

## **Assessment of oxidative stress in breast cancer patients: a hospital based study**

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### **ABSTRACT**

**Background:** Oxidative stress occurs as a result of the disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. This is regarded as a possible etiological factor in production of breast carcinoma, which is the most common cancer among the females both globally as well as in India. Oxidative stress also plays an important role in progression of cancer breast. This study was conducted to compare the levels of superoxide dismutase (SOD) and malondialdehyde (MDA) in breast cancer patients and normal age matched female participants.

**Methods:** This study was conducted in AVBR Hospital, Sawangi (Meghe) among 30 female breast cancer patients admitted in the surgery ward for treatment after being diagnosed with the disease, who attended the hospital from July 2016 to June 2017. Breast cancer patients of stage I and II were included in the study. Patients suffering from any other diseases apart from breast cancer like diabetes, hypertension, thyroid illnesses and other sort of cancers are excluded from the study. For each of the study participant, age and sex matched controls were selected who were mostly the relatives of patients attending the hospital and were devoid of any illnesses. Venous blood sample was collected from all the patients as well as the controls that were used for determination of the levels of SOD (superoxide dismutase) and MDA (malondialdehyde). Written informed consent was taken from all the study participants before drawing the blood samples.

**Results:** Authors have measured the markers of oxidative stress in breast cancer patients after diagnosis of breast cancer and compared these blood levels with those of the controls. The mean MDA (nmol/ml) levels for the breast cancer patients and the controls were 26.14 and 15.83 respectively, while the SOD (U/gm Hb) levels were 0.12 and 0.16 respectively and the difference of the mean were statistically significant ( $p$  value  $<0.01$ ).

**Conclusions:** There is presence of increased oxidative stress in breast cancer patients as compared to the healthy controls.

**Keywords:** Breast cancer, Malondialdehyde, Oxidative stress, Superoxide dismutase

### **INTRODUCTION**

Breast cancer is the most common cancer among women in both the developed and the developing countries in the world with an estimated 1,671,149 new cancer cases diagnosed in 2012, which accounts for 25 % of all the cancers. With an estimated 521,907 cases of deaths due to

breast cancer occurring in the world (2012), breast cancer ranks first as the cause of death from overall cancers in the world. For the less developed countries, breast cancer is the most frequent cause of cancer death among the women.<sup>1,2</sup> The risk of breast cancer increases with early menarche, late menopause, late age for the first pregnancy, obesity, dense breast tissue, oral contraceptive use,

hormone replacement therapy, addiction to alcohol and tobacco, positive family history.<sup>3</sup> Apart from these, a number of genes, like BRCA1 and BRCA2, and p53 have been linked to breast cancer susceptibility and development.<sup>4</sup>

Etiology of breast cancer is multifactorial which includes genetic, environmental, social, demographic and hormonal factors. Evidences are there to prove the occurrence of the disease as a result of increased oxidative stress.<sup>3,5</sup> Free radicals are the chemical species possessing an unpaired electron and are very reactive. They are produced in cells either as by-products of metabolism, or during phagocytosis in the extra-nuclear compartment by the mitochondrial respiratory chain and the mixed function oxidase system. These free radicals cause damage to the cellular structure like lips, proteins and nucleic acids leading to severe abnormalities.<sup>6,7</sup>

Antioxidants are compounds that react with free radicals either in vivo or in vitro and prevent oxidative damage. Endogenous antioxidant defenses are both enzymatic such as the superoxide dismutase, glutathione peroxidases, glutathione reductase and catalase; while non-enzymatic antioxidants namely vitamin C, vitamin E and vitamin A which play a main role in protecting the body against the harmful effects of free radicals and prevents excessive oxidative damage. Exogenous antioxidants are mostly the nutritional antioxidants that directly neutralize the free radicals, reduce the peroxide concentrations and repair oxidized membranes and ultimately decrease the production of reactive oxygen species via lipid metabolism.<sup>8</sup> Under normal circumstances, there is a steady state between the oxidants and the antioxidants inside the human body. Oxidative stress is a process which occurs due to a disturbance in the balance between the production of a reactive oxygen species (ROS) or free radicals (singlet oxygen, superoxide anions, hydrogen peroxide and hydroxyl radical) and the efficiency of the antioxidant defense systems like glutathione and enzymes such as superoxide dismutase (CuZn-SOD, Mn-SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRx) and glutathione-S-transferase (GST).<sup>9</sup> The oxidative damage to both the nuclear DNA and the mitochondrial DNA may lead to mutations that activate oncogenes or inactivate tumor suppressor genes.<sup>10</sup> Amin KA et al, have reported the involvement of ROS in etiology and development of breast cancer.

