1.1. Introduction

All living organisms are composed of cells which are programmed to fulfil various functions. The normal cell reproduction and multiplication are controlled and restrained. But sometimes certain factors initiate a genetic change which disrupts the normal cell function and forces it to behave abnormally. The control and restraints for normal growth and differentiation are vanished. The end result is that the new abnormal 'Cancer Cell'. Cancer cells are therefore derived from normal cells including skin, lung, intestine, brain, bone, breast, spleen or blood cells. Once the normal cells have been transformed into cancerous cells, they multiply at a relatively fast rate and lose all restraints and controls vital to coordinated growth and differentiation of normal cells. In the advanced stages, the cancer cells invade and destroy the surrounding normal tissue, often accompanied by metastasis via the blood (Franks et al., 1997).

Cancer may result from any one or a combination of chemical, physical, biological and genetic insults to individual cells. An important biological property shared by these agents is their ability to cause damage to or alteration of cellular DNA (Tannock & Hill, 1998). About 80% of human cancers are caused by environmental factors, principally chemicals (Doll & Peto, 1996).

1.2. Cancer of the Breast:

Carcinoma of the breast, the most common cancer in women, is the third most common cancer in the world, accounting for the highest morbidity and mortality in women. It is of serious concern owing to the rising
incidence of the disease in the last 5-10 years (Parkin et al., 1999). Women diagnosed with breast cancer have relative survival rates of 96%, 79%, 67%, and 60% for 1,5,10 and 15 years respectively (Wingo et al., 1998).

Many predisposing factors have been observed by Willett (1993). They are geographic distribution, genetic predisposition, increasing age, length of reproductive life, parity, age at the time of first child, obesity, exogenous estrogen, oral contraceptives and fibrocystic changes in atypical, epithelial hyperplasia and carcinoma of the contralateral breast or endometrium. Cancer of breast rarely occurs before the age of 25, but a steady rise has been observed at the time of menopause. The risk increases with early menarche and late menopause and is more frequent with nulliparous than multiparous women. Women with first child when older than 30 years are at increased risk.

Based on the degree of differentiation breast cancers are graded as non-metastasising, metastasising and moderately metastasising. The American joint committee on cancer classified tumors into different stages as: Stage I - Tumor size < 2 cm in diameter, with no nodal involvement. Stage II - Tumor size < 5cm, movable axillary node, with no distant metastasis. Stage III - skin involvement, pectoral chest wall fixation, axillary node and internal mammary lymph node involvement. Stage IV - any form of cancer with or without nodal involvement, pectoral fixation, but with disseminated metastasis.
1.3. Symptoms:

Breast cancer presents itself as a cancerous or non cancerous solitary, palpable mass. Cancerous lumps are hard, single and irregular in shape. Bleeding from nipple increase likelihood of presence of cancer. Other symptoms which indicate its presence include change in size and shape of breast, rash around the nipple, lump in arm pit, prominent veins around the breast, swelling of arms, ulceration of skin and symptoms of secondary tumors elsewhere. In advanced breast cancer in drawing of skin and dimpling, inversion of nipple is observed.

1.4. Incidence:

Annually, breast cancer is diagnosed in 910,000 women worldwide and 376,000 women die from the disease (WHO, 1997). There is a four to five fold variation in breast cancer incidence rates across different countries. Age adjusted rates for breast cancer are 176% higher in developed than in developing nations, with high incidence of expression in North America and Europe (Parkin, 1998).

In the United States, breast cancer accounts for highest incidence and second highest mortality rate of all cancers. According to the Surveillance, Epidemiology and End Results (SEER), 182,800 new cases and over 40,800 deaths from breast cancer have been reported among women in the United States (Greenlee et al., 2000). In Germany, breast cancer is responsible for 25% of female cancers and 18% of cancer deaths (Schleicher & Ammon, 1998).
Although the incidence of breast cancer is lower in Asia, the largest increase in incidence rates was recorded in Japan and Singapore. Furthermore, while no significant change in mortality rates have been reported in the U.S.A. England, Wales or Norway, there has been a 50-60% increase in Japan and Singapore (Ursin et al., 1994).

Migrant studies have shown a higher percentage of breast cancer in South African Indians when compared to ancestral Indians in the North West. In contrast, low rates of breast cancer have been recorded in Asian Indians from the Indian subcontinent (Walker & Halse, 1999).

In India, breast cancer is the second most common cancer among women (Schaier & Lubin, 2000). The age adjusted rates (AAR) of breast cancer in Chennai and Bangalore is 21.7, whereas in Mumbai it is 24.4 (Yeole & Jussawala, 1992). A population based survival study in Chennai has shown that the observed survival rates at 1, 3 and 5 years are 80%, 58% and 48% respectively, while the relative survival rates are 81%, 61% and 51% (Gajalakshmi et al., 1997). In Bangalore, the observed survival rate was 42.3% and the corresponding relative survival rate was 46.8% (Nanda Kumar et al., 1995).

