<td>49.2±7.8</td>
</tr>
<tr>
<td>3- Menopausal status:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>24 (60.0%)</td>
<td>18 (60.0%)</td>
<td>19 (63.3%)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>16 (40.0%)</td>
<td>12 (40.0%)</td>
<td>11 (36.7%)</td>
</tr>
<tr>
<td>4- Clinical stage:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I (T1,N0,M0)</td>
<td>17 (42.5%)</td>
<td>16 (53.3%)</td>
<td>15 (50.0%)</td>
</tr>
<tr>
<td>Stage II (T1-2,N1,M0)</td>
<td>20 (50.0%)</td>
<td>12 (40.0%)</td>
<td>12 (40.0%)</td>
</tr>
<tr>
<td>Stage III (T1-3,N0-2,M0)</td>
<td>3 (07.5%)</td>
<td>2 (6.7%)</td>
<td>3 (10.0%)</td>
</tr>
</tbody>
</table>

Fasting blood samples were collected from the patients and the controls by venous arm punctures into EDTA tubes. The activities of SOD were measured by using whole blood. The plasma were separated by centrifugation at 3000rpm for 10min at 4°C and used for MDA and CAT determination. After the separation of plasma, the buffy coat layer was removed and the erythrocytes were washed 3 times with normal saline. Aliquots of washed RBC were prepared for the GSH status assay. Hemolyzed samples were excluded from the study.

The experimental data were expressed as mean ± standard deviation (SD). In this study, p values of p<0.05 were considered significant. Statistical analysis was performed using the STATGRAPHICS plus statistical package.

**Measurement of malondialdehyde (MDA)** in plasma was estimated by the thiobarbituric acid (TBA) method [10,11]. MDA, which is a stable end product of fatty acid peroxidation, reacts with TBA at acidic conditions to form a complex that has maximum absorbance at 532nm. 300 µl of the sample was mixed with 1.5ml of 0.05mol/l HCl and 0.5ml of 0.67% TBA and then mixed and boiled well in heated water at (95°C) for 30min. After cooling, the products were extracted in 2ml of 15% butanol and centrifuged at 2500rpm at (4°C) for 30min. The absorbance of the supernatant was determined at 532nm.

SOD activity was estimated by the commercial Ransod kit (Randox Laboratories, UK). The method is based on the generation of O2•− produced by xanthine and xanthine oxidase (XOD), which react with phenyl tetrazolium chloride to form a red formazan dye. SOD activity was measured by the degree of inhibition of this reaction. The results were expressed as U/g Hb.
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CAT activity was measured by monitoring the decrease in absorption of $\text{H}_2\text{O}_2$ at 240nm [12]. 100ml of the cell lysate was added to a 0.5ml quartz cuvette containing 400 $\mu\text{l}$ of 20mM $\text{H}_2\text{O}_2$ in PBS (25°C) and mixed thoroughly by pipetting. The absorbance was monitored immediately at 240nm every 2s for 2min. Catalase activity was measured for each sample and the rate in mAU/min/mg protein was averaged.

GSH status analysis was assayed according to the method of Serru et al. and Ciriaco et al. [13]. Blood samples were obtained by a venous arm puncture and the plasma was separated by centrifugation. After the separation of plasma, the buffy coat was removed and the packed cells were washed 3 times with physiologic saline. One hundred microliter aliquots of washed RBC were added to 300 $\mu\text{l}$ of ice cold 5% metaphosphoric acid (MPA). To precipitate proteins completely, the samples were vortexed and incubated on ice for 10min. After centrifugation by 12,000rpm for 10min at 4°C, the supernatants were then filtered through a 0.2 $\mu\text{m}$ filter and diluted 5 times before being injected into the capillary electrophoresis system.

Results

A summary of descriptive statistics for the examined blood samples values is shown in Table (2). The results illustrated that the levels malondialdehyde (MDA) were found to be significantly higher in all groups of breast cancer patients than those of the controls ($p<0.05$). The MDA levels are (18.69±1.86) for preoperative, (16.01±1.22) for postoperative and (14.48±1.54) for after chemotherapy with increasing percentages 131.88%, 99.88% and 80.77% respectively (see Fig. 1). Similarly, there was significant increase in SOD activity in preoperative group ($p<0.05$). In addition, there were increasing in postoperative and after chemotherapy patients but not significant ($p>0.05$). The SOD activity are (1095±350) for preoperative, (958±112) for postoperative and (946±198) for after chemotherapy with increasing percentages 35.86%, 16.86% and 17.37% respectively (see Fig. 2). However, the levels of GSH and CAT activities were found significantly decreased in the all groups of breast cancer patients compared to control subjects ($p<0.05$). The CAT activity are (49.1±18.4) for preoperative, (51.6±19.8) for postoperative and (50.7±21.2) for after chemotherapy with decreasing percentages 32.73%, 29.30% and 30.53% respectively (see Fig. 3). The levels of GSH are (1.29±0.19) for preoperative, (1.44±0.21) for postoperative and (1.50±0.18) for after chemotherapy with decreasing percentages 31.75%, 23.81% and 20.63% respectively (see Fig. 4). In addition, there were no statistically significant differences in the parameters between preoperative, postoperative and after chemotherapy patients ($p>0.05$).