They have studied the effect of breast cancer on the level of various enzymes with antioxidant activities and biomarkers for oxidative stress and found an increased NO and lipid peroxidation in the patients of breast cancer with decreased antioxidant effects of catalase and reduced glutathione (GSH). This occurred as a result of free radical generation, which resulted in damage at cellular level. Similar observations were also found in the studies conducted by Kasapovic et al, and Prabasheela et al, where the blood levels of catalase and superoxide dismutase were found to decrease that indicated increased activity of the

free radicals in the breast cancer patients and decreased level of antioxidants as they are utilized for quenching free radicals.<sup>4,11,12</sup>

This study was thus undertaken to assess and compare the oxidative stress in breast cancer patients with age and sex matched controls. Aim was to assess the oxidative stress in breast cancer patients and to compare it with that of healthy controls.

## METHODS

It was observational study.

### *Locus of study*

The present study was carried out in the department of surgery, Acharya Vinoba Bhave Rural Hospital of Jawaharlal Nehru Medical College. All the biochemical procedures were carried out in Central research laboratory, DMIMS (DU), Sawangi (Meghe), Wardha.

### *Study subjects*

#### *Study group*

Thirty patients of carcinoma breast of operable stages (stage I and Stage II) of all the age groups attending the surgery department for treatment from 15th October 2015 to September 2017 were included in the present study.

#### *Control group*

Thirty age and sex matched healthy volunteers were enrolled as controls. Written informed consent was taken from all the study participants.

#### *Inclusion criteria*

Diagnosed cases of female breast cancer patients coming for treatment who consented were included. We have taken only Stage I and Stage II breast cancer in our study as we were interested to see the change in oxidative stress among the early breast cancer patients.

#### *Exclusion criteria*

Patients of cancer breast with associated chronic illness such as diseases altering antioxidant levels in healthy and cancer patients (like diabetes, hypertension, myocardial diseases, renal diseases, pancreatic diseases, pulmonary diseases, on hormone therapy and pregnancy) and patients with fibro adenoma breast or any previous treatment for breast carcinoma were excluded from this study. Patients who did not give written informed consent.

#### *Methodology*

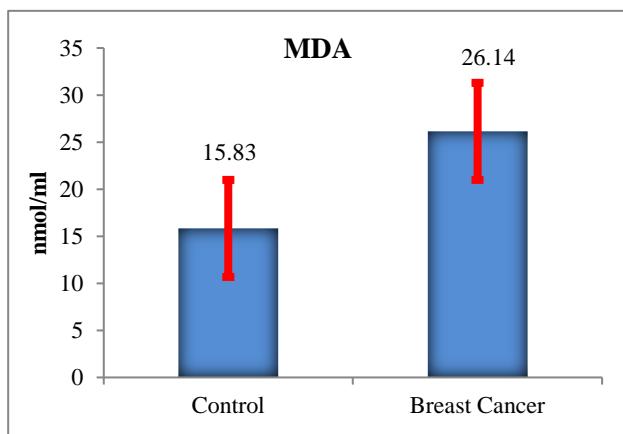
- Venous blood sample was collected from the breast cancer patients of stage I and II fulfilling our

inclusion criteria at diagnosis, and oxidative stress (Superoxide dismutase (SOD) and Malondialdehyde (MDA)) was measured.