1.5. Epidemiological Evidences of Breast Cancer:

The first observation regarding a familial association with breast cancer was performed more than five decades ago by Macklin (1954) who, in her pioneering work designed to look for the genetic basis of human breast cancer, found a significantly higher frequency of prostate cancer among relatives of women with breast cancer than among relatives of control groups.
Thiessen (1974), after analysis of the familial incidence and distribution of all malignancies in a group of 145 breast cancer patients, compared with that of 139 randomized control patients, reported that significantly higher incidences of only uterine, prostatic and breast cancer were found among both maternal and paternal relatives of the breast cancer patients. On this basis, he proposed that the mammary gland is a part of an integrated genital organ system whose different parts share unique biological and pathological characteristics, including hormone responsiveness and cancer susceptibility. He also hypothesized the existence of some common etiological factor that could operate in the development of tumors in diverse reproductive organs, including breast and prostate. Cannon et al. (1982), in a study of genetic epidemiology of prostate cancer in a population from the Utah Mormon genealogy, showed a significant coaggregation of prostate cancer with breast cancer. More recently, in case control studies based on anamnestic data, Andrieu et al. (1991) did not find evidence of association between these two tumors. By contrast, Tilinius et al. (1992) in a large cohort study including 1539 Icelandic women with breast cancer, reported that the risk of prostate cancer was significantly raised for all male relatives, as well as for first degree relatives, and second-degree relatives of breast cancer patients. Similarly, Anderson and Badzioch (1992) found that a family history of prostate cancer in male breast cancer patients resulted in a 4-fold increased breast cancer risk in first degree female relatives compared with that in male breast cancer families with no history of prostate cancer. By contrast, a family history of lung cancer, colon cancer or melanoma had no effect on increasing risk of breast cancer. Finally a series of recent studies
concerning the putative familial clustering of breast and prostate cancer have provided opposite results. Thus, Isaacs et al (1995) in a study of families selected because of the presence of prostate cancer did not find increased risks for cancer at other sites, such as breast, ovary or endometrium. Negri et al (1997) did not observe an elevated risk of prostate cancer in relatives of breast cancer patients.

Therefore, it seems clear that not all data on the potential association between breast cancer in females and prostate cancer in males are univocal. However, a number of studies preformed by different groups in populations of different geographic origin appear to indicate that a family history of breast cancer may have a significant influence on prostate cancer and vice versa.

1.6. Molecular Mechanisms and Genetic Abnormalities of Breast Cancer:
Breast cancer progression from normal tissue to invasive cancer takes place over 5-20 years. It is driven by a series of accumulating genetic changes that may be hereditary as well as somatic (Hollywood et al., 1995).

Two major classes of genes, namely oncogenes and tumor supressor genes (TSGs) have been implicated in cancer development (Macleod, 2000)). Normal mammalian cells contain cellular genes termed proto oncogenes that play a role in growth, development and differentiation. Proto oncogene activation to oncogene leads to uncontrolled cell growth and eventually neoplastic transformation. Oncogenes may be involved in different
stages of neoplasia. Some are involved in initiation, whereas others have a role in promotion, progression or metastasis; while some oncogenes cause uncontrolled growth by activating persistent growth stimulatory signal transduction pathways, others alter critical nodes in the cell cycle (Park, 1998).

Tumor suppressor genes maintain normal cellular homeostasis by controlling cellular proliferation, differentiation and apoptosis. Loss or inactivation of these genes is associated with the development of malignancy (Macleod, 2000). The TSGs are categorized into two classes. Growth inhibitory TSGs (GITSGs) and malignancy suppressor genes (MSGs) (Islam & Islam, 2000). GITSGs are cancer susceptibility genes that have an antagonist effect on cellular growth. These genes compete with oncogenes to bind a common factor to mediate their biological effects. MSGs have the ability to cause tumor suppression either by inducing cell differentiation or programmed cell death (Jansen Durr, 1996).

Genetic instability resulting from errors in DNA replication and repair may predispose a cell to the malignant phenotype. This can activate oncogenes or delete regions containing TSGs. Loss of heterozygosity (LOH) is a hallmark of TSG inactivation. Current evidence suggests that complex interactions between multiple TSGs with oncogene are required for cancer progression (Liotta & Liu, 2001).

