A data was collected from 130 women with newly diagnosed
breast cancer from the Department of Oncology Govt. General Hospital,
Kurnool and G.G Group of Hospitals, Kurnool, India. Patients were
interviewed to obtain information on general demographic data, smoking,
alcohol consumption, tobacco chewing, reproductive history, menopausal
status and family history of breast cancer. Informed consent was obtained
from all participants.

The association between known and suspected factors and the
development of breast cancer is shown in Table 1 The risk of breast cancer
in women was increased with a family history of breast cancer in mother, or
grand mother. A higher proportion of breast cancer patients were
nonvegetarians and tobacco chewers.

4.1. Hemoglobin & RBC Count :-

The most important function of the red cell is the transportation
of oxygen to the tissues and carbon dioxide to the lungs. To achieve this, the
red cell contains a large quantity of hemoglobin, a protein whose function
within the cell is modulated to serve this requirement effectively.

In the present study, we found that the RBC count and Hb
levels were decreased in all clinical stages of breast cancer patients, when
compared to the normal subjects. Significant decrease in Hb content was
observed in cancer patients, compared to control (Table 2).
### TABLE-1
GENERAL CHARACTERISTICS OF BREAST CANCER PATIENTS

<table>
<thead>
<tr>
<th>General characteristics</th>
<th>Breast cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range (years)</td>
<td></td>
</tr>
<tr>
<td>below 25</td>
<td>09</td>
</tr>
<tr>
<td>26 - 50</td>
<td>64</td>
</tr>
<tr>
<td>51 - 75</td>
<td>43</td>
</tr>
<tr>
<td>above 76</td>
<td>14</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Below 50</td>
<td>56</td>
</tr>
<tr>
<td>Above 50</td>
<td>74</td>
</tr>
<tr>
<td>Religion</td>
<td></td>
</tr>
<tr>
<td>Hindu</td>
<td>82</td>
</tr>
<tr>
<td>Muslim</td>
<td>28</td>
</tr>
<tr>
<td>Christian</td>
<td>20</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>119</td>
</tr>
<tr>
<td>Unmarried</td>
<td>11</td>
</tr>
<tr>
<td>Menopausal Status</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>69</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>61</td>
</tr>
<tr>
<td>Age at first pregnancy (years)</td>
<td></td>
</tr>
<tr>
<td>Below 20</td>
<td>69</td>
</tr>
<tr>
<td>20 - 30</td>
<td>40</td>
</tr>
<tr>
<td>above 30</td>
<td>04</td>
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<tr>
<td>Food habits</td>
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</tr>
<tr>
<td>Vegetarian</td>
<td>22</td>
</tr>
<tr>
<td>Nonvegetarian</td>
<td>108</td>
</tr>
<tr>
<td>Cancer site</td>
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<tr>
<td>Left breast</td>
<td>86</td>
</tr>
<tr>
<td>Right breast</td>
<td>44</td>
</tr>
<tr>
<td>Stage of cancer</td>
<td></td>
</tr>
<tr>
<td>I (T₁ N₀ M₀)</td>
<td>25</td>
</tr>
<tr>
<td>II (T₂ N₁ M₀)</td>
<td>45</td>
</tr>
<tr>
<td>III (T₃ N₁ M₀)</td>
<td>36</td>
</tr>
<tr>
<td>IV (T₃ N₂ M₀)</td>
<td>24</td>
</tr>
<tr>
<td>Types of habits</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>-</td>
</tr>
<tr>
<td>Alcohol</td>
<td>09</td>
</tr>
<tr>
<td>Tobacco chewing</td>
<td>57</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>92</td>
</tr>
<tr>
<td>Yes</td>
<td>38</td>
</tr>
</tbody>
</table>

T- tumor size, T₁≤2 cm; T₂ = 4 cm, T₃≥4 cm
N- nodal metastasis, N₀=no lymph node metastasis
N₁= nodal metastasis
N₂= bilateral lymphnode metastasis
M- distant metastasis, M₀= no distant metastasis

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Further the extent of decrease in Hb content was markedly increased from stage - I to stage - IV (19.2%, 29.3%, 37.6%, 50.5%). In our survey, it was also found that the cancer patients were reported the general symptoms of anemia like weakness, fatigue etc.

In cancer, anemia is a common hematological abnormality arising as a result of contributing factors such as the underlying chronic disease, chemotherapy, radiation therapy or bone marrow invasion with tumor (Ludwig & Fritz, 1998) Approximately 75% of all cancer patients reported symptoms of fatigue (Curt, 2000; Celia, 1998), which can present as weakness, listlessness, low energy and need to sleep during the day (Ludwig & Fritz, 1998; Sabbatini, 2000). While fatigue is the primary symptom of anemia in cancer patients, anemia can also cause a range of other symptoms, including palpitations, impaired cognitive function, nausea, reduced skin temperature, impaired immune function, headache, chest pain and depression (Cella, 1998).

Anemia also increases the risk of mortality in cancer patients. In a systemic review of 60 papers, Caro and colleagues (2001), examined the survival of cancer patients according to their Hb levels or the presence of anemia and found that the relative risk of death varied by cancer type. Overall, the presence of anemia in cancer patients increased the relative risk of death by 65% (Caro et al., 2001). One of the ways anemia increases mortality is by influencing treatment efficiency. Anemia influences response to radiation therapy because it limits the oxygen transporting capacity of the blood and consequently tissue oxygenation. Thus, anemia can contribute to
tumor hypoxia, which makes solid tumors resistant to sparsely ionizing radiation and some forms of chemotherapy (Vaupel et al., 2001).

Anemia related to the progression of cancer can result from activation of the immune and inflammatory systems, leading to an increased release of cytokines, including tumor necrosis factor, interferon – gamma, and interleukin – 1 (Bron et al., 2001; Cazzola, 2000). At least three mechanisms particulate in the cytokine mediated failure of erythropoiesis: impaired iron utilization, suppression of erythroid progenitor cell differentiation, and inadequate erythropoietin production (Bron et al., 2001). Patients with cancer have been shown to have inappropriately low levels of circulating erythropoietin for their degree of anemia (Miller et al., 2001), which could reflect a disruption of homeostatic mechanisms due to the inflammatory state associated with malignancy (Means Jr., 1995). In addition, the life span of red blood cells is reported to be shortened in cancer related anemia, and production of new cells could not compensate for the shortened survival time (Bron, 2001). Glaspy et al (2001), reported that anemia in cancer patients might be due to bleeding from the tumor bed or bleeding due to systemic coagulopathy.

