Chapter-IV
4.1. Introduction

Breast cancer comprises the most common malignant disease of all cancer diseases in the world. It has been estimated that 12% of women will develop breast cancer during their lifetime and the incidence rate is high in the western countries. In developing countries such as India, breast carcinoma accounts for 22.2% of all diagnoses and 17.2% of all cancer deaths among women (Ali et al., 2006). The development of breast tumor is a multistep process. It is usually thought that the induction of breast tumors due to accumulation of genetic modifications that result either by activation of tumor related genes (oncogenes) and/or inactivation of tumor suppressor genes, leading to an abnormal cellular proliferation and promoting the development of tumor in the breast tissue. Estrogen and progesterone receptors, epidermal growth factor receptor family members, CHEK2, ATM, p53, BRCA1, and BRCA2 are genetic risk factors for the development and progression of breast cancer (Keen and Davidson, 2003; Hou et al., 2003). Furthermore, several risk factors such as reproductive and hormonal factors, alcohol consumption, cigarette smoking, dietary factors and chronic inflammation have been identified as causative agents for breast cancer, but the underlying mechanism by which they increase risk of breast cancer is still unclear (Mitrunen and Hirvonen, 2003).

Several experimental, epidemiological and clinical studies have been reported the involvement of oxygen derived radicals or free radicals including singlet oxygen (\(^1\)O2), superoxide anions (O2’), hydrogen peroxide (H\(_2\)O\(_2\)), lipid peroxyl radical (LOO’), nitroxy radical (NO’), and hydroxyl radical (OH’) in the etiology of cancer (Taysi et al., 2003; Bedard and Krause, 2007). Free radicals are formed as by products of aerobic cellular metabolism in both physiological and pathological conditions in mammalian tissues. The endogenous sources of ROS includes mitochondria, cytochrome P450 metabolism, peroxisomes, and inflammatory cell activation (Inoue et al., 2003). In addition, the formation of ROS from exogenous source viz., xenobiotics, chlorinated compounds, environmental agents, radiation, UV light, the production of H\(_2\)O\(_2\), NO’ and O2’ by activating macrophages and phagocytes, metal catalyzed oxidation systems, air pollutants, and auto-oxidation of electron transport carriers (Stadtman and Levine 2000; Valko et al., 2006). At lower levels ROS are essential for the normal physiological process like cell differentiation (Abe et al., 2000), apoptosis (Ghosh and Mayers, 1998), and for cellular immunity (Golub et al., 1985). However, the
excess production of ROS and oxidants makes a phenomenon called oxidative stress. Furthermore, it causes oxidative damage to DNA, RNA and proteins, single and double-stranded DNA breaks, base and sugar modifications and DNA-protein cross-links, adduct formation (lipid peroxidation), mutagenesis and carcinogenesis (Nauseef, 2008; Ralph et al., 2010). The main target of free radicals is the polyunsaturated fatty acids (PUFAs) of cell membranes consequently leading to lipid peroxidation. Alterations in the levels of lipid peroxidation have been reported in breast cancer (Gonenc et al., 2001; Khanzode et al., 2004).

