

RESEARCH ARTICLE

BIOCHEMISTRY

**ANTIOXIDANT AND LIPID PEROXIDATION STATUS IN PRE AND POST OPERATIVE BREASTCARCINOMA.**



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**ABSTRACT**

Antioxidant enzymes considered as therapeutic agents for variety of disorders associated with oxidative stress. Serum Superoxide dis mutase, Catalase, Lipid peroxidation were quantified in 50 histopathologically proved Breast cancer patient in order to evaluate the changes in serum antioxidants status in pre and post breast carcinoma. When the data's were compared with age matched healthy female, the level of antioxidant were found to decrease in patients with Breast cancer due to increased oxygen species. The levels of Catalase & Superoxide dis mutase was found to increase after surgery, showing improvement in treatment except few of the patients which showed no change in level indicating a chance of recurrence. The level of lipid peroxidation was significantly higher in Breast cancer patients and decreased after surgery. However further elaborate clinical studies are required to evaluate the role of such antioxidant enzymes in Breast cancer management.

## KEY WORDS

Breast Carcinoma, Recurrence, Prognosis, Antioxidant, Oxidative stress, Metastasis.

## INTRODUCTION

Breast carcinoma is one of the most common neoplasm in women and is leading cause of cancer related death world wide. The aetiology of breast cancer is multi factorial. Significant breast cancer risk factor include age, early age at menarche, late age of menopause, late age of first pregnancy, obesity, oral contraception, hormone replacement therapy, diet, family history, lactation and prior history of benign breast disease<sup>1</sup>.

Antioxidant enzyme represents an important defense mechanism against oxidative stress. Moreover up regulation of antioxidant enzyme may confer a selective advantage for growth of tumor cells as compared to normal cells. Experimental evidence reveals that ROS (reactive oxygen species) are involved in initiation and promotion of carcinogenesis, where inactivation or loss of certain tumor suppressor genes is occurred<sup>2, 3</sup>. Biological system has evolved an array of enzymic & non-enzymic antioxidant defense mechanism to combat the deleterious effects of oxygen free radical. Excess generation of oxygen free radical can cause oxidative damages to biomolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis<sup>4</sup>. Superoxide dismutase (SOD) and Catalase (CAT) play a key role in the detoxification of superoxide anion and hydrogen peroxide respectively. We therefore examined the extent of lipid peroxidation, by the formation of thiobarbituric acid reaction substance and status of antioxidants SOD and CAT in the serum of pre and post operative breast carcinoma patients.

## MATERIALS AND METHODS

Blood samples were collected from

Aringar Anna Cancer Institute, Kancheepuram from 50 clinically and histopathologically proven breast cancer patients and compared with age matched control. The patients were not using hormones, oral contraceptives and were non-smokers. None of them had secondary disorder. Informed consent was obtained from all the participants. The human ethics committee of office of the Director, Govt. Aringar Anna Memorial cancer Hospital, Regional Cancer Center, Karapettai, Kancheepuram vide Ref.No.262/E1/08 has approved the study. Controls consisted of members of the public with no previous history of breast cancer and other cancer related diseases.

Blood samples were collected, centrifuged for 15 minutes at 3000rpm and the serum was separated and stored at 4°C for analysis. Lipid peroxidation was estimated by measurement of thiobarbituric acid reactive substances (TBARS), conjugated di- enes and hydro peroxides in serum by Ohkawa *et al*(1979) method. The pink colored chromogen formed by the reaction of two thiobarbituric acid with breakdown product of lipid peroxidation was read at 532 nm.

Superoxide dis mutase was assayed by following the inhibition of autooxidation of epinephrine spectrophotometrically at 480 nm by Misra Friedovich method.

Catalase activity was estimated by measurement of chromic acid in serum at 620 nm by Sinha method. The data for biochemical analysis are expressed as mean and standard deviation(SD). Statistical comparisons were performed by one way ANOVA.



## RESULT

### 1. Mean age and body weight distribution

Table 1

S.No	Parameters	Control	Patient
1	No, of Persons	50	50
2	Mean age	46 ± 4.3	44± 2.5
3	Mean body weight	61.8 ± 5.5	62.5 ± 5.7

The mean body weight of breast cancer patient was 61(range 50 - 74) which alters slightly during the study. Values are mean standard duration of 50 breast cancer patients and controls.