4.2. Iron:

Iron is an essential component of hemoglobin and myoglobin. It is also an important part of some enzymes, both in the form of heme (e.g. Xanthine oxidase). Non heme iron is also important in DNA synthesis as it is essential for the function of ribonucleotide reductase. Apart from the physiologically active iron, the body also contains significant stores of protein...
bound iron. Ferritin is the principal tissue iron storage protein and is found in all cells, although most iron is stored in the reticuloendothelial system (William & Stephen, 1995).

In present study, serum iron levels were estimated among breast cancer patients in relation to different clinical stages and in normal subjects. Serum iron levels were found to be increased in breast cancer patients, when compared to control. Serum iron levels were not significantly altered in stage-I breast cancer patients, whereas markedly elevated serum iron levels were observed in advanced stages of breast cancer. Increased iron content of serum from stage-II to IV of breast cancer patients compared to control, indicated that anemia observed in these patients is not due to iron deficiency. Knutson et al (2000), Patrick et al (2001) and Shigenaga et al (1994), reported significant increase in lipid peroxidation in high iron loaded experimental animals suggesting iron excess promote oxidative stress and may involve mitochondrial dysfunction as observed in aging and associated degenerative diseases.

Studies of severe iron overload modeling hemochromatosis have reported increased hepatic lipid peroxidation, nuclear DNA damage, and mitochondrial dysfunction (Britton et al., 1994). The severe iron overload of mitochondria has been found to induce mitochondrial DNA damage (Yaffe et al., 1996; Mitsuhashi et al., 2000). Iron can catalyze conversion of H$_2$O$_2$ into OH$^-$, the primary ROS responsible for DNA damage. Damage to mt DNA correlates with the mitochondrial dysfunction associated with oxidant stress and aging (Wallace, 1999). Thus the enhanced serum iron levels in the
present study may enhance the oxidative stress in the breast cancer patients. Iron has been recognized to potentiate carcinogenesis in several different organ systems and is an important risk factor for breast cancer (Reizenstein, 1991; Elliot et al., 1993; Thompson et al., 1991). For reasons not fully understood, iron accumulates in intracellular complexes with ferritin storage protein as a function of age. Thus, males and females reveal progressive iron accumulation with age, which is especially enhanced in post menopausal women (Giler & Moroz C., 1978), whose incidence of breast cancer is increased (Table 1).

Importantly, serum and breast ferritin levels are substantially elevated in breast carcinoma (Marcus & Zinberg, 1975; Wyllie & Liehr, 1997) and have been directly linked to mammary carcinogenesis through ROS (Wyllie & Liehr, 1997). It has been suggested that elevated ferritin/Iron complexes may supply the unusual needs for iron during proliferation of mammary tissue in normal or breast carcinoma cells. However, the need for iron in mammary gland cell growth may also substantially contribute to an unusual risk for ROS mediated injury. Treatment with ROS scavenging antioxidants, inhibition of critical ROS generating enzymes like xanthine oxidoreductase (XOR), reduction of iron intake, and reduced alcohol consumption by women in higher age dependent risk categories could significantly modulate the incidence of breast cancer.

4.3. Blood Sugar:

Blood glucose concentrations are maintained within very close limits in healthy people. Any given individual has a very strictly maintained
postabsorptive blood glucose concentration of 4.5-5.2 mmol/L, with
intraindividual coefficients of variation of 1-2%.

In present study, blood glucose levels were estimated among
breast cancer patients in relation to different clinical stages and in normal
subjects (Table 2). Blood glucose levels were found to be decreased
significantly in breast cancer patients, when compared to their normal
controls. Blood glucose levels are gradually declined with the progression of
breast cancer from stage-I to stage-IV. Very slight but no significant change
was observed in blood glucose levels of stage-I cancer patients, whereas
markedly and significantly reduced blood glucose levels were observed in
advanced stages of breast cancer (Stage-II, III and IV). Hypoglycemia,
induced in breast cancer patients may be due to increased utilization of
glucose to meet the needs of the growing tumors and increased catabolism of

The ability to maintain an increased rate of glucose utilization
and the capacity to sustain high rates of glycolysis under aerobic conditions
are the most common biochemical phenotypes of rapidly growing cancer cells
(Warburg, 1930; Weinhouse, 1966; Bustamante & Pedersen, 1977; Pedersen,
1978; Aisemberg, 1961). This elevated rate of glucose catabolism is
important for highly malignant tumors, which obtain over 50% of their energy,
and the anabolic precursors for biosynthetic pathways, via glycolysis
(Nakashima et al., 1984; Bustamante et al., 1981). The role of hexokinase,
which commits glucose to catabolism in the first step of the glycolytic
pathway, has come under increased scrutiny in efforts to understand the
molecular basis for the aberrant glycolytic phenotype (Arora & Peterson,
1988) and has been considered also as a potential target for arresting tumor cell growth (Floridi et al., 1981).

Parry & Pederson (1983) reported that in comparison to normal cells, the activity of hexokinase is markedly elevated in highly glycotytic, rapidly growing tumors (Parry & Pederson, 1983). Two factors are largely responsible for this enhanced activity, one of which involves of propensity for the tumor enzyme to bind to the outer mitochondrial membrane, and the other which involves the enzyme overproduction. Mitochondrial binding of the tumor enzyme has been studied in-depth (Rose & Warms, 1967) and has been shown to provide the enzyme with preferential access to mitochondrially generated ATP (Arora KK, and Pederson PL, 1988) and to reduce its sensitivity to product inhibition by glucose-6-phosphate (Bustamante & Pederson, 1977) an important regulator of hexokinase in normal cells (Gumma & McLean, 1969; Kurokawa et al., 1979). Tyrosine phosphorylation of the tumor enzyme (Arora & Pederson, 1993) may also play a role in this process but remains to be investigated.

The overproduction of hexokinase in cancer cells has been given only modest attention, but has nevertheless resulted in the important observation that mRNA levels for this critical key enzyme are also markedly elevated (Johansson et al., 1985; Paggi et al., 1991).

Blood glucose levels are declined in breast cancer patients due to an elevated glucose catabolic rate (Warburg, 1930), a property linked in large part to a marked elevation in the enzyme hexokinase (Bustamante &
Pederson, 1977; Nakashima et al., 1988) due to over expression of hexokinase gene. (Saroj et al., 1995).

4.4. Lipid Peroxidation:

Oxidative stress, especially lipid peroxidation, is known to be involved in carcinogenesis (Trush & Kensler, 1991). The polyunsaturated fatty acid side chains of membrane lipids are susceptible to oxidizing radicals with the formation of lipid peroxide. Later, it is eventually decomposed to a variety of end products including malondialdehyde (Trotta et al., 1982), which is not a normal constituent of erythrocytes and therefore its production provides a measure of the susceptibility of membrane lipid peroxidation.