A large number of factors, including oncogenes, tumor suppressor genes or hormonal receptors, may be altered in breast cancer. In fact, acquired or inherited abnormalities in a wide variety of genes have been
implicated in the pathogenesis of breast cancer (Isaacs, 1995; Jones et al.,
1995; Kallioniemi & Visakorpi, 1996; Walker et al., 1997). However, it
should be emphasized that most of these genetic abnormalities, including
those recently described in the PTEN / MMAC1 gene (Li et al., 1997; Steck
et al., 1997; Liaw et al., 1997), are not exclusive of breast cancer and
represents alterations in oneogenes or tumor suppressor genes commonly
mutated in human tumors from different origins. Nevertheless, mutational
studies on some genes, including AR gene and those involved in hereditary
breast cancer (BRCA1, BRCA2), have provided some results that may be of
relevance in the context of putative genetic abnormalities common to breast
and prostate cancer. The interest in AR as a potential factor common to both
tumors arises from recent observations indicating that genetic abnormalities in
this hormonal receptor are shared by these two hormonally dependent
tumors, but admittedly in only a small proportion of patients (Bentel & Tilley,
1996, Lobaccaro et al., 1993; Hall et al., 1996; Zhu et al., 1997).

1.7. AR alterations in breast cancer:

The AR is a transcription factor that plays an essential role in a
wide number of biological functions, from development and maintenance of
male reproductive functions to modulation of immune responses or
development of neural tissues (Chang et al., 1995). Like other nuclear
receptors, AR exerts its biological effects after binding of circulating
androgens mainly transported to target tissues by carrier proteins (Quigley et
al., 1995). Androgen binding induces a conformational change in the AR that
facilitates receptor homodimerization, nuclear transport, and interaction with
DNA. The binding of the AR to the hormone response element (HRE) present
in target genes results in the regulation of their transcriptional activity (Desl Pere et al., 1992). The structure of the AR is also similar to that of the other members of the steroid receptor family of ligand dependent transcription factors, with an N-terminal transactivating domain (exon A), a central DNA-binding domain (exon B and C), and a C-terminal hormone binding domain (exons D through H) (Chang et al., 1988).

The first indication that AR may also be altered in breast carcinoma was provided by Wooster et al., (Wooster et al., 1992), who reported an AR germline mutation in two brothers with breast cancer and Reifenstein syndrome, a partial androgen insensitivity syndrome originally described as an X-linked familial syndrome of hypospadias, infertility, and gynecomastia in association with normal 17-Ketosteroid excretion and high FSH levels (Quigley et al., 1995). The mutation results in the substitution Arg 607 Gln, within the region encoding the DNA-binding domain of the receptor. More recently, Lobaccaro et al., (Lobaccaro et al., 1993) identified another germline mutation in the AR gene, in a man with lobular carcinoma of the breast and partial androgen insensitivity syndrome. In summary, a series of recent studies performed by different groups has revealed that inherited and acquired AR alterations may occur in breast carcinomas.

1.8. BRCA1 and BRCA2 alterations in breast cancer:

Evidence for a genetic component in breast cancer risk was first noted by Paul Broca more than one century ago, when he described four generations of breast cancer in his wife's family (Broca, 1866). Since then extensive epidemiological analysis of breast cancer cases that appear to be
clustered in families have been reported. The results of this analysis suggest that about 5% of breast carcinomas may be explained by inherited mutations in one or more genes. Despite the genetic heterogeneity of breast cancer and the high prevalence of sporadic disease, several breast cancer susceptibility loci have been identified (Serova et al., 1997). The first of these genes, named BRCA1, was mapped in 1990 to chromosome 17q 21 by genetic linkage analysis of large families that included many cases of early-onset breast carcinomas (Hall et al., 1990) and has been recently identified by Miki et al (1994) using positional cloning methods. BRCA1 is composed of 22 coding exons distributed over more than 100kb of genomic DNA and encodes a 1863 amino acid protein, with two RING finger domains as its N-terminal part, which are thought to be involved in DNA-binding or in protein-protein interactions. In addition, BRCA1 shares a conserved region with 53 bpi (a p53 binding protein) and rad 9 (a yeast protein involved in the control of the DNA damage - induced cell cycle arrest), which has suggested that BRCA1 is likely to function in the cell nucleus and may be involved in one or more cell cycle check points (Koonin et al., 1996).

Mutations in the BRCA1 gene are thought to account for about half of the families susceptible to early onset breast cancer and for at least 80% of families with clustered breast and ovarian cancers (Couch et al., 1996, Szabo CI & King MC, 1997). To date, germline BRCA1 mutations have been reported in more than 200 families from different geographic origins. Germline BRCA1 mutations have also been found in young women with breast cancer who do not belong to families with multiple affected members (Langston et al., 1995). All classes of mutations are represented in these reported cases,
including missense mutations, nonsense mutations, deletions, insertions, or intronic mutations, although the majority result in the production of a truncated protein. The finding of this large percentage of loss-of-function mutation is consistent with the hypothesis that BRCA1 acts as tumor suppressor gene. It is also remarkable that most of the reported BRCA1 gene mutations have been identified in a single family, but a small number have been detected repeatedly. Of particular interest is a frameshift mutation caused by deletion of an AG dinucleotide, which has been identified in more than 20 families of Ashkenazi Jewish descents and is estimated to occur at a frequency of about 1% in this population (Struwing et al., 1995; Fitz Gerald et al., 1996).