The oxidative stress is defined as an imbalance between pro-oxidants (free radical species) and the body's defense system (antioxidants). It plays an important role in the development of many human diseases particularly breast cancer (Ray et al., 2000, Yeh et al., 2005; Aghvami et al., 2006; Gago-Dominguez et al., 2007). To compensate the harmful effects of ROS, cells have developed various defensive or reparative mechanisms. The most important of these protective mechanism is the antioxidant system which includes both enzymatic and non-enzymatic systems. Enzymatic system is the first line of defense that includes viz., SOD, CAT, GPx, GR and GST to combat ROS. SOD destroys the highly reactive superoxide anion by converting into the less reactive hydrogen peroxide, which can be decomposed to H₂O and singlet oxygen by CAT, GPX or PRX (Ray and Husain, 2002). Moreover, Catalase also detoxifies various phenols and alcohols (Nordberg and Arner, 2001). Also, GPx reduces lipid or nonlipid hydro-peroxides by oxidizing reduced glutathione (GSSH) to oxidized glutathione (GSSG). The GSSG is restored to a reduced (GSH) form by glutathione reductase (GR), the reaction serves to maintain a high GSH/GSSG ratio in the cell environment. Glutathione -\( \mathbf{S} \)-transferase (GST) is an enzyme involved in antioxidant defense system and also participate in the detoxification process. Alterations in GST levels in tumor tissue have been reported by various studies (Boyer et al., 1984; Matsui et al., 2000). The non-enzymatic defense system is the second line of defense system includes reduced glutathione (GSH), uric acid, vitamins A, C, E and transition metal binding proteins (metallothioneins) in scavenging of OH• radicals (Young and Woodside, 2001; Polat et al., 2002). The measurement of GSH in tissues and biological fluids has been used as an index of the oxidative stress under different physiological and pathological conditions (Mills et al., 1994). Particularly, GSH and thiol redox status regulate expression of genes involved in the etiology of different diseases, including cancer, AIDS, diabetes, or atherosclerosis (Sen and
Several studies have reported that increased lipid peroxidation and impairment in antioxidant defense system was observed in the breast cancer patients (Gonenc et al., 2001; Kumaraguruparan et al., 2002). During the recent past, there is less attention paid in the measurement of oxidative stress in breast cancer patients. Since, changes in the oxidant–antioxidant status with respect to different clinical stages and menopausal status have not been sufficiently documented.

The present study was designed to evaluate the association between the lipid peroxidation and antioxidant status in different clinical stages of menopausal condition in breast cancer patients.

4.2. Results

4.2.1. Lipid peroxidation (Lpx) levels in tumor tissue of breast cancer patients

The extent of lipid peroxidation in breast cancer tissues was estimated by the formation of thiobarbituric acid reactive substances (TBARS) is represented in Table 4.1. The levels of lipid peroxidation (µ moles of malondialdehyde formed/g wet wt of tissue) in the pre- and post menopausal non tumor tissues of breast at stage I were 9.69 ± 0.26 and 9.54 ± 0.21 respectively. Whereas, the levels of Lpx in pre- and post menopausal tumor tissues were 12.4 ± 0.28 and 11.06 ± 0.2 in stage I patients. In stage II, the levels of Lpx in pre- and post menopausal non tumor were 9.72 ± 0.22 and 9.66 ± 0.3 and the activity levels of Lpx in pre- and post menopausal tumor tissues were 12.26 ± 0.24 and 11.2 ± 0.18 respectively. In stage III, the levels of Lpx in pre- and post menopausal non tumor tissues were 9.7 ± 0.19 and 9.48 ± 0.2, and the levels of Lpx in pre- and postmenopausal tumor tissues were 12.06 ± 0.19 and 11.62 ± 0.24 respectively (Table 4.1). The percent change in the levels of Lpx in premenopausal women as compared to their respective non tumor tissue at stage I, II and III were 27.9%, 29.8% and 24.5% respectively. Whereas, the percent change in the levels of LPx in postmenopausal women as compared to their respective controls at stage I, II and III were 15.9%, 15.9% and 16.2 % respectively.

The concentration of TBARS in the breast tumor tissues is significantly higher (P<0.05) when compared to respective adjacent tissues (non tumor) in different clinical stages (I, II and III) of breast cancer patients. No significant change is observed in the levels of Lpx in the non tumor breast tissues of breast cancer. Whereas, a significant change was observed (p< 0.05) in the levels of Lpx in tumor tissue of breast cancer patients (Table 4.1).
In addition, the percent change in the levels of LPx was decreased to 10.9%, 11.25% and 8.77% respectively in postmenopausal breast tumor tissues when compared to premenopausal breast tumors. A significant increase in the levels of Lpx was observed in pre- and post menopausal breast cancer patients at all stages as compared to their respective controls. Thus, it clearly indicates that the concentration of lipid peroxidation was significantly increased as the stage progression of the breast cancer advances (Table 4.1).

