

# Lipid peroxidation and antioxidant status in patients with breast cancer

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## ABSTRACT

**Introduction:** The present study was undertaken to evaluate the status of lipid peroxidation and antioxidants as biomarkers in human plasma.

**Methods:** The extent of lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD) as well as the status of the antioxidants superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione S-transferase (GST) in serum samples of 40 breast cancer patients in and around Coimbatore, India, were studied. Controls consisted of members of the public with no previous history of breast cancer or other cancer-related diseases.

**Results:** The plasma samples of the breast cancer patients showed enhanced level of lipid peroxidation when compared to the corresponding controls. This was accompanied by a significant elevation in both enzymic and non-enzymic antioxidants.

**Conclusion:** These findings indicate the significant increase in lipid peroxidation as evidenced by the level of TBARS and antioxidant status such as elevated SOD, CAT, GPx, GSH and GST in samples from breast cancer patients compared to controls.

**Keywords:** antioxidants, breast cancer, glutathione peroxidase, lipid peroxidation

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## INTRODUCTION

Breast carcinoma is one of the most common neoplasms in women and is a leading cause of cancer-related deaths worldwide. The aetiology of breast cancer is multifactorial. 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.<sup>(1)</sup> In the United States, breast cancer is one of the most common malignant tumours in women. The American Cancer Society estimated that approximately 210,000 new patients would be diagnosed with breast cancer and 40,000 women would

die from this disease in 2004.<sup>(2)</sup>

The common risk factor in the development of breast cancer is the increased lifetime exposure to endogenous or exogenous oestrogens. A number of genes, including BRCA1 and BRCA2, HER-2/neu and p53, have been linked to breast cancer susceptibility and development.<sup>(3)</sup> Oxygen-free radicals (OFR) generated by a number of processes *in vivo* are highly reactive and toxic.<sup>(4)</sup> However, biological systems have evolved an array of enzymic and non-enzymic antioxidant defence mechanisms to combat the deleterious effects of OFR. Superoxide dismutase (SOD) and catalase (CAT) play a key role in the detoxification of superoxide anion and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), respectively, thereby protecting against OFR-induced damage. Reduced glutathione (GSH) in conjunction with glutathione peroxidase (GPx) and glutathione S-transferase (GST) plays a central role in the defence against free radicals, peroxides and a wide range of xenobiotics and carcinogens.<sup>(5,6)</sup>

Oxidative stress arises when there is an imbalance between OFR formation and scavenging by antioxidants. Excess generation of OFR can cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis. OFR-induced lipid peroxidation has been implicated in neoplastic transformation.<sup>(7)</sup> Although a number of studies have unravelled the role of oestrogens as well as the imbalance in oncogenes and tumour suppressor genes in breast cancer, the involvement of oxidative stress in breast carcinogenesis has not been extensively documented. We therefore examined the extent of lipid peroxidation, as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD), and the status of the antioxidants SOD, CAT, GSH, GPx and GST in the plasma of patients with carcinoma of the breast.

## METHODS

40 newly-diagnosed breast cancer patients from Coimbatore, India, with a mean age of 46.71 ± 3.85 years, and who had not undergone any previous treatment for their tumours, were chosen for the study. The patients were clinically categorised as stage II (19 patients) and stage III (21 patients) infiltrative ductal carcinoma of the breast. The patients were not using hormones, oral contraceptives and

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**Table I. General characteristics of breast cancer patients.**

General characteristics	Breast cancer patients
Total number of subjects	40
Age range (years)	25–60
Age at menarche (years)	12–15
Menopausal status	
Premenopausal	28
Postmenopausal	12
Cancer site	
Left breast	29
Right breast	11
Clinical status	
Infiltrative ductal carcinoma	40
Clinical stage	
Stage II T2N1M0	19
Stage III T3N1M0	21

T: tumour size; T1: < 2 cm, T2: 2–4 cm, T3: > 4 cm.

N: nodal metastasis, N0: no regional lymph node metastasis, N1: metastasis in a single ipsilateral node of < 3 cm diameter, M: distant metastasis, M0: no distant metastasis.

were nonsmokers. None of them had concomitant diseases such as diabetes mellitus, liver disease and rheumatoid arthritis (Table I). Informed consent was obtained from all the participants. The Human Ethics Committee, India approved the study. Controls consisted of members of the public with no previous history of breast cancer and other cancer-related diseases.