The observation that less than half the families with multiple cases of breast cancer showed linkage to BRCA1 led to the proposal that there was at least an additional gene associated with breast cancer susceptibility. This result prompted another genomic linkage search and a second breast cancer susceptibility gene, named BRCA2, was located on chromosome 13q12 (Wooster et al., 1994) and subsequently cloned (Tavtigian et al., 1996). BRCA2 is composed of 27 exons and encodes a protein of 3418 amino acid residues, which does not appear to be significantly similar to other proteins. Recent studies have shown that BRCA2 expression is coordinately regulated with BRCA1 expression during proliferation and differentiation in mammary epithelial cells, suggesting that both genes may act in the same pathway (Rajan et al., 1996). Similarly to BRCA1, BRCA2 interacts with Rad 51, providing additional support to the proposal that these proteins may be essential cofactor in the Rod 51 – mediated repair of double strand breaks (Sharan et al., 1997). In fact, Connor et al (1997) have found evidence of a
DNA repair defect in mice with a truncating BRCA2 mutation. Clinical studies have revealed that BRCA2 probably accounts for a proportion of early onset breast cancer roughly equal to that resulting from BRCA1, and it may be of special importance in families with a high incidence of male breast cancer, but not in those with multiple cases of ovarian cancer. Mutational analysis of the BRCA2 gene in different populations has revealed that as in BRCA1, the identified mutations are widely distributed throughout the coding sequence of the gene, although evidence of some recurrent mutations has also been found (Couch et al., 1996; Phelan et al., 1996). Also of interest is the finding that BRCA2 mutations in families with the highest risk of ovarian cancer relative to breast cancer and clustered in a single exon of this gene (Gayther et al., 1997). Finally, and also in common with BRCA1, diverse studies have shown that BRCA2 is a very infrequent target for somatic inactivation in breast and ovarian cancers (Miki et al., 1996; Teng et al., 1996; Friedman et al., 1997).

1.9. P53:

Over expression of p53 in breast tumors is positively correlated with mutations that increase the stability of the P53 protein (Hill et al., 1996). In the Li-Fraumeni syndrome, germline mutations are associated with early onset breast cancer (Sidransky et al., 1992). Mutations in the P53 gene are very common, highly associated with breast cancer and are indicators of early onset breast cancer as well as poor prognosis (Done et al., 2001). Specific alterations in P53 are maintained during progression from intraductal to infiltrating carcinoma as well as during metastasis. In a study on sporadic
breast carcinoma in Indian women, Kannan et al (2000), found significantly increased frequency of mutations in the P53 gene.

A target gene for P53, is WAF1/CiP1 involved in cell cycle regulation (EL-Deiry et al., 1993). Wild type P53 induces transcription of this gene, leading to cell cycle arrest at the G1/ S transition. In breast cancer cell lines, high levels of mutant P53 associated with low levels of WAF1/CiP1 gene product prevent the cells from arresting at G1/S phase (Seikh et al., 1994).

1.10. HER-2/neu :

The protooncogene HER-2/neu (C-erb B-2) encodes a transmembrane tyrosine kinase receptor that has considerable homology with epidermal growth factor receptor (Yamauchi et al., 2001). The HER-2/neu protein is a member of a family of growth factor receptors that includes epidermal growth factor or HER-1 (erb-B1), HER-3 (erb-3) and HER-4 (erb-4) (Plowman et al., 1993). Heregulins are a family of growth factors that bind to these receptors inducing transition of the downstream genes (Ross & Fletcher JA, 1998, Simon et al., 2001). Over expression HER-2/neu gene identified in 10-34% of breast cancer has been linked to faster disease progression metastasis and decreased survival. Tan et al (1998), have suggested that HER-2/neu may affect the invasive or metastatic properties of breast cancer by enhancing cell aggregation or homophilic adhesion. Heregulin is recognized to stimulate P21 activated kinase involved in the promotion of cell migration and signaling pathway. However, the cellular mechanisms of breast
cancer progression by the HER-2/neu heregulin pathway remain to be elucidated (Adam et al., 1998).