4.2.2. Antioxidant enzyme activities in the tumor tissues of breast cancer patients

4.2.2.1. Superoxide dismutase (SOD)

The activity levels of SOD (µ moles of NADPH oxidized/mg protein/ min) in pre- and post menopausal non tumor of breast tissues at stage I shows 4.92 ± 0.114 and 4.87 ± 0.1 and the activity levels of SOD in pre- and post menopausal tumor tissues were 6.98 ± 0.09 and 5.27 ± 0.01 respectively. In stage II patients, the activity levels of SOD in pre- and post menopausal non tumor were 4.95 ± 0.112 and 4.9 ± 0.106 and the activity levels of SOD in pre- and post menopausal tumor tissues were 7.36 ± 0.12 and 5.64 ± 0.11 respectively. In stage III, the activity levels of SOD in pre- and post menopausal non tumor tissues were 4.98 ± 0.15 and 4.88 ± 0.11. Whereas, the activity levels of SOD in pre- and post menopausal tumor tissues were 7.49 ± 0.1 and 7.16 ± 0.12 respectively (Table 4.2). The percent change in the activity levels of SOD in premenopausal women was 41.8%, 48.7% and 52.2% respectively when compared to their respective non tumors at stage 1, II and III. Whereas, the percent change in the levels of SOD in postmenopausal women was 8.21%, 15.1% and 46.7% respectively, when compared to their control tissues at respective clinical stages. (Table 4.2).

The activity levels of SOD were significantly elevated in tumor tissue when compared to their respective adjacent tissue at all clinical stages of breast cancer patients. No significant change was observed in the levels of SOD in the non tumor breast tissue of breast cancer. A significant increased (p< 0.05) SOD levels were observed in pre- and post menopausal breast cancer patients at all clinical stages as compared to their respective adjacent tissues (Table 4.2). Moreover, the percent change in the activity levels of SOD was decreased in postmenopausal breast tumors to 17.4%, 23.3% and 22.8% respectively when compared to premenopausal breast tumors in respective clinical stages.
4.2.2.2. Catalase (CAT)

The activity levels of CAT (µ moles of thioether formed/mg protein/ hr) in pre- and post menopausal non tumor and tumor tissues at stage I shows 0.412 ± 0.016, 0.398 ± 0.01 and 0.91 ± 0.016, 0.68 ± 0.015 respectively. In stage II patients, the activity levels of CAT in pre- and post menopausal non tumor and tumors were 0.4 ± 0.01, 0.399 ± 0.019 and 1.12 ± 0.01, 0.76 ± 0.018 respectively. In stage III, the activity levels of CAT in pre- and post menopausal non tumors are 0.397 ± 0.01 and 0.402 ± 0.017. Whereas, the activity levels of CAT in pre- and post menopausal breast cancer tumor tissues were 1.38 ± 0.015 and 0.81 ± 0.01 respectively (Table 4.2). The percent change in the activity levels of CAT in premenopausal women as compared to their respective non tumors at stage I, II and III were 120.8%, 180% and 247.6% respectively, and the percent change in the levels of CAT in postmenopausal women as compared to their respective controls at stage I, II and III were 70.8%, 90.5% and 101.5% respectively. There is a significant (p<0.05) increase in the activity levels of CAT was observed in breast cancer tumor tissue compared to their respective adjacent non tumor tissue at all clinical stages of breast cancer patients. No significant change was observed in the activity levels of CAT in the non tumor breast tissues of breast cancer. Whereas, significant change (p<0.05) was observed in the levels of CAT in pre- and post menopausal breast cancer patients at all clinical stages as compared to their respective adjacent non tumor tissues (Table 4.2). In addition, the percent change in the activity levels of CAT was decreased in postmenopausal breast cancer tumors to 25.2%, 32.1% and 41.3% respectively compared to premenopausal breast tumors in all clinical stages (Table 4.2).

