Lipid peroxidation and antioxidant enzymes status in pre and postmenopausal breast cancer

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ABSTRACT

The study was undertaken to assess the lipid peroxidation and antioxidant enzymes status in pre and post-menopausal breast cancer subjects. Three hundred fifty female human subjects (168 normal healthy & 182 breast cancer subjects) aged between 25-65 yrs. were selected for the study which were obtained from cancer hospital & research Institute (CHRI), Gwalior. All subjects were divided into four groups; group I (n=84) included premenopausal normal, group II (n=84) contained postmenopausal normal subjects, group III (n=91) included premenopausal breast cancer subjects and group IV (n=91) contained postmenopausal breast cancer subjects. The biochemical investigations e.g. GSH, lipid peroxidation, SOD, catalase & GPx were investigated in all group of subjects.

The GSH showed significant reduction (p<0.05) in group III & IV as compared to groups I & II respectively. The lipid peroxidation, SOD, catalase and GPx showed significant elevation (p<0.05) in group III & IV as compared to group I & II respectively.

The results of our study have shown higher oxygen free radical production & increased activities of antioxidant enzymes which may be a compensatory regulation of increased oxidative stress. Hence, the co-administration of antioxidants in the initial stages of the disease may be useful to the clinicians to manage the disease.

Key words: Breast cancer, premenopausal, postmenopausal, lipid peroxidation, antioxidants, Oxidative stress.

INTRODUCTION

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. It is caused by both external factors like tobacco, chemicals, radiation, and infectious organisms etc & internal factors like mutations, genetic factors, hormones, altered metabolic consequences etc. These factors may act together or in sequence to initiate or promote carcinogenesis. Breast cancer is a malignant (cancerous) tumor that starts from cells of the breast. It is the second commonest cancer of the world which comprises approx 19% of the total female cancers. According to American Cancer Society Report there will be more than 12 million new cancer cases every year worldwide, of which approximately 5.4 million will occur in economically developed countries and 6 to 7 million in economically developing countries.

The risk factors associated with breast cancer may exert their effects via generation of reactive oxygen species (ROSs), which are recognized to induce oxidative DNA damage and neoplastic transformation. ROS are being increasingly implicated in breast cancer development¹-³. Reactive oxygen species (ROS) such as superoxide anion radical (O₂⁻), hydroxyl (OH⁻), and hydrogen peroxide (H₂O₂) are produced in aerobic metabolism (Fang Yun-Zhong et al., 2002)⁴. An imbalance between the production and detoxification of ROS results in oxidative stress. 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.

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. Different antioxidant systems including nonenzymatic antioxidants such as glutathione (GSH), vitamins A, C, and E and various antioxidant enzymes defend against free radical attacks. Superoxide dismutase (SOD) catalyses the dismutation of the $\text{O}_2^-$ into $\text{H}_2\text{O}_2$. $\text{H}_2\text{O}_2$ is metabolized by catalase and glutathione peroxidase (GPx) is reduced into water and molecular oxygen. GPx reduces $\text{H}_2\text{O}_2$ and organic peroxides (ROO) while oxidizing GSH. Oxidized glutathione, GSSG, is reduced back to GSH by glutathione reductase (GRx) in the presence of NADPH.

As the etiology of breast cancer is multifactorial the significant breast cancer risk factors include age, early age at menarche, late age of menopause, late age at first pregnancy, obesity, oral contraception, hormone replacement therapy, diet, family history, lactation and prior history of benign breast disease. Some previous studies have shown alteration in above oxidative stress parameters with respect to the menopausal status of the breast cancer patients. The results remain complicated and controversial. Therefore the present study has been undertaken to view the Lipid peroxidation and antioxidant enzymes status in pre and postmenopausal breast cancer.

In the present study, the following parameters were assessed in the erythrocytes to elucidate the oxidant-antioxidant status in breast cancer patients. Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reacting substances (TBARS), which serves as an index of extent of lipid peroxidation. The reduced glutathione (GSH) level and the activities of antioxidant enzymes e.g. superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in erythrocytes were estimated.