1.11. Free Radicals And Cancer:

Epidemiological and experimental studies have implicated free radicals in the aetiology and development of cancer (Poulsen et al., 1998). A free radical is a highly reactive molecular species that contains one or more unpaired electrons. Free radicals are produced under physiological and pathological conditions. The four common reactive oxygen metabolites in biological systems include superoxide anion ($\text{O}_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH$^-$) and singlet oxygen ($^1\text{O}_2$). These are collectively known as reactive oxygen species (ROS) or oxy radicals or oxygen free radicals (OFR) (McDermott, 2000).

ROS are oxidants and highly toxic to all types of biological molecules including DNA, lipids, proteins and carbohydrates. They may be involved in processes such as mutagenesis, carcinogenesis, membrane damage, lipid peroxidation as well as carbohydrate damage (Datta et al., 2000). Oxidative destruction of polyunsaturated fatty acids (PUFA) initiates a self perpetuating chain reaction termed lipid peroxidation. (Cheeseman & slater,1993). They can cause tumour initiation by inducing DNA modifications including helical distortions, single and double strand breaks and interstrand crosslinks. OFR – induced DNA damage can block DNA replication, activate oncogenes and inactivate tumour supressor genes (Dreher & Junod, 1996). OFR generation activates protein kinase and induces DNA breakage. It leads to the induction of immediate early genes c-fos and c-myc, which stimulate
proliferation and tumour promotion (Sen, 1995). Overproduction of OFR can also cause alterations in DNA methylation in tumour cells resulting in aberrant gene expression thereby promoting tumourigenesis (Cerda & Weitzman, 1997). In addition OFR are recognised to stimulate tumour promotion by modulating apoptotic gene expression and poly ADP ribosylation of chromosomal proteins (Mates & Sanchez Jimenez, 2000). Excessive generation of OFR has been reported to influence tumour progression by causing genomic instability. Mutations in P53, a tumour suppressor gene which protects against OFR – induced carcinogenesis are the most frequent mutations in human cancer. This causes DNA damage and chromosomal rearrangements (Hussain et al., 1994).

1.12. Antioxidants:

Normal cells possess unique antioxidant defence mechanisms to combat the deleterious effects of OFR. An antioxidant is any substance which when found at low concentration compared to that of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1990). Antioxidants play a vital role in defense systems that deal with OFR – induced carcinogenesis. Inhibition of malignant transformation by antioxidants may be due to one or more of the following mechanisms, neutralization of OFR, preventing the metabolic activation of procarcinogens, inactivating carcinogens, inhibition of carcinogen binding to DNA, enhancing DNA repair mechanisms, inhibiting chromosomal aberrations, decreasing the expression of protooncogenes and reducing the expression of transcription factors and nuclear binding proteins involved in tumour progression (Shklar, 1998).
Antioxidants can be divided into three main groups – cellular enzymes, metal ion chelators, and a variety of other small molecules. The important enzymic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Metal ion chelators such as albumin, transferrin, haptoglobin, ceruloplasmin and metallothionein can sequestrate metal ions. The non enzymatic antioxidants and other small molecules with antioxidant function include reduced glutathione (GSH), ascorbic acid, α-tocopherol, β- Carotene, Uric acid and bilirubin (Weijl et al., 1997).

1.13. Superoxide Dismutase (SOD, EC 1.1.5.1)

Superoxide dismutase is the antioxidant enzyme that catalyzes the dismutation of the highly reactive superoxide anion to the less reactive species H₂O₂ which is subsequently destroyed by CAT or GPₓ reaction (Fridovich, 1995).

\[
O_2^\cdot + O_2^\cdot + 2H^+ \rightarrow H_2O_2 + O_2
\]

In humans, there are three forms of SOD - cytosolic cu/zn-SOD, mitochondrial Mn-SOD, and extracellular SOD that are involved in the process of destroying O₂ by successive oxidation and reduction reactions (Sandstrom et al., 1994)

1.14. Catalase (CAT, EC 1.11.1.16)

Catalase is present in all mammalian cell types. The enzyme is located in the sub cellular organelles such as the peroxisomes of the liver and kidney. Catalase consists of four protein subunits, each of which contains ferriprotoporphyrin group bound to its active site. CAT reacts with H₂O₂ to
form water and molecular oxygen and with H donors (methanol, ethanol, formic acid or phenols) with peroxidase activity (Eaton, 1991).

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

\[ \text{ROOH} + \text{AH}_2 \rightarrow \text{H}_2\text{O} + \text{ROH} + \text{A} \]

1.15. The Glutathione Redox Cycle:

1.15.1. Reduced Glutathione (GSH):

The glutathione redox cycle is one of the most important antioxidant systems. Glutathione, a low molecular weight tripeptide consisting of glutamate, cysteine and glycine, is the predominant cellular non-protein thiol in both prokaryotes and eukaryotes (Jernstorm et al., 1993). Eukaryotic cells have three major reservoirs of GSH – cytosol, mitochondria and endoplasmic reticulum (Anderson, 1996).