6.2.2.3. Glutathione S-transferase (GST)

Table. 4.3 represents the activity levels of GST (Units/mg protein/min) in pre- and post menopausal non tumor tissues of breast at stage I shows 1.82 ± 0.017 and 1.79 ± 0.01 and, the activity levels of GST in pre- and post menopausal tumor tissues were 2.56 ± 0.013 and 2.16 ± 0.011 respectively. In stage II patients, the activity levels of GST in pre- and post menopausal non tumor and tumor tissues were 1.801 ± 0.014, 1.82 ± 0.015 and 2.89 ± 0.01, 2.09 ± 0.016 respectively. In stage III, the activity levels of GST in pre- and post menopausal non tumors were 1.816 ± 0.011 and 1.809 ± 0.013, whereas, pre- and post menopausal tumor tissue were 2.96 ± 0.018 and 2.45 ± 0.01 respectively. The percent change in the activity
levels of GST in premenopausal women as compared to their respective non tumors at stage I, II and III were 40.6%, 60.4% and 63.5% respectively. Whereas, the percent change in the levels of GST in postmenopausal women as compared to their respective controls at stage I, II and III were 20.7%, 14.8% and 35.4% respectively. No significant change was observed in the levels of GST in the non tumor breast tissues of breast cancer. A statistically significant (p< 0.05) change was observed in the activity levels of GST in pre- and post menopausal breast cancer patients at all clinical stages as compared to their respective adjacent non tumor tissues (Table 4.3). The percent change was decreased to 15.6%, 27.6% and 17.2% in postmenopausal breast tumors when compared to premenopausal breast tumors (Table 4.3).

4.2.2.4. Glutathione peroxidase (GPx)

The activity levels of GPx (µ moles of NADPH oxidized/mg protein/min) in pre- and post menopausal non tumor tissues at stage I shows 1.22 ± 0.012 and 1.209 ± 0.01 and the activity levels of GPx in pre- and post menopausal breast cancer tumor tissues were 2.84 ± 0.013 and 2.26 ± 0.011 respectively. In stage II patients, the activity levels of GPx in pre- and post menopausal non tumors were 1.21 ± 0.012 and 1.208 ± 0.012, and the activity levels of GPx in pre- and post menopausal breast cancer tumor tissues were 3.02 ± 0.014 and 2.49 ± 0.012 respectively. In stage III, the activity levels of GPx in pre- and post menopausal non tumors and tumors were 1.21 ± 0.011 and 1.212 ± 0.013, whereas, the activity levels of GPx in pre- and post menopausal tumor tissues were 3.31 ± 0.011 and 2.68 ± 0.014 respectively (Table 4.3). The percent change in the activity levels of GPx in premenopausal women as compared to their respective non tumors at stage 1, II and III were 132.8%, 149.6% and 173.5% respectively, and the percent change in the levels of GPx in postmenopausal women as compared to their respective controls at stage 1, II and III were 86.9%, 106.1% and 121.1% respectively. The activity levels of GPx were increased significantly in breast cancer tumor tissues compared to their respective adjacent non tumor tissues at all clinical stages. No significant change in the levels of GPx was observed in the non tumor breast tissues of breast cancer. GPx was increased significantly (p> 0.05) in pre- and post menopausal breast cancer patients at all clinical stages as compared to their respective adjacent tissues. Whereas, the percent change was decreased to 20.4%, 17.5% and 19% in postmenopausal breast tumors compared to premenopausal breast tumors (Table 4.3).
4.2.2.5. *Glutathione reductase (GR)*

Table 4.4 summarizes the activity levels of GR (µ moles of NADPH oxidized/mg protein/min) in pre- and post menopausal non tumor tissues of breast at stage I shows 0.97 ± 0.012 and 0.96 ± 0.015. Whereas, the activity levels of GR in pre- and post menopausal tumor tissues were 2.95 ± 0.014 and 2.32 ± 0.012 respectively. In stage II patients, the activity levels of GR in pre- and post menopausal non tumor tissues were 0.97 ± 0.01 and 0.969 ± 0.012 and the activity levels of GR in pre- and post menopausal tumor tissues were 3.28 ± 0.015 and 2.66 ± 0.012 respectively. In stage III, the activity levels of GR in pre- and post menopausal non tumors were 0.966 ± 0.011 and 0.972 ± 0.014, whereas, the activity levels of GR in pre- and post menopausal breast cancer tumor tissue were 3.41 ± 0.011 and 2.7 ± 0.015 respectively. The percent change in the activity levels of GR was observed in premenopausal women compared to their respective non tumors tissues in stage I, II and III were 204.1%, 237.8% and 253% respectively, and the percent change in the activity levels of GR was observed in postmenopausal women as compared to their respective controls at stage I, II and III were 141.6%, 174.5% and 177.8% respectively (Table 4.4). A significant (p<0.05) increase in the levels of GR was observed in the breast cancer tumor tissues when compared to their respective non tumor tissues at all clinical stages of breast cancer patients. No significant change was observed in the levels of GR in the non tumor breast tissues of breast cancer. The activity levels of GR was significantly increased in pre- and post menopausal breast cancer patients at all clinical stages as compared to their respective adjacent non tumor tissues. The percent change was decreased in the activity levels of GR in postmenopausal breast tumors to 21.3%, 18.9% and 20.8% respectively compared to premenopausal breast cancer tumors (Table 4.4).