Plasma MDA levels have been studied as an indicator of lipid peroxidation in human. Different results with regard to the levels of lipid peroxidation products, especially MDA in various tumors including breast cancer have been reported. Gerber et al. (1997) have reported that patients with breast cancer have significantly lower plasma MDA levels when compared to healthy controls. However, Faber et al. (1995) have shown that the patients with breast cancer have higher MDA levels when compared to controls. Those reporting higher levels of oxidative stress, including increased MDA levels, in patients with various cancers have interpreted their data as the oxidative stress playing an important role in the process of carcinogenesis by means of including mutagenesis (Ray et al., 2000). However, some researchers have reported decreased levels of lipid peroxidation products and oxidative stress along with increased antioxidant status in cancer patients (Gerber et al., 1989; Gerber et al., 1997). So, in the
present study, lipid peroxidation was assessed in cancer patients and compared with control.

Table 3 shows the extent of lipid peroxidation in plasma of normal subjects and patients with breast cancer at four different stages. Lipid peroxidation was found to be significantly raised in breast cancer patients, when compared to control. The extent of lipid peroxidation was found to be increased with the progression of breast cancer from stage-I to stage-IV. Lipid peroxidation was enhanced by 25% and 50% in stage-I and stage-II breast cancer patients respectively when compared to the control. Markedly increased lipid peroxidation was observed in advanced stages of breast cancer (Stage-III & IV). The enhanced lipid peroxidation may be due to enhanced serum iron levels in these patients. Further the serum iron levels are correlated with the extent of lipid peroxidation observed in different stages of cancer (Figure 1).

Increased levels of lipid peroxidation products play a role in the early phases of tumor growth (Ames, 1983; Rice-Evans et al., 1993). Malondialdehyde (MDA), a natural product of lipid peroxidation and prostaglandin synthesis, is known to induce carcinogenesis (Trush & Kensler, 1991). Marnet (1999) reported that, the endogenous MDA has also been reported to cause mutagenesis in various tissues by forming DNA adducts.
Figure-1
Relationship between plasma lipid peroxidation and serum iron concentration in breast cancer patients and at four different stages.
Figure 2: Correlation between % of decrease in GSH and % of increase in lipid peroxidation at various stages of breast cancer compared to control.
The potential of MDA formation in formation of catecholestrogen metabolites have been proposed for its role in estrogen induced carcinogenesis (Kubutka et al., 2002; Sharma et al., 2001).

Damage to the breast epithelium by oxygen free radicals (OFR) can lead to fibroblast proliferation, epithelial hyperplasia, cellular atypia and breast cancer. Studies have shown increased lipid peroxidation in solid tumors (Zieba et al, 2001). Thangaraju et al (1994) reported that tamoxifen therapy in postmenopausal women with breast cancer reduced the increase in lipid peroxidation.

Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformability, reduced erythrocyte survival and lipid fluidity (Jain et al, 1983). Oxidative stress has been reported to increase osmotic fragility and reduce the whole cell deformability. Malondialdehyde cross linking and lipid peroxidation have been suggested to play a role in the immunological destruction of erythrocytes (Habbel & Miller, 1984).

Membrane lipid peroxidation is associated with shortened red cell survival in a number of hemolytic states due to either acquired or congenital defects such as oxidant drug induced hemolysis (Cohen & Hochstein, 1964) and B-thalassemia major (Kohane et al., 1978) as well as in the aging of red cells (Jain & Hochstein, 1979). Thus the observed decrease in RBC count in cancer patients compared to controls (Table 2) may be a consequence of enhanced plasma lipid peroxidation observed in these patients. Similar to our studies, Arivazhagan et al (1997) also related the
decreased erythrocyte count and increase in osmotic fragility in patients with gastric cancer to enhanced lipid peroxidation of erythrocytes.

In conclusion, MDA level seems an important biomarker for chemoprevention and can be used as a surrogate marker for chemoprevention studies. Additionally restoration of the endogenous levels of important potential carcinogenic factors, such as MDA might have a role in chemoprevention studies for cancer and warrants further research.

4.5. Reduced Glutathione (GSH):

Glutathione (gamma-glutamyl-cysteinyl-glycine ; GSH), the most prevalent non protein thiol in mammalian cells, protects against electrophiles, free radicals (Sies, 1990) and radiation induced cell damage (Guzman Barron, 1951; Meister, 1991).

Resistance of many cells against oxidative stress is associated with high intra cellular levels of glutathione (Meister, 1991; Mitchell et al, 1987; Estrela et al, 1995). GSH acts directly as a free radical scavenger by neutralizing OH⁻, restores damaged molecules by hydrogen donation, reduces peroxides, and maintains protein thiols in the reduced state (Sies, 1986).

GSH and thiol redox status regulate expression of genes involved in the pathogenesis of different diseases, including cancer, AIDS, diabetes, or atherosclerosis (Sen et al, 1996).
Table-2

Changes in the levels of Hb, RBC, Iron and Sugar in the blood of cancer patients at various stages and normal control (mean ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control n=10</th>
<th>Total Breast Cancer Patients n=40</th>
<th>Breast cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stage-I n=10</td>
<td>Stage-II n=10</td>
</tr>
<tr>
<td>Hb (grams/dl blood)</td>
<td>11.81 ± 1.36</td>
<td>7.71 ± 0.72</td>
<td>9.46 ± 0.74</td>
</tr>
<tr>
<td>RBC (Millions/ c mm)</td>
<td>4.71 ± 0.35</td>
<td>3.44 ± 0.36</td>
<td>4.24 ± 0.2</td>
</tr>
<tr>
<td>Iron (µg/dl serum)</td>
<td>156.9 ± 17.3</td>
<td>277.8 ± 20.3</td>
<td>163.7 ± 10.4</td>
</tr>
<tr>
<td>Sugar (mg/dl blood)</td>
<td>106.5 ± 15.8</td>
<td>71.85 ± 18.05</td>
<td>83.15 ± 31.5</td>
</tr>
</tbody>
</table>

a – Significantly different from controls (p<.05)