GSH spares membrane proteins and maintains ascorbic acid in the reduced form. Glutathione, a substrate for glutathione peroxidase and glutathione S-transferase, functions as a scavenger of free radicals such as $\text{O}_2^-$, $\text{OH}^-$ and lipid hydroperoxidases (Saez et al., 1990). GSH is known to play a crucial role in immunomodulation, remodelling of the extracellular matrix, apoptosis and mitochondrial respiration (Rahman & Macnee, 2000).

1.15.2. Glutathione Peroxidase (GPx EC 1.11.1.9)

This enzyme is found abundantly in the liver and erythrocytes. It consists of four protein subunits, each of which contains a single selenocysteine at its active site (Tappel, 1978).
Glutathione peroxidase catalyzes the reduction of hydroperoxides using GSH, thereby protecting mammalian cells against oxidative damage (Esterbauer et al., 1992).

\[ \text{ROOH} + 2\text{GSH} \rightarrow \text{ROH} + \text{GSSG} + \text{H}_2\text{O} \]

GPx removes H$_2$O$_2$ by coupling its reduction to H$_2$O with oxidation of reduced glutathione.

\[ \text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow \text{GSSG} + 2\text{H}_2\text{O} \]

1.15.3. *Glutathione S-Transferases (GSTs EC 2.5.1.8)*

GSTs, a multigene family of detoxification enzymes catalyze the conjugation of glutathione to electrophilic compounds thereby protecting cells against xenobiotics (Hayes & Pulford, 1995). Based on structural, physiochemical, enzymatic and immunological properties, GSTs are categorised into four classes, namely a, p, n and 0. GSTs are induced by several classes of compounds that offer protection against toxic and carcinogenic chemicals (Seidegard & Ekstrom, 1997).

1.16. *Vitamin E and Ascorbic Acid:*

Vitamin E reacts with lipid radicals generated in cell membranes to protect against further lipid peroxidation. \( \alpha \)-Tocopherol is most effective against carbon centred radicals and peroxyl radicals. It functions as a chain breaking antioxidant and prevents propagation of free radical reaction (Brigelius Flohe & Traber, 1999).

Ascorbic acid also has an important role in protection against lipid peroxidation. Ascorbic acid scavenges O$_2^-$, H$_2$O$_2$, Hocl and thiol radicals,
and is a potent quencher of singlet oxygen (Sauberlich, 1999). When vitamin E reacts with a radical, it is converted to a radical form, which can be recycled to vitamin E by reaction with vitamin C. Ascorbic acid spares GSH and together with vitamin E, prevents the oxidation of glutathione (Bayer, 1994). Tocopherol along with ascorbic acid has been shown to decrease the sensitivity to lipid peroxidation and increase glutathione levels (Rojas, et al 1996).

1.17. Risk Factors For Breast Cancer:

1.17.1. Age at menarche and menopause:

The incidence of breast cancer increases with age doubling about every 10 years until menopause. Women who start menstruating early in life or who have a late menopause have an increased risk of developing breast cancer (Rosner & Colditz, 1996).

1.17.2. Age at first pregnancy:

Nulliparity and late age at first child birth increases the lifetime incidence of breast cancer. The risk of breast cancer in women, who have their first child after the age 30 is about twice that of women who have their first child before the age of 30 (Albrektsen et al., 1999).

Obese postmenopausal women have a two fold increase in the risk of breast cancer when compared to premenopausal women (Thomas et al., 1997).
1.17.3. Oral Contraceptives:

Combination oral contraceptive use increases the risk of breast cancer in young women. The duration of use, age at first use, dose and type of hormone within the contraceptives appear to have no effect on breast cancer risk. Women who begin to use oral contraceptives before the age of 20 appear to have a higher relative risk than women who begin to use at an older age. Oral contraceptives play a major role in breast cancer occurrence because they exhibit their action by mimicking natural hormones which are major risk factors of breast cancer (Feigelson & Henderson, 1996).

1.17.4. Family History:

Breast cancer susceptibility is generally inherited as an autosomal dominant trait with limited penetrance. Breast cancer can be transmitted through either sex and some family members may transmit the abnormal gene without developing cancer themselves. Many families affected by breast cancer show an excess of ovarian, colon, prostatic and other cancers attributable to the same inherited mutation.

A woman's risk of breast cancer is two or more times greater if she has a first degree relative who developed the disease before the age of 50, and the younger the relative when she developed breast cancer, the greater the risk (Decker, 1993)

1.17.5. Previous benign breast disease:

Women with severe atypical epithelial hyperplasia have four to five times higher risk of developing breast cancer. Women with palpable
cysts, complex fibroadenomas, duct papillomas, sclerosis, adenosis and moderate or florid epithelial hyperplasia have slightly higher risk of breast cancer (McPherson et al., 2000).