4.2.2.6. *Glutathione reduced (GSH)*

The GSH content (µ moles of thiourea formed/mg protein/hr) in pre- and post menopausal non tumor tissues of breast at stage I shows 16.12 ± 0.20 and 15.28 ± 0.24. The content of GSH in pre- and post menopausal tumor tissues were 25.9 ± 0.22 and 19.2 ± 0.25 respectively. In stage II patients, the content of GSH in pre- and post menopausal non tumor and tumor tissues were 16.07 ± 0.24, 15.9 ± 0.2 and 26.8 ± 0.21, 19.95 ± 0.19 respectively. In stage III, the content of GSH in pre- and post menopausal non tumors were 15.9 ± 0.2 and 16.04 ± 0.19 and tumor tissue were shows 28.1 ± 0.22 and 20.9 ± 0.2 respectively (Table 4.4).
4.4) The percent change in the content of GSH was observed in premenopausal women as
compared to their respective non tumors in stage I, II and III were 60.6%, 66.7% and 75.6%
respectively, whereas the percent change in the content of GSH in postmenopausal women as
compared to their respective controls at stage I, II and III were 25.6%, 25.4% and 30.2%
respectively. The content of GSH were increased significantly in breast cancer tumor tissues
compared with their control counterparts. No significant change was observed in the content
of GSH in the non tumor breast tissues of breast cancer. The percent change was decreased to
25.8%, 25.5% and 25.6% in postmenopausal breast tumors when compared with
premenopausal breast tumors (Table 4.4).

4.3. Discussion

Oxidative stress plays a crucial role in the progression of carcinogenesis by means of
mutagenesis (Aghvami et al., 2006; Gago-Dominguez et al., 2007). It is defined as an
insidious condition in which there is imbalance between free radical generation and
scavenging antioxidant system. Excess generation of free radicals (ROS) can cause oxidative
damage to biomolecules resulting in lipid peroxidation and it has been implicated in
neoplastic transformation (Hristozov et al., 2001; Khanzode et al., 2004). Experimental
evidence reveals that ROS are involved in the initiation and progression of carcinogenesis,
where inactivation or loss of certain tumor suppressor genes takes place (Aghvami et al.,
2006). However, the etiology of human breast cancers is still unknown. In the present study
TBARS content (evidenced by lipid peroxidation) was found to be significantly increased in
pre and postmenopausal breast cancer tumors when compared to respective breast non tumors.
Similar results have been reported by several studies (Yeh et al., 2005; Rajneesh et al., 2008).
Studies of Gonenc et al. (2006) reported that lipid peroxidation is significantly increased in
the blood and malignant tumor and in benign breast tissue. On the other hand, the end
product of lipid peroxidation is malondialdehyde (MDA), due to its high cytotoxicity and
inhibitory effect on protective enzymes, and it is proposed to act as a tumor initiator and also
a co-carcinogenic factor. Increased lipid peroxidation may be imputed to overproduction of
reactive oxygen species. This is well supported by the observations of Portakal et al. (2000)
and Kumaraguruparan et al. (2002). In addition, studies of Kumar et al. (1991) reported that
the MDA levels elevated in the blood of postmenopausal women with breast cancer. Elevated
levels of lipid peroxidation product (MDA) plays a role in the early phases of tumor growth
(Ames, 1983), mutagenesis by forming DNA adducts (Marnett, 1999). Enhanced lipid peroxidation is counterbalanced by enhanced host antioxidant defense systems protecting against oxidative stress. Recent reports suggest that oxidative stress can cause up regulation of antioxidant enzymes in cells, which are showing more resistant to subsequent oxidative offense (Halliwell, 2000). Therefore, the lipid peroxidation levels may be an important biomarker for the evaluation of breast cancer and can also be used as a surrogate marker for chemo-preventive aspects.