Table-3

Changes in the levels of plasma lipid peroxidation in breast cancer patients at various stages and normal control (Mean ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control n=10</th>
<th>Total Breast Cancer Patients n=40</th>
<th>Breast cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stage-I n=10</td>
<td>Stage-II n=10</td>
</tr>
<tr>
<td>Lipid Peroxidation (nmole of MDA/ml plasma)</td>
<td>0.04 ± 0.01</td>
<td>0.087 ± 0.032</td>
<td>0.05 ± 0.04</td>
</tr>
</tbody>
</table>

a - Significantly different from controls (P<.05)
GSH is essential in the maintenance of enzyme SH groups in the proper redox state (Erickson, 1974). Moreover, GSH is a substrate or a cofactor for a number of protective enzymes, such as glutathione peroxidase, the glutathione S-transferase, or the glyoxalase (Sies, 1996). Moreover, glutathione status may be used as a biological marker of aging (Fletcher & Fletcher, 1994). The measurement of glutathione in tissues and biological fluids is used as an index of the oxidative stress that occurs under different physiological and pathological conditions (Sies, 1996; Jaeschke, 1990; Mills et al., 1994, Sastre et al., 1992). Griffith and Meister (1979) proposed the interorgan flow of glutathione. Therefore, GSH and GSSG levels in blood may reflect changes in glutathione status in other less accessible tissue (Sastre et al., 1992). Indeed, different studies have pointed out the interest of measuring blood glutathione for pathological and physiological purposes (Hercbergs et al., 1992; Menendez et al., 1974; Cimino et al., 1987; Lew & Pyke, 1985; Lew et al., 1985; Gohil et al., 1988; Fletcher, 1994). In addition, the erythrocyte has proved to be a valuable cell method for studying oxidative stress (Navarro et al., 1997).

Table 4 shows the changes in reduced glutathione levels in the erythrocytes of breast cancer patients at various stages and normal control. GSH levels were found to be significantly reduced in all clinical stages of breast cancer patients, when compared to control. GSH levels were found to be decreased by 9.4% to 68% from stage-I to Stage-IV of cancer patients compared to control, indicating the percentage of decrease in GSH advances from stage-I to stage-IV (9.4%, 37%, 61%, 68%). Thus the deceased GSH levels in different stages of breast cancer coinsides with the enhanced lipid
peroxidation observed in corresponding stages of breast cancer patients. Decreased GSH levels in breast cancer patients may be attributed to increased utilization of GSH to scavenge lipid peroxides as well as sequestration by tumor cells. Navarro et al (1999) reported that, reduced GSH level in patients was attributed to decreased availability of substrate for GSH synthesis.

Cancer patients are more likely to respond to treatment if their erythrocyte GSH and, by inference, tumor GSH concentrations are low (Herbergs et al, 1992).

The decreased GSH levels in different stages of breast cancer correlated positively with the enhanced lipid peroxidation observed in the corresponding stages of the cancer patients (Table 2).

4.6. Vitamin C:

The role of antioxidant vitamins, diet and lifestyle modifications in modulating human cancer incidence have drawn significant attention from basic and clinical scientists. The issue had been critically reviewed with respect to cancer prevention (Prasad et al, 1998).

Vitamin C (ascorbic acid) is an essential micro nutrient required for normal metabolic functioning of the body (Jaffe, 1984). Humans and other primates have lost the ability to synthesize vitamin C as a result of a mutation in the gene coding for L-gulonolactone oxidase, an enzyme required for the biosynthesis of vitamin C via the glucuronic acid pathway (Woodall & Ames, 1997).
Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine, and neurotransmitters (Burri & Jacob, 1997; Tsao, 1997). Other proposed activities of vitamin C include maintenance of enzyme thiols in a reduced state and sparing of glutathione, an important intracellular antioxidant and enzyme cofactor (Meister, 1994), and tetrahydrofolate, a cofactor required for catecholamine biosynthesis (Tsao, 1997).

Vitamin C is an important water soluble antioxidant in biological fluids (Frei et al., 1989). Vitamin C readily scavenges reactive oxygen and nitrogen species, such as superoxide and hydroperoxyl radicals, aqueous peroxyl radicals, singlet oxygen, ozone, peroxynitrite, nitrogen dioxide, nitrooxide radicals, and hypochlorous acid thereby effectively protecting the cells from damage (Halliwell, 1996). Although vitamin C also reacts rapidly with hydroxyl radicals, it is nevertheless unable to preferentially scavenge this radical over other substrates (Niki & Noguchi, 1997). Vitamin C can also act as a coantioxidant by regenerating α-tocopherol (vitamin E) from the α-tocopheroxyl radical, produced via scavenging of lipid soluble radicals (Bowry et al., 1995). This is a potentially important function because invitro experiments have shown that α-tocopherol can act as a pro-oxidant in the absence of coantioxidants such as vitamin C (Neuzil et al., 1997).

Vitamin C may protect against cancer through several mechanisms in addition to inhibition of DNA oxidation. One potential mechanism is chemoprotection against mutagenic compounds such as nitrosamines (Hecht, 1997; Tannenbaum & Wishnok, 1987). In addition,
vitamin C may reduce carcinogenesis through stimulation of the immune system.

Table 4 shows the alterations in the levels of vitamin C in all clinical stages of breast cancer and normal control. Vitamin C levels were found to be decreased in breast cancer patients, when compared to control. Its concentrations were decreased by 36% to 39% from stage-I to stage-IV of cancer patients. No significant decrease was observed in stage-I, whereas 24% decrease was observed in stage-II and, 30.9% and 39% in stage-III and stage-IV respectively, when compared to control. Reports of Thangaraju et al (1994), and Manoharan et al (1995), are in support of our present observation of decreased Vitamin C levels in breast cancer patients and intensification of vitamin C deficiency as the disease advanced to stage III and IV. Lower concentration of vitamin C may be one of the possible factors for higher plasma lipids, which modulate tumor cell proliferation. Since vitamin C levels are decreased, they are likely not sufficient enough to counter ROS thereby resulting in higher oxidative stress, which may be the cause of cellular and molecular damage thereby leading to cell proliferation and malignant conversion in the development of breast cancer. The significant decrease in vitamin C level at stage-III and its further intensification in stage-IV of breast cancer indicate the consequences of vitamin C deficiency leading to cell proliferation and malignant conversion are intensified in stage-III & IV compared to stage-I and II.

Epidemiologic studies have shown an inverse association between vitamin C intake, mainly from fruit and vegetables and cancer (Block, 1991). A multitude of epidemiologic studies have shown that increased
consumption of fresh fruit and vegetables is associated with a reduced risk of most types of cancer (Block, 1991; Fontham, 1994). Over the years, numerous case control studies have been carried out to investigate the role of vitamin C in cancer prevention; these have been revised by Block (1991) and Fontham (1994). Of the hormone dependent cancers, only breast cancer was inversely associated with vitamin C intake, in contrast with ovary and prostate cancers (Fontham, 1994).

