 

# A Study on the Antioxidant Defense System in Breast cancer Patients.

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All the authors have contributed towards the work carried out in this study .

**Abstract:**
Hospital based case control study carried out for reduced Glutathione levels (GSH), Superoxide dismutase activity (SOD), Total Antioxidant Potential (AOP), Malondialdehyde (MDA) and Nitrate levels in a major referral hospital in Kashmir, North India involving patients with breast cancer (N=40) and healthy Controls (N=20). Patients with history of drug use or some other similar disorder which might influence antioxidant enzyme activity were not included in the study. The results were analyzed statistically using the Student's *t*-test for unpaired variables. MDA, SOD and nitrite levels were increased in breast cancer patients as compared to controls ($p<0.005$, $P<0.01$ and $P<0.05$) respectively. GSH, and AOP levels in plasma of breast cancer patients were low as compared to healthy voluntary controls ($P<0.005$ and $p<0.005$). Increased oxidative stress in patients is indicated by increased SOD activity and is evident by depleted GSH levels and AOP.
**Key words:** Reduced glutathione (GSH), thiobarbituric acid (TBA), superoxide dismutase (SOD), Antioxidant potential (AOP), Malondialdehyde (MDA).

## Introduction:

Breast cancer is one of the most common cancers in women of developed and developing countries (1). Oxidative stress caused by increased free radical generation and/or decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis (2-4). Free radicals are formed in major physiological and pathological conditions in mammalian tissues wherein structural changes in lipids,

 

proteins and nucleic acids are consistently brought (5). In healthy conditions at cellular level, a subtle balance exists between the free radical generation and the antioxidant defence systems. Reactive Oxygen Species (ROS) are essential for various normal physiological processes like cell differentiation (6), apoptosis (7), cell immunity (8) and cellular defense against microorganisms at low concentrations (9). Excess generation of these oxygen free radicals generate a phenomenon called oxidative stress which cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis. In mammalian systems the prime targets of ROS are the polyunsaturated fatty acids in cell membranes which later lead to its lipid peroxidation and resultant neoplastic transformation of the cell (10). There is accumulating evidence from animal and human systems implicating a role of oxidative stress and lipid peroxidation in the development of breast cancer (11). Experimental investigations as well as clinical and epidemiological studies confirm the involvement of singlet oxygen ($1O_2$), superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($OH^{\bullet}$) in the etiology of cancer (12-13). However there are conflicting reports of tissue concentrations of malondialdehyde (MDA), nitrite and Vit. E in breast cancer patients and only a few studies on their blood concentrations have been reported so for. The present study aimed to determine the extent of oxidative stress by measuring malondialdehyde (MDA), nitrate, lipid hydroperoxide and total antioxidant capacity (TAC) in blood and tissue of patients with malignant breast tumor and benign breast disease.

## MATERIAL AND METHODS

### Subjects:

This study was conducted in Division of Veterinary Biochemistry, SKUAST-K and in the Department of Biochemistry, Govt. Medical College Srinagar, Kashmir India. The patient group consisted of 40 persons with Breast Cancer who were treated at the department of the Medical Oncology SMHS Hospital Srinagar and 20 healthy controls. Patients with past or present disorders which may have influenced antioxidant enzyme activity were not included in the study. The fasting blood samples of both groups were drawn in to citrate (3.5mg/ml blood) containing glass tubes and centrifuged at 480 × g for 11 minutes, and plasma samples were stored at -20 $^{o}$C until analysis.

### Procedures:

Standard procedures were adopted for the estimation of GSH, SOD, AOP and MDA levels as a measure of LP in the current work.

GSH levels were estimated within one hour by method of Moron et al**. (19)**. 25% Trichloroacetic acid (TCA )was added to precipitate out and proteins were separated by centrifugation at 2000 g for 15 min. GSH in the supernatant fraction formed a complex with 2,2'-Dinitro -5,5'- Dithio- bis- Nitrobenzoic acid (DTNB ) which was measured at 412 nm .

SOD activity was determined as described by Kono et al. (20). Photo oxidation of Hydroxylamine Hydrochloride was used to generate the superoxide anion. This anion reduces nitroblue tetrazolium (NBT) to formazan, which is monitored at 560 nm. SOD of the sample removes the superoxide anion and inhibits the reduction. The level of this reduction was used as a measure of SOD activity.