- Blood samples were collected from the 30 healthy female controls for measuring the level of MDA and SOD.
- MDA level was measured by thiobarbituric acid (TBA) method and SOD level was measured by inhibition of autoxidation of Pyrogallol (Marklund M).

## RESULTS

The mean age of the breast cancer patients was  $52.53 \pm 9.43$  years and those of the control group was  $53.97 \pm 10.06$  years respectively. There was no statistically significant difference in between the mean ages of control and study group. We have measured the markers of oxidative stress in breast cancer patients after diagnosis of breast cancer and compared these blood levels with those of the controls. The MDA (nmol/ml) levels for the breast cancer patients and the controls were 26.14 and 15.83 respectively (Figure 1), while the SOD (U/gm Hb) levels were 0.12 and 0.16 respectively (Figure 2). The difference of the mean between the MDA and Sod levels of the breast cancer patients and the controls are found to be statistically significant ( $p$  value <0.01).

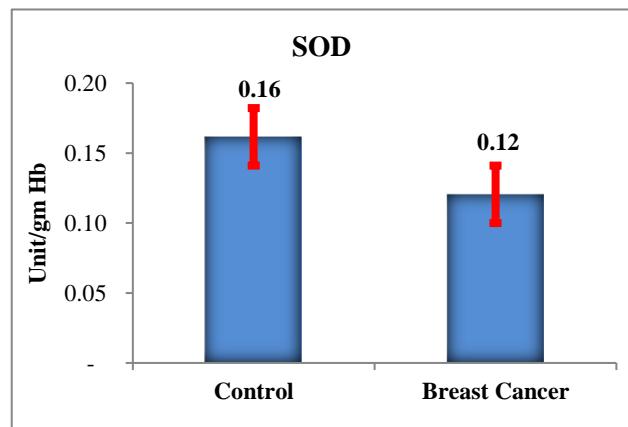


**Figure 1: Levels of MDA in the blood of breast cancer patients and control representing healthy subjects. Values represent the mean  $\pm$ SEM.**

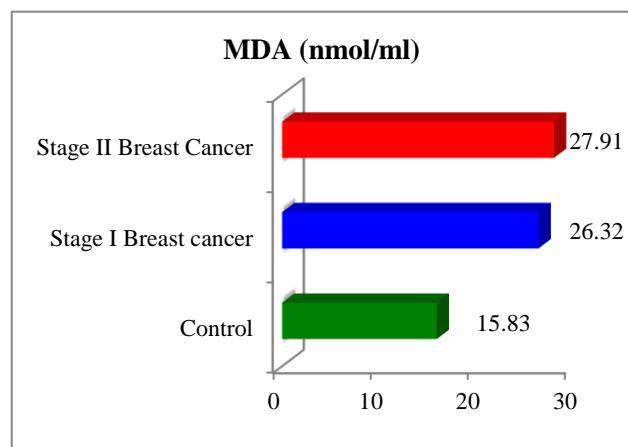
Authors have taken cases of early breast cancer in our study to see the changes in the oxidative stress occurring in hem as compared to the healthy controls. Figure 3 shows the comparison of the MDA levels among stage I and stage II of breast cancer as compared to controls, where we found the mean MDA values are 26.32, 27.91 and 15.83nmol/ml respectively. The levels of MDA are increasing as the stage of breast cancer increases, but this difference was not found to be statistically significant ( $p$  value >0.05).

Similarly, Figure 4 shows the comparison of the SOD levels among stage I and stage II of breast cancer as