Cellular redox balance is maintained by a powerful antioxidant system that “counteracts” reactive oxygen species. The antioxidant system act as a protective mechanism against free radicals, and it consists of SOD, catalase, the glutathione system (glutathione, glutathione reductase, peroxidase and transferases), thioredoxin system (thioredoxins, thioredoxin peroxidase and peroxiredoxins), vitamin E and C are major antioxidants besides the antioxidant enzymes. Increased generation of free radicals can induce SOD and CAT, and these are the backbone of the cellular antioxidant defense system. In the present study the activity levels of antioxidant enzymes such as SOD and CAT significantly increased in pre and post menopausal breast tumor tissue compared with non tumor tissue. SOD and CAT are considered primary antioxidant enzymes since they are involved in the elimination of ROS such as O$_2^-$ and H$_2$O$_2$. SOD provides the first line of cellular defense and protects the cell against superoxide anion mediated by lipid peroxidation. Whereas, higher activity levels of catalase could be a protective mechanism for the cells due to the tumor induced elevated production of reactive oxygen species. Moreover, higher activity of CAT found to be common in tumor cell lines compared to controls (Ripple and Henry, 1997). The CAT decomposes H$_2$O$_2$ and protects the cells from the accumulation of H$_2$O$_2$ by dismutation form water and oxygen (Lenzi et al., 1993; Mueller et al., 1997). In contrast, Studies of Ray and Husain (2002) reported that SOD and CAT role as anticarcinogens by inhibiting initiation, promotion and progression or transformation stages in carcinogenesis. The increased activity of SOD and CAT enzymes are in agreement with numerous reports of antioxidant enzyme over expression in breast tumors (Liu et al., 1997; Li et al., 1998).

The Glutathione -S- transferase is a group of multifunctional proteins, which play a central role in detoxification of electrophilic chemicals, xenobiotics and their compounds (Smith et al., 1977). GSTs catalyze the conjugation of glutathione to cytotoxic products
Glutathione peroxidase is an enzyme induced by oxidative stress, plays a significant role in the peroxyl scavenging mechanism thereby sustaining the functional integrity of the cell membranes (Chandra et al., 2000). In the present study, the activity levels of the GPx were observed at higher levels in pre and post menopausal breast tumors in different clinical stages. Similar results were also observed i.e., higher levels of GPx in breast tumors by Kumaraguruparan et al. (2002). Studies of Abou Ghalia and Fouad (2000) reported that GPx and Glutathione reductase (GRx) activities were increased in the blood and tumor tissues from the patients with breast cancer disease. In addition, elevated levels of SOD, glutathione peroxidase and glutathione reductase and a decrease in GSH content was observed in cancer tissue suggesting an increased defense against oxidative damage in colorectal cancer (Skrzydelwska et al., 2001). Moreover, higher activity level of GPx has been suggested to result from increased expression of GPx cDNA in breast cancer cell lines (Doroshow, 1995). Furthermore, GPx is well-known to play an important role in tumorogenesis by altering the lipoxygenase and cyclooxygenase pathways (Capdevila et al., 1995).

Glutathione reductase catalyzes the NADPH-dependent reduction of glutathione disulfide (oxidized glutathione - GSSG) reduced to GSH (Sweet and Blanchard, 1991) and maintains at optimum levels of intracellular glutathione in reduced form (Schafer and Buettner, 2001; Soo et al., 2006) and prevents high oxidative stress by counteracting oxidation. The present results also portend the above where significantly increased GR content was observed in the pre and post-menopausal malignant breast tumors as an attempt to convert more GSSG to GSH in order to raise GSH content. Reduced glutathione (GSH) plays an important role in the antioxidant defense mechanisms and protects cells from toxins (aldehydes, lipid hydroperoxides) of lipid peroxidation induced by ROS and thus protect normal mammary epithelial cells (Rundle et al., 2000). It plays an essential role in protecting tissues from oxidative stress (Jokanovic, 2001). However, higher levels of GST may promptly detoxify anticancer agents, thereby preventing their cytotoxic action. In the present study, the activity levels of GST was statistically significant in pre and post menopausal breast tumors with respect to all clinical stages. Recent studies reported that increased GST activity in patients with breast cancer compared to their respective controls (Oguztuzun et al., 2011). Therefore, higher activity levels of GST could be a cellular compensatory mechanism to counteract the effect against increased oxidative stress.
such as free radicals. It also plays a vital role in maintaining cellular redox status of thioredoxin that activates ribonucleotide reductase, a key enzyme in the DNA synthesis. The over expression of GSH have been reported in both animal and human tumors (Skrzydlewska et al., 2001; Rajneesh et al., 2008).