4.7. Antioxidant Enzymes:

**Glutathione Peroxidase (GPx) & Catalase:**

In the erythrocytes $H_2O_2$ produced can be detoxified by the action of various enzymes such as GPx and CAT. Of the enzymatic detoxification pathways, selenium dependent glutathione peroxidase play an essential part in the metabolism of $H_2O_2$, fatty acid hydroperoxides, and phospholipid hydroperoxides in mamalian cells (Chu et al., 1990 & 1993; Esworthy et al., 1991; Maiorino et al., 1991).

GPx catalyzes the reduction of hydroperoxides with GSH to form GSSG and the reduction product of the hydroperoxide (Chance et al., 1979) thus affording a major defending role in cells against peroxidative damage (Meister & Anderson, 1983). GPx initiates a repair mechanism whenever an oxidative challenge could result in peroxidation of complex biochemical compounds such as lipids and nucleic acids (Uday Bhandyopadhyay et al., 1994). So, it plays an important role in number of cellular defense mechanisms essential for the survival of the cell.
Table 5 shows the alterations in the activity of erythrocyte GPx in breast cancer patients compared to normal subjects. The activity of GPx was found to be increased significantly in breast cancer patients. GPx activity was elevated significantly from stage - II to stage -IV breast cancer patients (4% to 25%), whereas no significant change was observed in stage I breast cancer patients, when compared to controls.

The elevation of erythrocyte GPx activity in breast cancer patients and progression of its elevation from stage II to stage IV may be a marker of cell proliferation by eliminating H$_2$O$_2$ and other hydroperoxides. Similar to our study, Doroshow (1995), Ican et al (2002) and Kumaraguruparan et al (2002), were also reported enhanced GPx in breast tumors. The enhanced activity of GPx in the present study also correlated with decreased GSH levels of plasma. Inspite of enhanced GPx activity the plasma lipid peroxidation was found to be enhanced in breast cancer patients indicating that the protection provided by GPx is not sufficient to counterbalance the oxidative stress observed in these patients. GPx plays a key role in tumorigenesis by altering the lipooxigenase and cyclooxygenase pathways (Bryant et al., 1982; Capdevila et al, 1995). Contrary to our reports, Robinson et al (1979) reported decreased GPx activity with progression of neoplastic transformation and in erythrocytes of gastric cancer patients. Hong et al (2001) reported that GPx was a key enzyme in the defense against oxidative damage and cell survival was correlated directly with the GPx activity.

The antioxidant enzyme, catalase, is widely distributed in all animal tissues and high activity is found in red blood cells. Studies have
shown that the administration of catalase results in protection against $H_2O_2$ mediated lipid peroxidation (Corrocher et al, 1986; Holbach, 1977).

$H_2O_2$ is considered a key metabolite because of its relative stability, its diffusion (Fridovich, 1976), and its involvement in cell signaling cascade (Khan & Wilson, 1995; Fridovich, 1995; Sujuki et al., 1997; Pantopoulso et al., 1997). For these reasons the erythrocyte has been a traditional target for studying the metabolism of $H_2O_2$. Since the first description of glutathione peroxidase in 1957, an intense debate was created on whether catalase or GPx was the primary enzyme in the removal of $H_2O_2$ (Mills, 1957; Cohen, 1963; Jacob et al, 1965; Kirkman & Gaetanic, 1984; Scott et al., 1991). A major role of GPx in decomposing $H_2O_2$ especially derived from studies on erythrocytes with a reduced capacity to generate NADPH (Cohen et al, 1963). Experiments with acatalasic erythrocytes provided more evidence that catalase are a first line of defense against $H_2O_2$. Erythrocyte provided more evidence that catalase is a first line of defense against $H_2O_2$ (Jacob et al, 1965). Using a mixture of GPx and catalase in a cell free system which corresponding activities to erythrocytes, it was previously concluded that catalase accounts for more than 50% of the removal of $H_2O_2$ (Gaetani et al., 1595; Scott et al, 1993). Catalase decomposes $H_2O_2$ without generation of free radicals by minimizing one electron transfers. Hence, a protective role against free radicals may be the main physiological function in these cells (Sebastain Mueller et al., 1997).

In present study, catalase activity was estimated in all clinical stages of breast cancer and normal controls. Catalase activity was found to be increased significantly from stage - II to stage - IV of breast cancer patients.
when compared to the normal controls (Table 5). Catalase activity was increased by 26% in stage II and 38% in stage III & IV, compared to controls. No significant change was observed in stage I breast cancer patients. The increase in catalase activity could be a protective mechanism for the cells due to the tumor induced hyperproduction of reactive oxygen intermediates. Over expression of antioxidants have been documented in wide varieties of malignant tumors including breast cancer (Skrzydlewska et al, 2001; Iscan et al., 2002; Saydam et al., 1997; Kumaraguruparan et al, 2002). Halliwell (2000) reported that oxidative stress can cause upregulation of antioxidant enzymes that render cells more resistant to subsequent oxidative insult. Further Lu et al (1997) reported that cancer cells with increased activity of antioxidant enzymes are presumed to escape recognition by cytotoxic lymphocytes. Unlike to our reports, decreased erythrocyte catalase activity was reported in patients with stomach cancer and in cancerous tissue in human hepatoma (Corrocher et al., 1986) and liver tumor bearing mice (Kaplan & Groses, 1972), others have reported reduced antioxidant activities in lung tumors (Jaruga et al., 1994; Guner et al., 1996; Coursin et al., 1996). The increase in lipid peroxidation observed in breast cancer patients in the present study was not counterbalanced by the enhanced antioxidant defense enzymes such as GPx and catalase. Moreover, these enzymic changes may suggest possible cancer induced alterations in the regulation of gene expression in the pluripotent stem cells, or may reflect a more rapid turnover of the red blood cells.
### Table-4

Alterations in the levels of GSH and Vitamin C in the Breast Cancer patients at various stages and normal control (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control n=10</th>
<th>Total Breast Cancer Patients n=40</th>
<th>Breast cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stage-I n=10</td>
</tr>
<tr>
<td>GSH (µg/dl Plasma)</td>
<td>53.05 ± 3.0</td>
<td>29.77 ± 3.43</td>
<td>48.05 ± 2.1</td>
</tr>
<tr>
<td>Vitamin C (mg/dl serum)</td>
<td>1.10 ± 0.39</td>
<td>0.82 ± 0.25</td>
<td>1.06 ± 0.39</td>
</tr>
</tbody>
</table>

a- significantly different from control (P<.05)