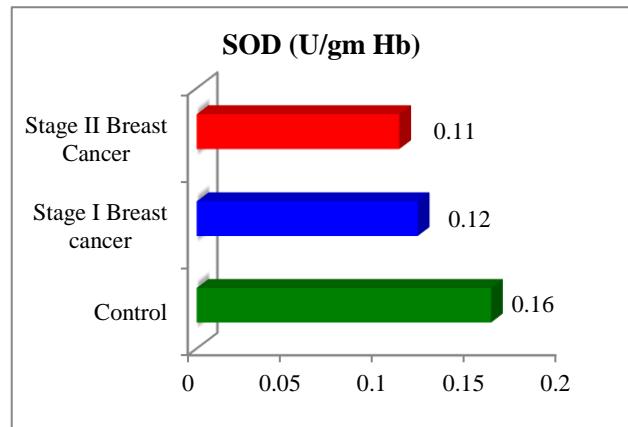
compared to controls, where we found the mean SOD values are 0.12, 0.11 and 0.16U/gm Hb respectively. These levels of SOD are decreasing as the stage of disease increases, and this difference was found to be significant statistically ( $p$  value <0.05).



**Figure 2: Levels of SOD in the blood of breast cancer patients and control representing healthy subjects. Values represent the mean  $\pm$ SEM.**



**Figure 3: MDA (nmol/ml) level in the early breast cancer as compared to control.**



**Figure 4: SOD (U/gm Hb) level in the early breast cancer as compared to control.**

## DISCUSSION

Oxidative stress produced through either increased free radical generation and/or a decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis. It has been found that the reactive oxygen species are involved in initiation and promotion of cancer.<sup>14,15</sup>

In our study to compare oxidative stress in carcinoma breast patients we have included 30 healthy age matched females in control group (mean age 53.97 years) and 30 patients of carcinoma breast of which stage I patients were 57 % and stage II patients were 43%. Mean age of study group was 52.53 years. In the current research, we studied both the MDA and SOD levels in the blood of breast cancer patients at diagnosis of breast cancer, we have estimated the SOD and MDA level in normal subjects.

In this study, we found that MDA levels were elevated in the serum of patients with breast cancer. As far as breast cancer is concerned, enhanced oxidative stress in tissues and serum has previously been reported. Some authors have demonstrated increased lipid peroxidation and increased MDA-DNA adducts have been detected in mammary tissues of breast cancer patients.<sup>16,17</sup>

In the study, conducted by Kumaraguruparan R et al, and Portakal O et al, 66 found that the MDA levels were significantly raised in the breast cancer patients.<sup>18,19</sup> Hristozov D et al, also came up with a similar finding, where the MDA levels increased significantly among the cancer patients when compared with the control.<sup>20</sup> A way of analyzing the oxidative stress is by quantification of the lipid peroxidation by measuring the different biomarkers of oxidative stress like MDA or Malondialdehyde, which is a low molecular weight aldehyde, which is produced as a result of the lipid peroxidation in the aerobic organisms that is generated through several enzymatic and non-enzymatic reactions.<sup>19</sup>

The mean level of antioxidant SOD in the controls was 0.16 Unit/gm Hb and the breast cancer patients at the time of diagnosis was 0.12 Unit/gm Hb in the present study and this reduction in SOD may be related to carcinoma breast induced oxidative stress. This difference of mean is also significant statistically (p value <0.01), similar results of reduced level of SOD was observed by Hristozov D et al, who compared the SOD level in cancer patients at different stages with the control and benign disease. Though they did not find any significant difference between the benign disease and the control group, but the SOD levels decreased significantly in all the stages of carcinoma breast when compared with the normal individual.<sup>20</sup>

Super oxide dismutase (SOD) is an enzyme that helps in catalyzing the potentially harmful superoxide radical ( $O_2^-$ ) in the cells that prevents tissue damage.<sup>21,22</sup> Under the conditions of oxidative stress, as in cases of breast cancer, increased concentration of reactive oxygen species

initiates damage to many biomolecules, catalysis of these harmful radical by SOD leads to decreased levels of this protective antioxidant.<sup>23</sup>

The knowledge of significantly increased oxidative stress in the breast cancer patients as compared to the healthy individual that we have gathered from the present study may be utilized in developing strategies for preventive and curative aspects of breast cancer.

## CONCLUSION

Our study concluded that the oxidative stress in patients of carcinoma breast is increased as compared to healthy controls as evidenced by increased malondialdehyde (MDA) and decreased levels of superoxide dismutase (SOD) in breast cancer patients.

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