Oxidative stress may be involved in breast cancer which depends on an overall balance between the generation of ROS and the scavenging mechanism of antioxidants (Ray et al., 2001; Behrend et al., 2003). In the present study increased lipid peroxidation was observed in breast cancer patients and it is associated with enhanced antioxidant defense mechanisms reflecting the ability of the tumor cells to counter oxidative stress. These changes were more strongly associated with tumors in premenopausal than in postmenopausal breast cancer women. This may be because premenopausal breast tumors tend to be usually more aggressive than those that develop after menopause. Alterations in the lipid peroxidation (Lpx) and antioxidant status (SOD, CAT, GST, GPx, GR and GSH) were high in stage III, stage II and followed by stage I compared to their respective non tumor counterparts, suggesting that the involvement of ROS in tumor initiation, promotion and progression. The protective mechanisms through which cancer cells prevent ROS overproduction may be through marked improvement in the antioxidant levels thereby minimizing the rapid rate of cell proliferation. However, the increased activities of antioxidant enzymes may be a compensatory counterbalancing protective regulation in response to augmented oxidative stress. So, the treatment with antioxidants in the early stages of the disease may be useful as secondary treatment to prevent the oxidative damage. From the present results, it can be suggested that the antioxidant levels and lipid peroxidation may be considered as biomarkers in the clinical prognosis/diagnosis of breast cancer so as to formulate the therapeutic strategies in the treatment of most dreaded disease, the breast cancer. An indepth research is certainly warranted to ascertain the role of above biomarkers in breast cancer diagnosis.
Table 4.1. Lipid peroxidation (LPx) levels in pre and post-menopausal breast cancer patients

<table>
<thead>
<tr>
<th>Stages</th>
<th>Stage I (n=12/12)</th>
<th>Stage II (n=12/12)</th>
<th>Stage III (n=12/12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non tumor</td>
<td>Tumor</td>
<td>Non tumor</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid peroxidation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µ moles of malondialdehyde formed/g wet wt. of tissue)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.26</td>
<td>0.28</td>
<td>0.2</td>
<td>0.22</td>
</tr>
<tr>
<td>9.54±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.21</td>
<td>27.9</td>
<td>15.9</td>
<td>11.25*</td>
</tr>
</tbody>
</table>

Each Value is mean ± S.D of twelve individuals. Pre: premenopausal, Post: postmenopausal
Mean values that do not share same superscript differ significantly from each other at p< 0.05
Values in the parentheses are the percent change over the respective control
Values in the parentheses* are the percent change in the postmenopausal over the premenopausal breast cancer patients.
Table 4.2. Activity levels of superoxide dismutase (SOD) and catalase (CAT) in pre and post-menopausal breast cancer patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stages</th>
<th>Stage I (n=12/12)</th>
<th>Stage II (n=12/12)</th>
<th>Stage III (n=12/12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group</td>
<td>Non tumor</td>
<td>Tumor</td>
<td>Non tumor</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>SOD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Units/mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protein/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.92(^{a}) ± 0.114</td>
<td>4.87(^{a}) ± 0.10</td>
<td>6.98(^{b}) ± 0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(41.8)</td>
<td>(-24.4)</td>
<td>(8.21)</td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td>0.412(^{a}) ± 0.016</td>
<td>0.398(^{a}) ± 0.011</td>
<td>0.91(^{b}) ± 0.016</td>
</tr>
<tr>
<td>(µ moles of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>metabolized/mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protein/ hr)</td>
<td></td>
<td>(120.8)</td>
<td>(70.8)</td>
<td>(70.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.397(^{a}) ± 0.01</td>
<td>0.402(^{a}) ± 0.017</td>
<td>1.38(^{f}) ± 0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(120.8)</td>
<td>(70.8)</td>
<td>(70.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.76(^{e}) ± 0.018</td>
<td>0.01</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(90.5)</td>
<td>(90.5)</td>
<td>(90.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-32.1)</td>
<td>(-32.1)</td>
<td>(-32.1)</td>
</tr>
</tbody>
</table>