### Table-5

Changes in the activities of erythrocyte Glutathione peroxidase and Catalase in the Breast Cancer patients at various stages and normal control (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control n=10</th>
<th>Total Breast Cancer Patients n=40</th>
<th>Breast cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stage-I n=10</td>
</tr>
<tr>
<td>GPx (U^A)</td>
<td>177.3 ± 31.0</td>
<td>2.03.36 ± 25.57</td>
<td>170.06 ± 15.0</td>
</tr>
<tr>
<td>Catalase (U^B)</td>
<td>0.05 ± 0.001</td>
<td>0.063 ± 0.007</td>
<td>0.05 ± 0.007</td>
</tr>
</tbody>
</table>

U^A - µg of GSH consumed/min/g Hb

U^B - µg of H₂O₂ decomposed/min/g Hb

a- significantly different from control (P < .05)
4.8 Uric Acid:

Uric acid is an end product of the metabolism of purines. It is accordingly formed endogenously from nucleoprotein metabolism, and exogenously from the metabolism of purines taken in the food. Uric acid has been demonstrated to be an important antioxidant and a free radical scavenger in humans. It is one of the major radical trapping antioxidants in plasma and is reported to protect the erythrocyte membrane against lipid peroxidation (Wayaner et al., 1987). Urate also possesses antioxidant activity. In has been found to protect ascorbate against oxidation by cupric ion and iron. Uric acid interacts with peroxynitrite to form a stable nitric oxide donor, thus promoting vasodilation and reducing the potential for peroxynitrite induced oxidative damage (Skinner et al., 1998). Thus uric acid could be expected to protect against oxidative stress.

Table 6 shows the changes in uric acid levels in breast cancer patients at various stages and in normal controls. Uric acid concentrations were found to be significantly increased in all clinical stages of breast cancer patients, when compared to controls. Serum uric acid levels were increased by 16% to 45% as the disease progresses from stage - I to stage - IV (16%, 39%, 41% and 45%), when compared to normal control. The elevated concentration of serum uric acid level in breast cancer patients may be viewed as an index of increased antioxidant defense to compensate the loss of other antioxidant mechanisms. In contrast to our results Nagini et al (1997) reported decreased serum uric acid levels in oral cancer patients.
Uric acid levels are increased due to increased catabolism of purines, increased nucleic acid turnover and decreased uric acid excretion. Increased levels are also found in leukaemia. This appears to be due to the considerable breakdown of cell nuclei, with resulting increased metabolism of nucleoprotein (Harold Varley, 1969). Even though, uric acid may appear to make a significant contribution to serum antioxidant capacity it has been found to stimulate granulocyte adherence to the endothelium (Boogaerts et al., 1983), and peroxide and superoxide free radical liberation (Falasca et al., 1993). Therefore, uric acid may have a deleterious effect on the endothelium through leukocyte activation and interestingly a consistent relationship and circulating inflammatory markers (Hutton et al., 1985; Leyva et al., 1998).

4.9. Mucoproteins:

Many of the genetic alterations selected during malignant transformation disrupt paracrine signaling networks, resulting in the release of tumor cells from normal growth constraints or the establishment of new signaling pathways that confer growth and survival advantages on cancer cells (Hanahan & Weinberg, 2000). Many mucoproteins have been identified as highly expressed in cancer, with potential clinical utility for the therapeutic intervention or as diagnostic or prognostic biomarkers. Recent genomic analyses have identified an increasingly large number of genes over expressed in cancers, of which several encode mucoproteins (Welsh et al., 2001).

Pettingale and Tee (1997) stated that the patients with breast cancer had significantly higher levels of beta 2 glycoprotein and ceruloplasmin. There were a number of significant correlations between serum
protein levels and the progression of breast cancer as measured by the clinical score. A longer follow-up may be required to establish the value of mucoprotein changes which could predict the development of metastasis in patients with breast cancer.

Table 6 shows the alterations in the serum mucoprotein levels in breast cancer patients at various stages and normal control. Mucoprotein concentrations were found to be increased in breast cancer patients, when compared to control. Slightly but not significantly increased serum mucoprotein levels were found in stage - I breast cancer, whereas from stage-II to stage - IV, serum mucoprotein levels were elevated markedly (15%, 41%, 70%), when compared to normal control.

Of 74 different genes identified as over expressed in cancer tissues, several encode glycoproteins with demonstrated clinical diagnostic application. For example α-Fetoprotein (AFP) levels are elevated in liver carcinoma. Except in the pregnant patient, AFP levels greater than 1000 μg/L are indicative of cancer. At these levels of AFP, about half of hepatocellular carcinomas may be detected (Kelsten et al., 1988). AFP is also useful for determining prognosis and in the monitoring of therapy for hepatocellular carcinoma. Elevated AFP levels after surgery may indicate incomplete removal of the tumor or the presence of metastasis. Falling or rising AFP levels after therapy may determine the success or failure of the treatment.

Sleat et al (1983) explained that all carcinomas contained 4-fold higher levels of mannose-6-phosphate mucoproteins than did benign tumors or normal breast samples. In about 15% of the carcinomas, levels of
mannose – 6 - phosphate mucoproteins were highly elevated (7-10 fold). Multiple mannose-6-phosphate glycoproteins were detected, suggesting a general alteration in the synthesis or processing of many lysosomal enzymes in carcinomas.

4.10. Sialic Acid:

Sialic acids comprise of N - or O - acyl derivatives of 9-carbon sugar neuraminic acid. Sialic acids are terminal sugar components of the oligosaccharide chains of glycoproteins and glycolipids. In human beings it is present in body fluids (blood plasma, breast milk, synovial fluid, sweat, gastric juices and urine) and tissues (erythrocytes, leucocytes, platelets, salivary glands, throat, stomach, cervix, colon, cartilage etc. (Sillanaukee et al., 1999) In blood plasma a large quantity of sialic acids are present in fibrinogen, ceruloplasmin, antitrypsin, complement proteins and transferrin( Taniuchi et al., 1981; Petren & Vesteberg, 1989). It is also present as constituent of membrane glycoproteins of erythrocytes, leucocytes and platelets.

Sialic acid perform important functions in biological recognition phenomena, including cell-cell interactions (Munday et al, 1999; Yednock & Rosen, 1989) and binding of toxins, viruses, bacteria and parasite to their cellular receptors (Karlsson, 1995; Kelm & Schemer, 1997). Similarly, sialic acids can be key determinants for the stability and biological activity of hormones or enzymes in vivo (Ulloa-Aguirre et al., 1999). Interest in sialic acid has rapidly increased in recent years, especially since the recognition of their involvement in the regulation of a variety of biological
phenomena. The structure, occurrence and general functions of sialic acids have been extensively reviewed (Shauer, 1982). Some important functions attributed to sialic acids are: (1) sialic acids contribute significantly to the overall negative charge of cell surface and glycoproteins. The negative charge contributes to, cell to cell repulsion (antiadhesion effect), functioning stability and survival of glycoproteins in blood circulations; (2) due to the shielding effect, sialylated glycans protect parts of a glycoprotein from proteolytic attacks; (3) membrane sialic acids assist in tissues and body fluids; (4) it serves as a component of cell surface receptors.