Blood was collected by venous arm puncture in patients and controls. The collected blood was injected into EDTA vacutainers and the plasma was separated by centrifuging at 1,000 g for 15 minutes. All the chemicals and reagents used in the study were of analytical grade and purchased in Hi-Media Laboratories (Mumbai, India) and Sigma (St Louis, MO, USA). Lipid hydroperoxides were estimated by the method of Jiang et al.<sup>(8)</sup> The reaction mixture in a total volume of 2.0 ml containing 0.2 ml of plasma and 1.8 ml of Fox reagent, was incubated for 30 minutes. Hydroperoxides are detected by their ability to oxidise ferrous iron, leading to the formation of a chromophore with an absorbance maximum at 560 nm.

Lipid peroxidation was estimated by measurement of thiobarbituric acid reactive substances (TBARS) in plasma by the method of Yagi.<sup>(9)</sup> The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated at 532 nm. CDs were estimated by the method of Rao and Recknagel.<sup>(10)</sup> This method is based on the arrangement of the double bonds in polyunsaturated fatty acids to form CDs with an absorbance maximum at 233 nm.

GSH was determined by the method of Ellman.<sup>(11)</sup> GSH estimation was based on the development of a yellow colour when 5,5-dithio (2-nitrobenzoic acid) was added to compounds containing sulfhydryl groups. GPx activity was assayed by the method of Rotruck et al.<sup>(12)</sup> with

modification. A known amount of enzyme preparation was incubated with H<sub>2</sub>O<sub>2</sub> in the presence of GSH for a specified time period. The amount of H<sub>2</sub>O<sub>2</sub> utilised was determined by the method of Ellman.<sup>(11)</sup> The activity of GST was estimated by the method of Habig et al,<sup>(13)</sup> by following the increase in absorbance at 340 nm using 1-chloro-2,4-dinitrobenzene as substrate.

SOD was assayed by the method of Kakkar et al.<sup>(14)</sup> based on the 50% inhibition of the formation of nicotinamide adenine dinucleotide (NADH)-phenazine methosulfate-nitroblue tetrazolium formazan at 520 nm. Haemoglobin in the haemolysate was measured according to the method of Drabkin and Austin.<sup>(15)</sup> Blood was diluted in an alkaline medium containing potassium cyanide and potassium ferricyanide. Haemoglobin oxidised to methaemoglobin combines with cyanide to form cyanmethaemoglobin which was measured at 540 nm. The data for biochemical analyses are expressed as mean and standard deviation (SD). Statistical comparisons were performed by Student's *t*-test using the Statistical Package for Social Sciences version 10.0 (SPSS Inc, Chicago, IL, USA).

## RESULTS

The extent of lipid peroxidation in the breast cancer samples as evidenced by the formation of TBARS, CDs and lipid hydroperoxides, is represented in Table II. The concentration of TBARS in the cancer samples was significantly higher ( $p < 0.05$ ) when compared to the corresponding control samples. Values for CDs and lipid hydroperoxides showed a similar but significantly greater response ( $p < 0.001$ ) in cancer samples compared to the corresponding control samples. Table III indicates the antioxidant profile in the cancer plasma samples. The concentration of GSH and the activities of SOD, CAT, GPx and GST in the cancer samples showed a marked elevation compared to the controls. The respective concentrations of SOD and CAT were significantly increased compared to the controls ( $p < 0.001$ ). The activity of GPx was 1.65-fold higher in breast tumours compared to the controls. GSH and GST exhibited nearly a 2–3-fold increase ( $p < 0.001$ ) in the patient plasma samples compared to the corresponding controls.

## DISCUSSION

Damage to the breast epithelium by OFR can lead to fibroblast proliferation, epithelial hyperplasia, cellular atypia and breast cancer. Studies have shown increased lipid peroxidation in solid tumours.<sup>(16,17)</sup> Tamoxifen therapy in postmenopausal women with breast cancer reduced the increase in lipid peroxidation. Damage to the

**Table II. Lipid peroxidation in breast cancer patients (mean  $\pm$  SD, n = 40).**

Parameters	Controls	Breast cancer patients
TBARS (nmol/100 mg protein)	126.58 $\pm$ 11.62	133.24 $\pm$ 12.94*
CD ( $\mu$ mol/haemoglobin)	0.46 $\pm$ 0.04	0.61 $\pm$ 0.05**
LOOH (nmol/100 mg protein)	0.44 $\pm$ 0.08	0.59 $\pm$ 0.09**

\*As compared with breast cancer controls,  $p < 0.05$ .