**MATERIAL AND METHODS**

The present study was based on one seventy five newly diagnosed breast cancer patients (Ranging 25 years to 65 years). Age- and sex-matched healthy volunteers served as the control subjects, the breast cancer subjects were histopathologically proved. None of the subjects had concomitant disease such as diabetes mellitus, rheumatoid arthritis, liver disorder or any other malignancies. The subjects were clinically classified into four groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Eighty four pre-menopausal women serve as control</td>
</tr>
<tr>
<td>II</td>
<td>Ninety one post-menopausal women serve as control</td>
</tr>
<tr>
<td>III</td>
<td>Eighty four pre-menopausal breast cancer subjects</td>
</tr>
<tr>
<td>IV</td>
<td>Ninety one post-menopausal breast cancer subjects</td>
</tr>
</tbody>
</table>

**Chemicals and Kits**

The following chemicals were used in the study; Di thionitrobenzoic acid, phenazine methasulphate, nitroblue tetrazolium, NADH,

<table>
<thead>
<tr>
<th>Physical</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
<td>(n=84)</td>
<td>(n=91)</td>
<td>(n=84)</td>
<td>(n=91)</td>
</tr>
<tr>
<td>Age</td>
<td>30.71± 1.47</td>
<td>54.66±1.95</td>
<td>33.43±1.08</td>
<td>57.33±1.76</td>
</tr>
<tr>
<td>Weight (kg.)</td>
<td>65.33 ± 5.21</td>
<td>56.9± 3.65</td>
<td>60.8±3.9</td>
<td>58.8±4.12</td>
</tr>
</tbody>
</table>

Values are expressed mean ±SE
sodium dodecyl sulphate, Folin-cholchatae reagent (Sisco Research Laboratories Pvt. Ltd.), choloroform, ethanol, n–butanol, pyridine, acetic acid, , sodium azide, trichloro acetic acid , tris-HCl, sodium pyrophosphate, sodium potassium tetrata , sodium carbonate (Ranbaxy Fine Chemicals Ltd), , thiobarbituric acid, sodium chloride, potassium dichromate, sodium dihydrogen phosphate, disodium hydrogen phosphate, reduced glutathione, sulphosalicylic acid (Himedia Laboratories. Pvt. Ltd.).

Sample Collection and preservation
The blood Samples were collected from the patients and controls by venous arm punctures in to plain and EDTA vials. (an anticoagulant, 2 mg/ml) and stored at –20°C for biochemical investigations.

Haemolysate preparation
The plasma and buffy coat were removed from whole blood by centrifugation at 3000 rpm for 10 minutes at 4°C. The red cells were washed thrice with normal saline and haemolysate was prepared by mixing packed cell volume (5%) in distilled water.

Biochemical assays
Blood reduced glutathione (GSH) by Ellman17, Thiobarbituric acid reactive substances (TBARS) Lipid peroxidation by Ohkawa et al.,18, Catalase activity by Sinha19, Superoxide dismutase (SOD) activity by Winterbourn et al.,20, Glutathione peroxidase by Rotruck et al., 21.

Statistical analysis
Data are expressed as means ± SE. Data comparisons were carried out using one way analysis of variance (Sigma Stat, statistical software, version 3.5). The values were considered to be significant when the p value was less than 0.05.

RESULTS
Table 1 shows status of physical parameters in pre & postmenopausal normal and breast cancer subjects.

Fig. 1 shows that comparison of GSH and lipid peroxidation level in pre & postmenopausal normal and breast cancer subjects. The GSH level was significantly reduced (p<0.05) in group III & IV subjects as compared with control group I & II respectively. Whereas Lipid peroxidation level was significantly increased (p<0.05) in group III & IV in comparison with group I & II respectively.

Fig.II indicates the comparison of SOD, Catalase and GPx activity in both pre & post menopausal stages of normal and breast cancer subjects. The group III and IV subjects showed significant elevation (p<0.05) in SOD, catalase and GPx as compared with group I & II .

DISCUSSION
The clinical study revealed comparison of oxidative stress markers in pre and postmenopausal normal and breast carcinoma subjects. The GSH level reduced in both (pre & postmenopausal) subjects as compared with normal control. Glutathione, as a reductant, is very important in maintaining the stability of erythrocyte membranes. It is implicated in the cellular defense against xenobiotics and deleterious compounds, such as free radicals and hydroperoxides22. Thus, GSH contributes to the reduction of mitochondria-damaging peroxides (catalyzed by GSH peroxidase), which results in prevention of the injury caused by oxidative stress. A decrease in blood GSH in circulation has been reported in several diseases including malignancies23. The results of our study are in support of several previous studies, according to which, the lower GSH levels in breast cancer patients support the hypothesis that the glutathione status is inversely related to malignant transformation.