In present case-control study, sialic acid concentrations were estimated among breast cancer patients in relation to different clinical stages (Table 6). Sialic acid concentrations in serum were significantly elevated in all clinical stages of breast cancer patients compared to controls. Sialic acid concentrations were increased by 14%, 29.8%, 40%, 85% from stage - I to IV respectively, when compared to the normal control.

Increased sialic acid concentrations have been observed in several diseases eg. Tumors, myocardial infarction, diabetes, inflammatory disorders, and alcoholism (Schauer et al., 1995; Sillanaukee et al., 1999; Romppanen et al., 1997; Parzkowska et al., 1998; Crook et al., 1994; Crook et al., 1996). Manoharan et al (1995) reported increased plasma lipid bound sialic acid in patients with oral squamous cell carcinoma, whereas enhanced plasma ceruloplasmin, a sialoglycoprotein was reported in oral cancer patients (Nagini et al., 1997).
4.11. Copper:

Copper is an essential trace element. From the gut, copper is carried to the liver bound to albumin and there it is incorporated into ceruloplasmin. Ceruloplasmin is then secreted into the blood and accounts for 80-90% of the circulating copper. The main role of copper is as a component of copper metalloenzymes. In the synthesis of collagen and elastin, the cross linking reactions require various copper containing amine oxidases and copper is also involved in the oxidation of Fe$^{2+}$ to Fe$^{3+}$ during hemoglobin and transferrin formation (William & Stephen, 1995).

Reduction of H$_2$O$_2$ by copper ion produces highly reactive DNA damaging species (Chevion, 1988). Copper ion induces significantly more DNA base damage in the presence of H$_2$O$_2$ than does ferrous ion (Aruoma et al., 1991; Dizdaroglu et al., 1991), the other biologically relevant transition metal ion. Copper is an important structural metal ion in chromatin being present at about one copper ion per kilobase (Bryan et al., 1981). For these reasons, there is an increased interest in the ability of copper ion to participate in DNA damaging reactions in vivo (Stoewe & Prutz, 1987).

In present study, serum copper levels were estimated among breast cancer patients in relation to different clinical stages and in normal subjects (Table 6). Serum copper levels were found to be increased significantly in stage - II, III and IV breast cancer patients, when compared with controls. But in stage - I patients the serum copper levels were not altered significantly. Markedly elevated serum copper levels (69.4%) were observed in stage - IV cancer patients, when compared to stage III (14%). Similar to our studies enhanced serum copper levels are reflected in oral
cancer patients by observing enhanced plasma ceruloplasmin concentration in these patients (Gutteridge et al., 1981).

Kinetic inhibitor and sequence context studies have all suggested that DNA damages induced by copper ion in the presence of H₂O₂ occurs site specifically at sites of DNA – associated copper (Kazakov et al., 1988). However, the nature of the ultimate DNA oxidizing species produced by interaction of the DNA - Cu (I) complex with H₂O₂ remains uncertain. Some investigators have suggested that the hydroxyl radical is the principal reactive intermediate (Stoewe & Pruty, 1987), while others have proposed less reactive intermediates, such as copper – oxo complex, or cupryl ion (Johnson et al., 1988). Regardless of the true reaction mechanism, exposure of DNA to copper ions has been reported to result in single and double stranded breaks, modified bases, abasic sites, and DNA protein cross links (Halliwell & Aruoma, 1991; Dizdaroglu, 1992).

DNA damage induced by copper ion mediated reduction of H₂O₂ has been studied with respect to reaction mechanism, reaction kinetics (Stoewe & Prutz, 1987), damage products (Aruoma et al, 1991) and DNA sequence context effects (Yamamoto & Kawanishi, 1989). DNA damage induced by copper ion mediated reduction of H₂O₂ is site specific for instance, it occurs only at sites of DNA bound copper ion. The kinetic data of Goldstein and Czapski (1986), suggest that the sequence of events leading to DNA damage begins with reduction of cu(II) in solution. Free cu(I) is rapidly bound to DNA with high affinity (Prutz et al., 1990). H₂O₂ then enters the coordination complex of DNA-cu (I), resulting in oxidation of cu (I) and DNA.
damage. Failed repair of damaged DNA and an inability to regulate an essential check points in cell cycle may result in cancer.

4.12. Acid Phosphatases:

Acid phosphatases (ACP : EC 3.1.3.2) are a family of enzymes that can be differentiated according to structural, catalytic and immunological properties, tissue distribution and subcellular location. So far three isoenzymes of extra cytoplasmic acid phosphatases have been identified that belong to the group of orthophosphoric monoesters with an acidic pH optimum (Drexler & Gignac, 1994).

Human ACPs are normally found at low concentrations. However, pronounced changes in their synthesis occur in particular diseases, where usually high or low enzyme expression is seen as part of the pathophysiological process. This observation suggests that acid phosphatases could be diagnostically useful as serological and histological markers of disease and could also be of use in the investigation of the pathophysiology of the associated disease.

In present study, serum ACP activity was estimated in all clinical stages of breast cancer and in normal subjects (Table 7). The serum ACP activity was found to be increased in breast cancer patients, when compared to the normal subjects. Slight increase was observed in the activity of ACP in stage I & II breast cancer patients, whereas markedly increased ACP activity was observed in advanced stages of breast cancer stage - III and IV (59%, 102%) when compared to control.
Hayman et al (1994) found that ACP could catalyze the formation of free radicals. Taking this observation further, Halleen et al (1999) incubated ACP invitro with H2O2 which produced ROS in the form of hydroxyl radicals. Hydroxyl radical is of paramount concern for breast carcinogenesis because OH' can modify DNA to produce several OH' adduction products, base deletions, single strand and double stand breaks (Write et al., 1995; Lubec, 1996; Brawn & Fridovich, 1981). Enhanced serum ACP activity (59%, 102%) and increased lipid peroxidation (190%, 200%) at stage III and IV of breast cancer may promote more free radical generation in the advanced stages of breast cancer, compared to earlier stages (I and II) of breast cancer.