Total amount of lipid peroxidation products in the plasma of 40 patients & 20 healthy volunteers was estimated using the thiobarbituric acid (TBA) method (21), which measures the TBA reactive products chiefly malondialdehyde (MDA). In brief, 1.0 ml of plasma, 1.0 ml of normal saline and 1.0 ml of 25% TCA were mixed and centrifuged at 2000 for 20 min. One ml of protein free supernatant was taken, mixed with 0.25 ml of 1% TBA and boiled for 45 min at 95$^{o}$ C. After cooling, the intensity of the pink color of the product obtained was read at 532 nm.

Nitrite as a constituent in the estimation of lipid peroxidation was measured using Griess reagents (22). The 100 µL of serum were added to 50 µL 1% sulfanilamide (Griess reagent 1), followed by the addition of 50 µL 0.1% N-(1-napthyl) ethylenediamine (Griess reagent 2), and later absorbance was measured at 550 nm after 15 min.

### Statistical analysis:

The results were analyzed statistically using the Student's *t*-test for unpaired variables to evaluate the significance of differences between the mean values of the two studied groups.

### Results:

The results of different biochemical parameters estimated in the study are presented in Table-1.

## DISCUSSION:

Damage to the breast epithelium by reactive oxygen species can lead to fibroblast proliferation, epithelial hyperplasia and breast cancer. Studies have shown increased lipid peroxidation in solid tumours (23,

 

24). Increased generation of oxygen free radicals, such as $O_2$ and $H_2O_2$, can induce the activity of superoxide dismutase. An increase in total and mitochondrial SOD activities due to over expression were observed from patients with breast carcinoma has been reported (25, 26). Glutathione, an important substrate for glutathione Peroxidise and Glutathione–S-Transferase has been documented to have regulatory effects on cell proliferation (27). GSH is recognized as a potent antioxidant and enzyme cofactor and is under tight homeostatic control for its synthesis, recycling and utilization .Free radical and other oxidative agents in the biological systems are known to deplete GSH and this study revealed that in glutathione cycle depletion has outpaced synthesis of glutathione.

Lipid peroxidation and Oxidative stress caused by increased free radical generation and decreased antioxidant level in the target cells and tissues is suggested to play an important role in carcinogenesis (28-31). Potentially cytotoxic agents that are important in the etiopathogenesis of several diseases are generated by the interaction of ROS and RNS. Earlier studies showed the involvement of ROS induced lipid peroxidation in cancer initiation, progression and promotion (32,33). In recent years, using MDA as a marker of oxidative stress, there has been a growing interest in studying the role played by lipid peroxidation in cancer progression. MDA is low-molecular weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids. Some studies reported that increased plasma MDA levels in breast cancer compared with the levels in benign breast disease (34). In our study, serum MDA levels were elevated in the breast cancer patients as compared to controls which confirm the role of lipid peroxidation in the promotion of breast cancer.

Presence of NO is well-known in human biological fluids, suggesting its role in physiological and pathological processes. The NO readily oxidized to nitrite and nitrate in biological systems. It exhibits a dual role, with regard to the complex mechanism of tumour invasion and metastasis and could either mediate tumorocidal activity or promote tumour growth (35). In this study, we demonstrated that in breast cancer patients, the serum and tissue levels of nitric oxide are significantly increased compared to benign breast disease which depicts its role in the carcinogenesis of breast tissue.

**Conclusion :**

The study reveals that there is increased oxidative stress related damage leading to increased LP is Breast cancer patients, same is responded by increased antioxidant enzyme activity.

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**Table-1**—Status of GSH, SOD, AOP, MDA and Nitrate levels as a measure of LP in breast cancer patients and controls.

| **Parameter** | **Patients** | **Controls** | **P** |
|---|---|---|---|
| **GSH (µM/l)** | 2.58 ± 0.22 | 4.76 ± 0.52 | P<0.005 |
| **SOD (U/ml)** | 5.24 ± 0.69 | 4.14 ± 0.56U | P<0.01 |
| **AOP(nmol$^{-1}$/ml.h)** | 0.020± .011 | 0.042 ± 0.018 | P<0.005 |
| **MDA (nmol/ml)** | 0.88 ± 0.20 | 0. 53 ± 0.16 | P<0.005 |
| **Nitrite (µmol/L)** | 14.70±3.93 | 5.58±1.08 | P<0.05 |