Table (2): Levels of Lipid peroxidation and antioxidant status in the blood of patients with breast cancer

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=35)</th>
<th>Preoperative (n=40)</th>
<th>3 weeks postoperative (n=30)</th>
<th>Three weeks after Chemotherapy (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA ($\mu\text{mol/ml}$)</td>
<td>8.06±2.33</td>
<td>18.69±1.87*</td>
<td>16.01±1.22*</td>
<td>14.48±1.54*</td>
</tr>
<tr>
<td>GSH ($\mu\text{mol/ml}$)</td>
<td>1.89±0.12</td>
<td>1.29±0.19*</td>
<td>1.44±0.21*</td>
<td>1.50±0.18*</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>806±149</td>
<td>1095±350*</td>
<td>958±112*</td>
<td>946±198*</td>
</tr>
<tr>
<td>CAT (U/g Hb)</td>
<td>72.98±9.26</td>
<td>49.1±18.4*</td>
<td>51.6±19.8*</td>
<td>50.7±21.2*</td>
</tr>
</tbody>
</table>

*Values are expressed as mean±SD. *$p<0.05$; as compared to controls.

Fig. (1): Levels of MDA in the blood of patients with breast cancer.

Fig. (2): Levels of SOD in the blood of patients with breast cancer.
In correlation analysis, negative correlations were found between MDA and GSH levels ($r=-0.637$, $p<0.05$), MDA and CAT ($r=-0.665$, $p<0.05$) and a positive correlation was seen between CAT and GSH ($r=0.726$, $p<0.05$), MDA and SOD ($r=0.328$, $p<0.05$) in breast cancer patients.

**Discussion**

Increased oxidative stress and lipid peroxidation are implicated in carcinogenic processes. The magnitude of this damage (called oxidative stress) depends not only on ROS levels but also on the body’s defense mechanisms against them mediated by various cellular antioxidants [8,14].

Malondialdehyde (MDA), a product of lipid peroxidation which is the oxidation of polyunsaturated fatty acids in membranes induced by free radicals, is an indicator of oxidative damage. Many studies have examined the possibility of a connection between lipid peroxidation and cancer [6,15]. Higher plasma MDA levels have been reported in cancer patients than those in the controls [15]. However, lower lipid peroxidation measured by plasma thiobarbituric acid-reactive substances has also been reported in the breast cancer group compared to controls [16]. In the present study, our findings are in agreement with most of the earlier studies which suggested that there was a possibility of the accumulation of ROS which might result in significantly higher lipid peroxidation cellular and molecular levels.

Glutathione (GSH), as a reductant, is very important in maintaining the stability of erythrocyte membranes. It is implicated in the cellular defence against xenobiotics and a deleterious compound, such as free radicals and hydroperoxides [17]. GSH in the nucleus also maintains the redox state of critical protein sulphhydryls that are necessary for DNA repair and expression [8]. A decrease in blood GSH in circulation has been reported in several diseases including malignancies [18]. The lower GSH levels seen in breast cancer patients support the hypothesis that the glutathione status is inversely related to malignant transformation [19]. Several researches have reported decreases in the levels of GSH in the blood of the patients with breast cancer compared to those of the control subjects [20,21]. Our results showed that there were significant decreases in the levels GSH in the blood of the patients with breast cancer compared to those of the control subjects. The decrease in GSH concentration can be explained by decreased GSH synthesis and/or increased GSH consumption in the removal of peroxides and xenobiotics.

The cell has strong endogenous antioxidant defenses against increases in lipid peroxidation and ROS, among them, superoxide dismutase (SOD) and catalase (CAT) enzymes, which are the first line of defense against superoxide and hydrogen peroxide [22,23]. The significant increase in SOD activities indicates the formation of more superoxide radicals and their removal as SOD metabolizes superoxide radicals [24]. However, the significant decrease in CAT activities indicates the decomposition of $H_2O_2$ (forming $H_2O$ and $O_2$) [25]. Studies showed that oxidants may activate gene expression through the antioxidant responsive
elements (ARE) [26], which may explain the observation in the enhanced enzyme activities. Our data showed that there were significant increase in SOD activities and significant decrease in CAT activities in the patients with breast cancer than those of the controls. A substantial increase in GSH levels and CAT activity were found in the postoperative or/and after chemotherapy patients, which might be due to the free radical scavenging activity. Our data showed that, there was no difference in the lipid peroxidation and antioxidant status in the blood of breast cancer patients before and after therapy.

In Conclusions, breast cancer is related to increase of lipid peroxidation in plasma with concomitant decrease of antioxidant defense capacity. Overall, our data support the importance of endogenous antioxidant in the etiology of breast cancer across all levels of predicted risk. There no difference in the lipid peroxidation and antioxidant status in the blood of breast cancer patients before and after therapy. Prospective studies in a larger population should be carried out to demonstrate our present findings.

References
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