Prostate acid phosphatase (PACP) has been used extensively as a serum marker for cancer of the prostate. It is released into the serum from the prostate gland in increasing amounts as malignant tissue proliferates (Bull et al., 2001). The histological detection of an acid phosphatase enzyme is also exploited to diagnose leukaemic reticuloendotheliosis, otherwise known as hairy cell leukaemia (Yam, 1974).

4.13. Alkaline Phosphatase:

Alkaline phosphatases (ALP; Orthophosphoric monoester phosphohydrolase. EC 3.1.3.1) are a group of phosphotidylinositol – anchored membrane proteins with wide substrate specificity (Fishman, 1987; Horris, 1990). In humans at least four distinct classes of this enzyme have been identified using genetic and biochemical analysis (Harris, 1982; Stigbrand et al, 1982). The heat stable isozyme from placenta (PALP) and the partially heat stable isozyme from intestine (IALP) are tissue specific in terms of their
expression (Millan, 1986). The heat labile isozyme represents the liver – bone – kidney or tissue nonspecific (TNALP) forms and is present in most cell types (Harris, 1982; Stigbrand et al., 1982; Weiss et al., 1986). The fourth isozyme is also heat stable and is present at low levels in germ cells (GCALP) (Gum et al., 1990). To date, several studies have indicated the involvement of ALP in bone mineralization, transport of inorganic phosphate (Hui et al., 1984; Hiranon et al., 1984) and cellular events such as the regulation of protein phosphorylation, cell growth, apoptosis and cellular migration during embryonic development (Chan & Stinson, 1986; Change et al., 1994).

In the present work, serum alkaline phosphatase activity was estimated in all clinical stages of breast cancer patients and in normal subjects (Table 7). Serum alkaline phosphatase activity was found to be raised in breast cancer patients, when compared to the normal subjects. Serum ALP activity was not significantly altered in first two clinical stages of breast cancer, where as in stage III and IV serum ALP activity was found to be elevated by 21% and 29% in stage III and IV respectively, when compared to controls.

ALP genes are regulated by distinct signals as shown by clear differences in their expression profiles (Millan, 1992). Ectopic expression of ALPs has been associated with a variety of human cancers, although the mechanism has yet to be elucidated. The expression pattern of ALP isozymes are altered in malignant tissues, for example, PALP and GCALP are over expressed in cells derived from breast cancer and chorio carcinoma, respectively (Change et al., 1994; Watanabe et al., 1989). Enhanced
expression of ALP has also been reported in hepato cellular carcinoma (Higashino et al., 1989).

The aberrant expression of ALP genes in cancer cells has led to the suggestion that ALP isoenzymes may be involved in tumorigenesis. ALP genes are shown to be highly inducible in a number of cell types and by a variety of agents. Among them, steroid hormones such as glucocorticoids, progesterone and estradiol have been shown to alter the expression of ALP genes in cell lines of various origins, including cervical cancer, endometrial adenocarcinoma and breast cancer cells (Chou & Takahashi, 1987). In addition to steroids, retinoic acid (RA), an active metabolite of vitamin A, is also shown to regulated ALP activity (Scheibe et al., 1991; Ng et al., 1989; Gianni et al., 1993).

The serum alkaline phosphatase activity is often markedly elevated in cancer patients due to over expression of ALP genes (Kaye & Rankin, 1988). However, elevated plasma concentrations are also found in a number of malignancies, including those involving lung, testis, ovary and uterus. It appears to be a good marker for testicular seminomas.

4.14. Inorganic Phosphate:

Phosphorus in the form of inorganic or organic phosphate is an important and widely distributed element in the human body (Bilezikian, 1990). Serum inorganic phosphate levels are dependant on metals and variation in the secretion of hormones such as parathyroid hormone and may very widely in various pathological conditions (David & Robert, 1988).
Table-6
Changes in the levels of Uric acid, Mucoproteins, Sialic acid and Copper in the Breast Cancer patients at various stages and normal control (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control n=10</th>
<th>Total Breast Cancer Patients n=40</th>
<th>Breast cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uric acid (mg/dl serum)</td>
<td>Stage-I n=10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.92 ± 1.21</td>
<td>8.03 ± 0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mucoproteins (mg/dl serum)</td>
<td>69.8 ± 5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sialic acid (µg/dl plasma)</td>
<td>1.84 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Copper (mg/dl serum)</td>
<td>160 ± 6.1</td>
</tr>
</tbody>
</table>

a-significantly different from control (P< 0.05)

Table-7
Changes in the activity of serum Acid phosphatase, Alkaline phosphatase and Inorganic phosphorus at various stages of Breast Cancer patients and normal control (mean ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control n=10</th>
<th>Total Breast Cancer Patients n=40</th>
<th>Breast cancer patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ACP (UA)</td>
<td>Stage-I n=10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46.08 ± 3.5</td>
<td>68.9 ± 8.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALP (UA)</td>
<td>1.86 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inorganic Phosphorus (mg/dl serum)</td>
<td>4.14 ± 0.21</td>
</tr>
</tbody>
</table>

a-significantly different from control (P< 0.05)

UA: n moles of PNP liberated / min / dl serum
Inorganic phosphate has several diverse actions in cells. It has a critical role as a high energy phosphate bond in adenosine triphosphate (ATP) and other high energy compounds. It is also a component of cyclic adenine and guanine nucleotides as well as nicotinamide adenine dinucleotide phosphate (NADP), which are important in many enzyme systems (Yu & Lee, 1987).

In the present study, serum inorganic phosphate concentrations were estimated in breast cancer patients at various clinical stages and normal control (Table 7). The serum inorganic phosphate levels were found to be elevated in breast cancer patients, when compared to control. The extent of increase in serum inorganic phosphate was increase gradually from stage - I to IV (9%, 27.5%, 36%, 63%), compared to control. Increased ACP and ALP activities in breast cancer patients may be a reason for increased serum inorganic phosphate concentrations in breast cancer patients.

Increased phosphate levels are usually associated with acute or chronic renal failure, hypoparathyroidism, leukemia and lymphoma. The most acute problem associated with rapid elevation of serum phosphate levels is hypocalcemia with tetany, seizures and hypertension (Knochel et al, 1986). Inorganic phosphate residues are linked by the high energy phosphoanhydride bonds found in ATP, to form inorganic polyphosphates (Poly P) found in all bacteria, fungal, plant and animal cells (Kornberg et al, 1999). Inorganic phosphates are the substrates for protein Kinases, which regulate cell growth in large part by its ability to phosphorylate the substrates like ribosomal S6 Kinase and PHAS-I (Finger et al, 2002). Thus, activation of protein kinases stimulates protein synthesis and proliferation in mammalian cancer cells.