\*\*As compared with breast cancer controls,  $p < 0.001$ .

**Table III. Antioxidant status in breast cancer patients (mean  $\pm$  SD, n = 40).**

Parameters	Controls	Breast cancer patients
SOD <sup>a</sup>	18.13 $\pm$ 1.40	21.24 $\pm$ 2.42*
CAT <sup>b</sup>	8.59 $\pm$ 0.94	10.21 $\pm$ 0.85*
GSH <sup>c</sup>	7.50 $\pm$ 0.30	17.45 $\pm$ 0.26*
GPx <sup>d</sup>	17.61 $\pm$ 0.91	29.19 $\pm$ 1.21*
GST <sup>e</sup>	2.72 $\pm$ 0.63	5.83 $\pm$ 0.72*

<sup>a</sup> Amount of enzymes required to give 50% inhibition of NBT reduction/mg protein.

<sup>b</sup>  $\mu$ mol H<sub>2</sub>O<sub>2</sub> utilised/s/mg protein.

<sup>c</sup> mg/dl plasma

<sup>d</sup>  $\mu$ mol GSH utilised/min/mg protein.

<sup>e</sup>  $\mu$ mol CDNB-GSH conjugate formed/min/mg protein.

\*As compared with breast cancer control.  $p < 0.001$ .

mammary epithelium by reactive oxygen species can lead to fibroblast proliferation epithelial hyperplasia, cellular atypia and breast cancer.<sup>(18,19)</sup>

The increase in plasma lipid peroxidation in breast cancer seen in the present study was associated with enhanced antioxidant capacities. Increased generation of OFR, such as O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>, can induce SOD and CAT. An increase in total and mitochondrial SOD activities due to over expression has been reported.<sup>(20)</sup> Increased SOD mRNA expression was observed in cancer samples from patients with carcinoma of the breast.<sup>(21)</sup> Higher activity of CAT has been documented in tumour cell lines compared to controls.<sup>(22)</sup> Our results lend credence to these reports. Glutathione, an important substrate for GPx and GST, has been documented to have regulatory effects on cell proliferation.<sup>(23)</sup> Over expression of GSH has been reported in both animal and human tumours by us as well as by other workers.<sup>(17,24,25)</sup>

A significant increase in the activity of GPx, the first step of enzyme defence against H<sub>2</sub>O<sub>2</sub> and other hydroperoxides, has been reported in tumours.<sup>(26,27)</sup> The higher activity of GPx in breast cancer cell lines was suggested to result from an increased expression of genomic DNA.<sup>(28)</sup> GST, which is involved in the detoxification of electrophilic toxins and carcinogens, is increased in most of the human tumours studied. High concentrations of GST may rapidly detoxify anticancer agents, thereby preventing their cytotoxic action. Enhanced GST activity in breast cancer samples in our study supports ubiquitously-reported induction of GST, especially the isoenzyme GST-P in various cancer tissues and cell lines.<sup>(29,30)</sup> Overproduction of OFR coupled with

antioxidant depletion is recognised to result in oxidative stress.<sup>(5,6)</sup>

The increase in lipid peroxidation in breast cancer patients in the present study was counterbalanced by enhanced host antioxidant defence systems protecting against oxidative stress. Recent reports suggest that oxidative stress can cause upregulation of antioxidant enzymes that render cells more resistant to subsequent oxidative insult.<sup>(31)</sup> Prolonged exercise generates oxidative stress, which results in increased endogenous antioxidants. Exercise-trained mice showed increased levels of hepatic SOD and CAT.<sup>(32,33)</sup> Several researchers reported decreases in the antioxidant level and increases in the lipid peroxidation level.<sup>(1,2)</sup>

Overexpression of antioxidants has been documented in a wide variety of malignant tumours, including breast cancer.<sup>(17,27,29)</sup> Cancer cells with increased activities of antioxidant enzymes are presumed to escape recognition by cytotoxic lymphocytes.<sup>(34)</sup> From the results of the present study, we suggest that increased lipid peroxidation and host antioxidant defences associated with the development of breast cancer may offer a selective growth advantage to tumour cells over their surrounding normal counterparts.

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