### 2. Lipid peroxidation status (mean ±SD, n=50)

Table 2

Parameters	Controls	Before Surgery	After Surgery
TBARS(nmol/100mg protein)	124.32 ± 9.45	139.24 ±14.9*	131.58 ±11.2*
CD(µmol/hemoglobin)	0.48 ±0.02	0.64± 0.05**	0.58± 0.04**
LOOH(nmol/100mg protein)	0.44± 0.06	0.60± 0.08**	0.51± 0.02**

\* $p < 0.005$ ; \*\* $p < 0.001$  as compared to respective controls.

### 3. Antioxidant status (mean ±SD, n=50)

Parameters	Controls	Before Surgery	After Surgery
SOD(Unit/mL)	3.2 ± 0.51	1.7 ± 0.16**	2.1 ±0.52**
CAT(Unit/min/dL)	5.2± 0.94	2.8 ± 0.85**	4.0 ± 0.64**

\*\* $p < 0.001$  as compared to respective controls.

## DISCUSSION

Oxidative stress is considered to be implicated in the path of physiology of breast cancer<sup>5</sup>. Oxidative stress is caused by imbalance between the production of reactive oxygen and biological systems ability to readily detoxify the reactive intermediate or easily repair the resulting damage<sup>6-10</sup>. However it is also postulated that transformed cells appeared to generate more reactive oxygen species (ROS) than their normal counter part and in fact cancer cell exist in condition of increased oxidative stress associated with oncogenic transformation,

alteration in metabolic activity and consequently increased generation of ROS. Studies showed increased lipid peroxidation in solid tumors<sup>11,12</sup>. Peroxidation of membrane lipids affect fluidity between intracellular and extra cellular environment, integrity of other molecules in cell membrane and if the perturbation is severe, the cell wall collapse, causing apoptosis. The increase in lipid peroxidation in pre and post operative breast cancer patient in the present study was associated with reduction in antioxidant capacities. Protection against lipid peroxidation and other damage consist of two major part enzymatic and non enzymatic antioxidant<sup>13</sup>.



Increased oxidative stress and lipid peroxidation are implicated in carcinogenesis process<sup>14</sup>. Our results lend credence to these reports. Elevated levels of lipid peroxidation products supported the hypothesis that the tumoral cell produces the large amount of free radicals and that there exist a relationship between free radical activity and carcinogenesis. In the present study lipid peroxidation level after surgery was significantly lower than level of sample before surgery indicating disease prognosis.

The antioxidant enzyme Superoxide dismutase and Catalase are the backbone of the cellular antioxidant defense system<sup>15</sup>. The low activity of these antioxidant enzymes might be due to depletion of antioxidant defense system. The activity of the hydrogen peroxide detoxifying enzymes Catalase was significantly decreased in the more metastatic line<sup>16</sup>. In the present study also the catalytic activity was found to be lower in breast cancer patients before surgery when compared to normal subject. The level of Catalase showed a significant increase in breast cancer patient after surgery. Zigman and Colleagues<sup>17</sup> suggested that O<sub>2</sub> and OH might be involved in the decreased Catalase activity elicited by UV-A exposure. Keno and Fridovich<sup>18</sup> have reported inactivation of Catalase by O<sub>2</sub>.

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SOD provides the first line of cellular defense and protects the cell against superoxide anion mediated lipid peroxidation. SOD activity was significantly lower in high grade tumor than low grade tumor<sup>19</sup>. Increased SOD mRNA expression was observed in cancer samples from patients with carcinoma of the breast<sup>20</sup>; in contrary to our study, which showed decreased level in breast cancer patient before surgery and significantly the level increased in 45 of patient after surgery except 5 of the patient which showed no significant change indicating a chance of recurrence. Further clinical studies are required before a definitive conclusion to be drawn.

## CONCLUSION

From the data analyzed in our study, it was found that the antioxidant level decreased in breast cancer patient due to increased ROS. The levels were found to increase after surgery, except 5 of the patient which showed no change in their level indicating a chance of recurrence. Thus the Antioxidant status in breast cancer patient proved to be the best biomarker for early diagnosis, prognosis and metastasis.



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