In the present study, the lipid peroxidation product i.e. MDA levels have been increased significantly in erythrocytes of the patients with breast cancer compared to controls. MDA is an indicator of oxidative damage. Many studies have examined the possibility of a connection between lipid peroxidation and cancer24-25. Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. Similar reports of elevated MDA levels have been reported
Table 1: Comparative values of reduced glutathione and lipid peroxidation levels in pre and post menopausal normal and breast cancer subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (U/gmHb)</th>
<th>Group II (U/gmHb)</th>
<th>Group III (U/gmHb)</th>
<th>Group IV (U/gmHb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced glutathione (GSH)</td>
<td>2.3</td>
<td>1.94</td>
<td>1.47 *</td>
<td>1.57 #</td>
</tr>
<tr>
<td>Lipid peroxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed mean ± SE
Units: GSH- (U/gmHb); Lipid peroxidation-µg/ml
* p<0.05 (values significantly differ when compared with Group I)
# p<0.05 (values significantly differ when compared with Group II)

Fig. 1: Comparison of GSH and lipid peroxidation levels in pre and postmenopausal normal and breast cancer subjects

Table 2: Comparative values of SOD, catalase, and GPx activities in pre and postmenopausal normal and breast cancer subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I (IU/mg protein)</th>
<th>Group II (IU/mg protein)</th>
<th>Group III (IU/mg protein)</th>
<th>Group IV (IU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>209.41</td>
<td>221.37</td>
<td>301.25 #</td>
<td>334.29 *</td>
</tr>
<tr>
<td>Catalase</td>
<td>44.20</td>
<td>39.11</td>
<td>53.43 *</td>
<td>51.72 #</td>
</tr>
<tr>
<td>GPx</td>
<td>27.3</td>
<td>26.4</td>
<td>33.4 *</td>
<td>31.4 #</td>
</tr>
</tbody>
</table>

Values are expressed mean ± SE
Units: Catalase-(IU/mg protein); SOD- (U/gmHb); GPx- (U/ 10 mg Hb)
* p<0.05 (values significantly differ when compared with Group I)
# p<0.05 (values significantly differ when compared with Group II)

Fig. 2: Comparison of SOD, catalase, and GPx activity in pre and postmenopausal normal and breast cancer subjects

Abbreviations: GSH- Reduced glutathione
SOD- Superoxide dismutase
GPx- Glutathione peroxidase
in patients with malignant breast tumour\textsuperscript{26-29}. 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 at cellular and molecular levels. Hence it may be considered for use as a surrogate biomarker for cancer risk.

SOD, CAT and GPx are considered as primary antioxidant enzymes, since they are involved in direct elimination of ROS. They can also act as anti carcinogens and inhibitors at initiation, promotion/ transformation stages in carcinogenesis. Superoxide dismutase (SOD) and catalase (CAT) catalyze the detoxification of superoxide anion ($O_2^-$) and hydrogen peroxide ($H_2O_2$), respectively, and protect the cell against ROS-induced damage\textsuperscript{30}. Glutathione peroxidase (GPx, EC1.11.1.9) plays a central role in the defense against free radicals, peroxides, and a wide range of xenobiotics and carcinogens\textsuperscript{31}. In our study, SOD, Catalase and GPx activities were found to be significantly elevated in pre & post menopausal breast cancer subjects as compared to their respective control groups. The antioxidant enzyme activity may be elevated to counter oxidative stress.

**CONCLUSION**

The results of our study have shown higher oxygen free radical production & increased activities of antioxidant enzymes, which may be a compensatory regulation in response to increased oxidative stress. As Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions hence, the treatment with antioxidants in the initial stages of the disease may be a useful therapy to prevent the oxidative damage. The results suggest the necessity for therapeutic co-administration of antioxidants along with conventional drugs. However, more studies may be required to substantiate the results and arrive at a definite conclusion in terms of safety and efficacy of adding antioxidant therapy for the treatment of carcinoma of breast.

**ACKNOWLEDGMENTS**

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**REFERENCES**