Each Value is mean ± S.D of twelve individuals. Pre: premenopausal, Post: postmenopausal
Mean values that do not share same superscript differ significantly from each other at p< 0.05
Values in the parentheses are the percent change over the respective control
Values in the parentheses\(^*\) are the percent change in the postmenopausal over the premenopausal breast cancer patients.
Table 4.3. Activity levels of Glutathione s- Transferase (GST) and Glutathione Peroxidase (GPx) in pre and post-menopausal breast cancer patients

<table>
<thead>
<tr>
<th>Stages</th>
<th>Stage I (n=12/12)</th>
<th>Stage II (n=12/12)</th>
<th>Stage III (n=12/12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non tumor</td>
<td>Tumor</td>
<td>Non tumor</td>
</tr>
<tr>
<td>Group</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td><strong>Parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GST</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µ moles of thioether formed/ mg protein / min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.82&lt;sup&gt;a&lt;/sup&gt; ± 0.017</td>
<td>1.79&lt;sup&gt;a&lt;/sup&gt; ± 0.01</td>
<td>2.56&lt;sup&gt;b&lt;/sup&gt; ± 0.013</td>
</tr>
<tr>
<td></td>
<td>(40.6)</td>
<td>(-15.6)</td>
<td>(20.7)</td>
</tr>
<tr>
<td><strong>GPx</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µ moles of NADPH oxidized/mg protein/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.22&lt;sup&gt;a&lt;/sup&gt; ± 0.012</td>
<td>1.209&lt;sup&gt;a&lt;/sup&gt; ± 0.01</td>
<td>2.84&lt;sup&gt;b&lt;/sup&gt; ± 0.013</td>
</tr>
<tr>
<td></td>
<td>(132.8)</td>
<td>(-20.4)</td>
<td>(86.9)</td>
</tr>
</tbody>
</table>

Each Value is mean ± S.D of twelve individuals. Pre: premenopausal, Post: postmenopausal
Mean values that do not share same superscript differ significantly from each other at p< 0.05
Values in the parentheses are the percent change over the respective control
Values in the parentheses* are the percent change in the postmenopausal over the premenopausal breast cancer patients.
Table 4.4. Activity level of Glutathione Reductase (GR) and Glutathione Reduced (GSH) content in pre and post-menopausal breast cancer patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stages</th>
<th>Stage I (n=12/12)</th>
<th></th>
<th>Stage II (n=12/12)</th>
<th></th>
<th>Stage III (n=12/12)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group</td>
<td>Non tumor</td>
<td>Tumor</td>
<td>Non tumor</td>
<td>Tumor</td>
<td>Non tumor</td>
<td>Tumor</td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>GR (µ moles of NADPH oxidized/mg protein/min)</td>
<td>0.97±0.012</td>
<td>0.96±0.015</td>
<td>2.95±0.014</td>
<td>2.32±0.012</td>
<td>0.971±0.01</td>
<td>0.969±0.012</td>
<td>3.28±0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH (µ moles of thiourea formed/mg protein/hr)</td>
<td>16.12±0.2</td>
<td>15.28±0.24</td>
<td>25.9±0.22</td>
<td>19.2±0.25</td>
<td>16.07±0.24</td>
<td>15.9±0.2</td>
<td>26.8±0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each Value is mean ± S.D of twelve individuals. Pre: premenopausal, Post: postmenopausal
Mean values that do not share same superscript differ significantly from each other at p< 0.05
Values in the parentheses are the percent change over the respective control
Values in the parentheses* are the percent change in the postmenopausal over the premenopausal breast cancer patients.