1.17.6. Diet:

Dietary factors are widely believed to play an important role in determining the risk of many cancers, including breast cancer. Vitamin A and carotenoids are considered anticarcinogenic in experimental systems. Fruits and vegetables seen to confer protection (Trichopoulou et al., 1995). Heterocyclic amines, consumed with charbroiled food, have carcinogenic potential (Sugimura et al., 1994). Plant estrogens found in soy products such as tofu have been suggested to confer protection against breast cancer in Asian populations (Adlercreutz, 1995; Messina & Barnes, 1991). Vitamin D has been proposed as an anticarcinogenic compound for breast cancer (Colston et al., 1989). High circulating levels of 1, 25 - dihydroxy vitamin D were associated with low incidence of breast cancer.

The current epidemiological data suggests that the epidemic of breast cancer may be partially attributable to increased fat consumption, increased caloric intake during growth, low fiber, vegetable and fruit consumption, and other lifestyle factors including exercise, alcohol, and smoking (Prentice et al., 1990; Hunter & Willett, 1996; Rose & Hender Sone, 1994). Refinements in our knowledge regarding fat consumption and its connection to cancer suggest that specific fatty acids (e.g., the n-6 polyunsaturated fatty acids) may be more potent tumor enhancers than other unsaturated or saturated fatty acids (Rose, 1996, 1997; Fay et al., 1997; Willett, 1997).
Studies on breast cancer and diet have shown a positive correlation between saturated fatty acids and breast cancer risk and a significant negative association for unsaturated fatty acids (Hunter, 1999).

1.17.7. Life Style:

Although studies have shown a positive correlation between alcohol consumption and the incidence of breast cancer, the results are inconsistent. (Mcpherson K et al., 2000).

1.17.8. Estrogens and Breast Cancer:

The common risk factor in the development of breast cancer is the cumulative exposure to 17B-estradiol. This exposure may be endogenous or exogenous.

1.17.8.1. Endogenous Estrogens:

Increased lifetime exposure to estrogens especially estrone and estradiol has long been linked to the promotion and progression of breast cancer because of their physiological actions on the mammary gland (Dorgan et al., 2001; Clemons & Goss, 2001).

Estrogens promote the development of mammary cancer in rodents and exert both direct and indirect proliferative effects (Lupulescu, 1995). The direct effect may involve activation of oncogenes and induction of enzymes and proteins that may play a role in nucleic acid synthesis. Stimulation of prolactin secretion and production of growth factors and non growth factor peptides and some of the indirect effects of estrogens on cell proliferation. Although the exact mechanisms of estrogen induced breast
tumorigenesis remain to be elucidated, the alkylation of cellular molecules and generation of active radicals that can damage DNA, together with potential genotoxicity of estrogen and some of its metabolites have been implicated (Nandi et al., 1995; Yager & Liehr, 1996).

1.17.8.2. Exogenous estrogens:

The impact of exogenous estrogens on development, reproduction and health has attracted the focus of recent attention. These are a group of chemicals with widely diverse structures that exhibits estrogenic properties. They are referred to as estrogenic xenobiotics or xenoestrogens (Olea et al., 1998). Exposure to xenoestrogens may be from the diet or the environment. Animal fat is most common dietary source of xenoestrogens because these compounds accumulate in adipose tissue. Environmental estrogens are being increasingly implicated in breast carcinogenesis. These include organo chlorine pesticides such as dichlorodiphenyl trichloroethane (DDT), toxaphene, dieldrin and endosulfan, phenolic derivatives and poly chlorinated biphenyls (PCBs), antioxidants such as t-butyl hydroxyanisole and alkylphenol, and plasticizers such as benzylbutylphthalate, and compounds used in dental restorations such as bisphenol – A (Longnecker et al., 1997; Olea & Olea Serrano, 1996).

Humans may be inadvertently exposed to xenoestrogens in several ways including water, food, air, medical or personal products. Due to their lipophilic nature, xenoestrogens accumulate in adipose tissues during pregnancy and lactation causing steady accumulation in the foetus and infants (Olea et al., 1998).
Certain plant derived dietary constituents also have estrogenic activity. These include soy and legumes rich in isoflavone phytoestrogen (genistein, dieldrin, and coumestrol). These exert anticarcinogenic effects by functioning as estrogen antagonists, antioxidants, inhibitors of aromatase enzymes and by altering hormone levels (Wolf & Weston A., 1997). Diets rich in vegetables, fruits and grain products can modulate breast carcinogenesis (Devis et al., 1997). Although higher concentrations of phytoestrogens inhibit cell cycle proliferation, lower concentrations are known to induce proliferation effects on human breast cancer cells (Dees et al., 1997).

Studies have found, small to moderate increase in breast cancer risk after long term estrogen replacement alone (Feigelson & Henderson, 1996), combined estrogen and progestin therapy could be more carcinogenic than estrogen alone (Schairer et al., 2000).

1.17.8.3. Estrogen receptors:

Estrogens play an important role in the aetiology of breast cancer through the expression of their receptors. Estrogens diffuse passively through the cell and nuclear membranes and bind to estrogen receptors. The ligand receptor complex binds to and activates estrogen response elements in the upstream promoter region of target genes (Clemons & Goss, 2001).

1.18. Management of Breast Cancer:

Three major treatments are surgery, radiation and drug therapy. Surgery is used for initial management stage-I tumors <2 cm. In stage-II
tumour, surgery and radiation therapy are employed. In stage III inoperable lesions, high dose chemotherapy, stem cell transplantation and hormonal therapy are effected. In stage-IV chronic, incurable tumors as distant metastases all three modes are utilized but with little effect.

Surgery involves mastectomy and minimal invasive techniques employ laser, deep freezing of cancer cells, high intensity ultrasound. Radio frequency ablation employs enough heat to destroy cancer cells. Radiation therapy uses γ- rays to kill cancer cells. Chemotherapy utilizes cyclophosphamide, methotextrate and 5’ fluorouracil. Metastatic disease irrespective of hormone receptor status utilises paclitaxel and metalloproteinase inhibitors eg. Marimastat (Rassmussen & Mc Cann, 1997). Hormone therapy involves blocking estrogen binding to receptor in case of estrogen positive cells. Hormone therapy involves employment of steroids or non-steroids. Tamoxifen is a selective estrogen receptor modulator, which unlike estrogen binds to receptor and does not stimulate cancer cell growth (Fischer et al., 1992). Aromatases are enzymes that are major source of estrogens. Aromatase inhibitors are also used in management of metastatic hormone receptor positive tumors. Aminoglutethimide is a non steroidal aromatase inhibitor. Steroidal inhibitors are also used.

High dose chemotherapy along with peripheral blood stem cell rescue or bone marrow transplantation is used for breast cancer that has metastasized. The objective is to kill cancer cells with very high toxic doses of chemotherapy without destroying the ability to regenerate new healthy blood cells. Monoclonal antibodies genetically designed to target specific antigens.
Trastuzumab (herceptin) is designed to target and block protein encoded by HER-2/neu gene which are responsible for 30% of breast cancer cell growth (Gilewski et al., 2000). Bisphosphonates prevent fractures and reduce pains in patients whose cancer has spread to bone (Ralston et al., 1989). In addition to trastuzumab, a number of drugs that use patients own immune system to prevent or fight off cancer, are used as vaccine that boost host factors in the immune system specifically attack breast cancer cells are being developed.

1.19. Antioxidants In Cancer Therapy:

Dietary and endogenous antioxidants prevent cellular damage by reacting with the elimination of free radicals. However, cancer chemotherapeutics involves generation of free radicals to cause cellular damage and necrosis of malignant cells. So a concern has logically developed as to whether exogenous antioxidant compounds taken concurrently with drugs are beneficial for chemotherapy. A number of data show reduction in adverse effects of chemotherapy when given concurrently with antioxidants (Weijl et al., 1997). Conflicting views in the use of antioxidants to cancer therapy have also been presented. Therefore the cautious and judicious use of antioxidants along with chemotherapy is recommended. The argument that antioxidants could blunt the effect of standard therapies prevails (Labriola and Livingston, 1999).

1.20. Outcome of Therapies:

Surgery: Short term pain and tenderness may appear. Removal of lymph node causes numbness, tending and oedema occur.
**Radiation Therapy:** Radiation increases risk of developing other cancers such as soft tissue malignancies like sarcoma, cause loss of appetite, fatigue and burns on skin appear. Lymphoedema develop impaired mobility.

**Systemic Therapy:** Nausea, hairloss, fatigue, weight loss, supression of immune system, allergic reactions occur to taxols as well as drop in white blood cells.

1.21. **Selective estrogen receptor modulators (SERMS):**

Recent studies have suggested that breast cancer could be prevented by developing drugs to block estrogen action in the breast (Brown et al., 2000). Selective estrogen receptor modulators (SERMS), such as tamoxifen, reloxifen etc., which are competitive inhibitors of estrogen binding at estrogen receptors alpha and beta, play a major role in the prevention and treatment of breast cancer. Each estrogen receptor complex has an unique structure that influences its activity in different body tissues. Although tamoxifen appears to decrease the risk of breast cancer, it has been reported to induce endometrial cancers (Jordan et al., 2001; Lippman et al., 1998).